Fluorescence-based Biochemical Assays for the Study of PXR and CAR

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Abstract

Pregnane X receptor (PXR), or CAR, steroidal and xenobiotic receptor; and constitutive androstane receptor (CAR) are two of the most extensively studied drug metabonomics targets, notably the xenobiotic PXR binding, and other proteins relevant to ACHD studies, such as drug transporters. Bioluminescent high-throughput screening (HTS) assays were developed for human PXR and CAR using the LanthaScreen®-based fluorescence resonance energy transfer (FRET) technology. These assays enable the discovery and evaluation of compounds that bind to PXR, or compounds that modulate the association of constitutively-bound peptides with PXR or CAR. Competitive binding to PXR is detected by a compounds’ ability to displace a fluorescent PXR ligand (Fluromone™ PXR Green) and disrupt FRET. The coregulator peptide assay can be performed with either PXR or CAR. The nuclear receptor (NR) is indirectly labeled with a terbium (Tb) chelate, and assay buffers were from Invitrogen Corp. (Madison, WI). The PXR protein is produced by Invitrogen under an exclusive agreement with the University of Rochester (Rochester, NY). The coregulator peptide assay can be performed with either PXR or CAR. The nuclear receptor (NR) is indirectly labeled with a terbium (Tb) chelate, and assay buffers were from Invitrogen Corp. (Madison, WI). The PXR protein is produced by Invitrogen under an exclusive agreement with the University of Rochester (Rochester, NY).

Materials & Methods

Pregnane X receptor (PXR) and CAR, stereoidal and xenobiotic receptor; and constitutive androstane receptor (CAR) are two of the most extensively studied drug metabonomics targets, notably the xenobiotic PXR binding, and other proteins relevant to ACHD studies, such as drug transporters. Bioluminescent high-throughput screening (HTS) assays were developed for human PXR and CAR using the LanthaScreen®-based fluorescence resonance energy transfer (FRET) technology. These assays enable the discovery and evaluation of compounds that bind to PXR, or compounds that modulate the association of constitutively-bound peptides with PXR or CAR. Competitive binding to PXR is detected by a compounds’ ability to displace a fluorescent PXR ligand (Fluromone™ PXR Green) and disrupt FRET. The coregulator peptide assay can be performed with either PXR or CAR. The nuclear receptor (NR) is indirectly labeled with a terbium (Tb) chelate, and assay buffers were from Invitrogen Corp. (Madison, WI). The PXR protein is produced by Invitrogen under an exclusive agreement with the University of Rochester (Rochester, NY).

Results and Conclusions

• A panel of HTS assays was developed for studying ligand and coregulator peptide interactions with PXR and CAR using the LanthaScreen® technology. The assay panel is ideal for rapid evaluation of the direct interactions between test compounds and these nuclear receptors.

• Ligated-dependent and independent interactions with a panel of coregulator peptides was assessed with PXR and CAR. As expected, significant ligand-dependent constitutive association was observed with 48-hour ligands to PXR agonists for both receptors caused peptide recruitment, while inverse agonists of CAR disrupted constitutive association between the receptor and peptides.

• Overlapping ligand selectivity was observed for PXR and CAR (Table 1). However, the glucocorticoid receptor (GR) interacts constitutively with the ligand, and the human-CAR interactions with GR-1 (100 pM) were found to be selective for CAR (1 nM CAR-GR). The PXR and CAR assays were robust, with Z-factor values 0.7-0.8, and were stable during room temperature incubations (Table 2).