Combined Analysis of Nucleic Acids and Protein for Cancer Research

Jared Isaac1, Mazi Mohiuddin2, David Sarracino2, Mousumi Rath3, Amol Prakash4, Haiping Liu5, Sharmi Muralitharan5, Jennifer Freeland5, Shen Lu5, and Colin Toombs6

1Anatomical Pathology Division (APD) Kalamazoo, MI; 2Chromatography Mass Spectrometry Division Cambridge, MA; 3Optys Boston, MA; 4APD Fremont, CA; 5APD Runcorn, GB
Contact: jared.isaac@thermofisher.com

Poster Note 64806

ABSTRACT
This study was undertaken to determine the feasibility of isolation of RNA, DNA and Protein from a single formaldehyde fixed paraffin embedded (FFPE) section to detect lung cancer biomarkers. Correlation of RNA expression and DNA mutations by Next Generation Sequencing (NGS) and Protein expression by liquid chromatography mass spectrometry (LC-MS) from the same tissue section is expected to empower researchers with confirmatory, quantitative data.

A proprietary Unified Method (UM) (Thermo Fisher Scientific, Kalamazoo, MI) for isolating RNA, DNA and Proteins from a single FFPE section was developed and used to analyze RNA expression, DNA mutations and Protein expression from a clinical sample. The RNA and DNA were analyzed using the Thermo Scientific™ Ion AmpliSeq™ NGS platform and the Proteins were analyzed on a Thermo Scientific™ LC-MS system. A Proprietary Integrated Bioinformatics Software (PIBS) (Thermo Fisher Scientific, Kalamazoo, MI) was developed to correlate RNA expression, DNA mutations and Protein Expression. The PIBS correlates RNA and Protein Quantification, DNA mutations and morphological information, and it is scalable to handle replicate datasets from Proteogenomics workflows. Researchers will be able to correlate data across profiles and identify differentially expressed markers. Proteogenomics data can be analyzed with histological subtypes as well as the presence of drug targets for research use. Development of targeted LC-MS research methods is expected to increase the correspondence between FF and FFPE datasets as well as the detection of future biomarkers of interest.

INTRODUCTION

Immunohistochemistry (IHC) probes are a commonly used testing technology. 20-35% of IHC qualitative testing is equivocal, leading to repeated testing and increased costs (1, 2). Evaluation of lung cancer (LCa) is dependent on determination of cancer subtype: Adenocarcinoma (AC) or Squamous Carcinoma (SC) (3). This study was performed to determine if the use of a modified Liquid Chromatography Mass Spectrometry (LC-MS) for protein detection will improve the identification of LCa biomarkers.

MATERIALS AND METHODS

Sample Preparation

LCa Adenocarcinoma purchased from Asterand Biosciences, Detroit, MI. Tissue was grossed into 3 cm sections and fixed with 10% neutral buffered formalin (NBF) for 24 hr at RT. After fixation, tissues were processed under standard conditions, paraffin embedded (FFPE) and stored at 4 C ~30 ng fresh frozen (FF) pieces were used as controls (CTRL). 7 μm curls were sectioned and processed with the UM conditions and 4 μm sections adhered to glass slides for IHC staining.

IHC

IHC was performed on the Thermo Scientific™ Lab Vision™ Autostainer 360-2D and PT Module and Thermo Scientific™ UltraVision™ Quanto Detection System HRP DAB per manufacturer’s recommendations.

Nucleic Acid (NA) and Protein Quality Control

Extracted NA and Protein were QC assayed using the Thermo Scientific™ Qubit® 3.0, Thermo Scientific™ Nanotip® 2000c, and the 2100 Bioanalyzer (Agilent Technologies, Santa Clara, CA).

NGS

NA were processed on the Ion AmpliSeq workflow using the Thermo Scientific™ Ion AmpliSeq™ RNA and DNA Cancer 50 gene Panels on the Thermo Scientific™ Ion Personal Genoma Machine™ (PGM).

LC-MS

Peptides from digested proteins were cleaned with Thermo Scientific™ HyperSep™ Ratain CX SPE cartridges and injected in 98:2 Water Methanol Formic Acid into a Thermo Scientific™ Dionex™ Ultimate™ 3000 HPLC with Thermo Scientific™ Dionex™ Acquity™ 120 C18 Reversed-Phase LC column. Data were acquired in 60 minute runs with data dependent analysis on a Thermo Scientific™ Q Exactive™ HF (QEHF) hybrid quadrupole-orbitrap. Using Pierce PRTK standards run before and after each sample. The QEHF was used with the following settings: FT 100 ms, AGC=3x106, 120,000 resolution, and a mass range of 350-1500 m/z.

Bioinformatics

NGS sequencing was processed using Thermo Scientific™ Ion Reporter™ software and LC-MS data were analyzed with Thermo Scientific™ Proteome Discoverer™ software. A Proprietary Integrated Bioinformatics Software (PIBS) was developed to perform an integrated analysis of Laser Capture Microdissection (LCM) and Proteogenomic data.

RESULTS

Figure 1. Nucleic Acid and Protein Yield

In comparison to CTRL extraction, UM extraction of RNA (400 ng) and Protein (10 μg) yield is comparable, however DNA (50 ng) yield is much lower.

Figure 2. RNA NGS QC Parameters

Using the RNA Cancer 50 Panel, a median read length of 112 bp, 99.2% reads on target and 46/50 reads were detected at 100X for FFPE samples extracted using the UM in comparison to FF.

Figure 3. IHC Detection of LCa Biomarkers

Figure 4. Concordance of FF and FFPE Identified peptides and proteins by Discovery LC-MS.

Figure 5. Concordance of FF and FFPE Identified peptides and proteins by Discovery LC-MS.

Figure 6. Comparison of IHC and LC-MS Detection of LCa Biomarkers.

Table 1. AmpliSeq RNA Cancer 50 gene Targeted Panel (NGS) % reads on Target Targets detected at 100X

<table>
<thead>
<tr>
<th></th>
<th>UM</th>
<th>FF</th>
<th>FFPE</th>
</tr>
</thead>
<tbody>
<tr>
<td>% reads on Target</td>
<td>99.39%</td>
<td>46/50</td>
<td>99.20%</td>
</tr>
</tbody>
</table>

Diagnostic Errors More Common, Costly, and Harmful than Treatment Mistakes
Proteins from a single FFPE section was developed and used to analyze RNA expression, DNA mutations and chromatography mass spectrometry (LC-MS) from the same tissue section is expected to empower researchers.

Bioinformatics

Extracted NA and Protein were QC assayed using the Thermo

IHC was performed on the Thermo

Combined Analysis of Nucleic Acids and Protein for Cancer Research

controls (CTRL). A proprietary Unified Method (UM) (Thermo Fisher Scientific, Kalamazoo, MI) for isolating RNA, DNA and with confirmatory, quantitative data.

Cancer 50 gene Panels on the Thermo

MATERIALS AND METHODS

This study was undertaken to determine the feasibility of isolation of RNA, DNA and Protein from a single

with Thermo

NGS platform and the Proteins were analyzed on a Thermo

Scientific™

Machine™

software. A Proprietry Integrated Bioinformatics

Q

yield is comparable, however DNA (30 ng) yield is much lower.

Figure 1. Nucleic Acid and Protein Yield

CTRL    UM    CTRL    UM    CTRL    UM

Figure 6. Comparison of IHC and LC-MS Detection of LCa Biomarkers

<table>
<thead>
<tr>
<th>Protein</th>
<th>IHC</th>
<th>LC/MS</th>
<th>AC</th>
<th>SC</th>
</tr>
</thead>
<tbody>
<tr>
<td>CK5/CK6a/6b</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>CK7</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>CK20</td>
<td>-</td>
<td>+</td>
<td>?</td>
<td>?</td>
</tr>
<tr>
<td>c-MET</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>EGFR</td>
<td>+</td>
<td>-</td>
<td>?</td>
<td>?</td>
</tr>
<tr>
<td>HER2</td>
<td>-</td>
<td>-</td>
<td>?</td>
<td>?</td>
</tr>
<tr>
<td>KRAS</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Napsin A</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>p63/p40</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>TTF1</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
</tbody>
</table>

Figure 4. IHC Detection of LCa Biomarkers

Her2

Her2

KrAS

KrAS

c-Met

p63

TTF-1

Figure 5. Concordance of FF and FFPE Identified peptides and proteins by Discovery LC-MS

Figure 5. Concordance of FF and FFPE Identified peptides and proteins by Discovery LC-MS. Three FF and FFPE LCa samples each were processed with the UM and analyzed by discovery LC-MS. Violin diagrams show the triplicate overlap in FF (A) and FFPE (B) identified protein groups. FF (C) and FFPE (D) identified peptide groups. and FF versus FFPE identified protein groups (E, 753 and 688, respectively) and identified peptide (F, 3688 and 2746, respectively).

Figure 7. Schematic of UM LCM Workflow with PIBS

Figure 7. Schematic of UM LCM Workflow with PIBS. Demonstration of developed PIBS illustrating spatial localization of UM gene and protein expression color coded to LCa tissue micro dissected using the Archuna LCM. The PIBS provides correlation between RNASeq expression and Protein Quantitation coupled with the DNA mutations, and it is scalable to handle replicate datasets both from the proteomics and genomics workflows. Users will be able to correlate data across profiles, identify differentially expressed markers and generate interpretation reports.
Combined Analysis of Nucleic Acids and Protein for Cancer Research

Protein expression from a clinical sample. The RNA and DNA were analyzed using the Thermo Scientific™ Exactive™ Orbitrap MS with Pierce PRTK standards run before and after each sample. The QEHF was used with the Qubit® 3.0, Thermo Scientific™, and HyperSep™ Protein & DNA purification. The potential of this technology is to provide information for a clinical sample.

Scientific™. The proprietary Unified Method (UM) for extraction of biomolecules prior to Next Generation Sequencing (NGS) and Mass Spectrometry (LC-MS) from the same tissue section is expected to empower researchers to leverage the power of these technologies.

The PIBS correlates RNA and Protein Quantification, DNA mutations and morphological information, and it is intended to encourage use of these products in any manner that might infringe the intellectual property rights of others. Specifications, terms and pricing are subject to change. Not all products are available in all countries. Please consult your local sales representative for details.

FIGURE 1. Nucleic Acid and Protein Yield

FIGURE 2. RNA NGS QC Parameters

FIGURE 5. Concordance of FF and FFPE Identified peptides and proteins by Discovery LC-MS

FIGURE 6. Comparison of IHC and LC-MS Detection of LCa Biomarkers

FIGURE 7. Schematic of UM LCM Workflow with PIBS

MATERIALS AND METHODS

IMMUNOHISTOCHEMISTRY (IHC) PROBES ARE A COMMONLY USED TESTING TECHNOLOGY. 20-35% OF IHC QUALITATIVE TESTING RESULTS WERE INCORRECTLY READ AS POSITIVE.

INTRODUCTION

IMMUNOHISTOCHEMISTRY (IHC) PROBES ARE A COMMONLY USED TESTING TECHNOLOGY. 20-35% OF IHC QUALITATIVE TESTING RESULTS WERE INCORRECTLY READ AS POSITIVE.

FIGURE 1. Nucleic Acid and Protein Yield

FIGURE 2. RNA NGS QC Parameters

FIGURE 3. Concordance of FF and FFPE Identified peptides and proteins by Discovery LC-MS

FIGURE 4. IHC Detection of LCa Biomarkers

FIGURE 6. Comparison of IHC and LC-MS Detection of LCa Biomarkers

FIGURE 7. Schematic of UM LCM Workflow with PIBS

CONCLUSIONS

• A unified method (UM) for extraction of RNA, DNA and Proteins has been developed.
• Data generated using LCa tissue indicates reproducible and biologically interpretable information.
• The PIBS empowers cancer researchers by providing detailed information about Protein, DNA & RNA markers along with the morphological information about the tissue samples.
• The PIBS can provide confirmation of protein expression of multiple targets as opposed to a single antibody based IHC stain.
• Combined extraction of Nucleic Acid and Protein from the same section of FFPE tissue enables Proteogenomics & Translational Research.

REFERENCES