Proteomic Analysis of Perineural Invasion in Pancreatic Adenocarcinoma Reveals Up-Regulation of Neurosecretory Protein VGF in Invaded Nerves

Richard Jones1, Wasfi Alrawashdeh2, Michael Ford1, Ravi Amunugama1, David Allen1, Pedro Cuillas2, Nilukshi Wijesuriya3, Tatjana Crnogorac-Jurcevic2
1MS Bioworks, LLC, Ann Arbor, MI; 2Barts Cancer Institute, Queen Mary University of London, London, UK; 3Department of Pathology, Royal London Hospital, London, UK

1. Introduction

Pancreatic adenocarcinoma (PDAC) is one of the most lethal human malignancies. Perineural invasion (PNI) is a characteristic feature of PDAC reported in 60-100% of cases. PNi is associated with tumor recurrence, poor prognosis and pain in pancreatic cancer patients. However, the mechanisms underlying this process and its influence on both nerves and cancer still remain poorly understood. Here, the aim was to characterise the molecular changes at protein level in both cancer cells and nerves within PNi lesions using laser microdissection (LM) and mass spectrometry.

2. Sample Preparation and Mass Spectrometry

- 1D/LC
  - Peptide Digest A x5
  - Peptide Digest C x5
  - Peptide Digest B x5
  - Peptide Digest D x5

- 2D/LC
  - Peptide Digest A x3 Pooled
  - Peptide Digest C x3 Pooled
  - RP-PR 12 fractions per group in duplicate

Hierarchical Cluster Analysis (HCA) using Ln NSAF values showed grouping of IN and NIN. HCA is shown for all 1,013 proteins and those 133 proteins with p<0.05. 61 proteins (6%) had p<0.05 and fold change >2 (red spots in volcano plot).

3. Non-Invaded Nerves vs. Invaded Nerves (B vs. D)

Data were searched at 1% protein and peptide FDR and requiring at least two unique peptides per protein. Match between runs was selected. Detection metrics are summarized below:

<table>
<thead>
<tr>
<th>Total Proteins</th>
<th>B1</th>
<th>B2</th>
<th>B3</th>
<th>B4</th>
<th>B5</th>
<th>D1</th>
<th>D2</th>
<th>D3</th>
<th>D4</th>
<th>D5</th>
</tr>
</thead>
<tbody>
<tr>
<td>1D/LC (1,013 Total Proteins)</td>
<td>875</td>
<td>932</td>
<td>917</td>
<td>945</td>
<td>939</td>
<td>917</td>
<td>963</td>
<td>900</td>
<td>920</td>
<td>928</td>
</tr>
<tr>
<td>Total Unique Peptides</td>
<td>4112</td>
<td>4880</td>
<td>4891</td>
<td>5015</td>
<td>5108</td>
<td>4981</td>
<td>4811</td>
<td>4834</td>
<td>4786</td>
<td>5027</td>
</tr>
<tr>
<td>Total PSMs</td>
<td>6155</td>
<td>7441</td>
<td>7436</td>
<td>7529</td>
<td>7558</td>
<td>7414</td>
<td>6369</td>
<td>7364</td>
<td>7216</td>
<td>7403</td>
</tr>
</tbody>
</table>

Hierarchical Cluster Analysis (HCA) using Ln NSAF of 1D analysis showed individual patients clustering and not sample groups. This is reflected by only 3 proteins (0.4%) having p<0.05 and fold change >2 (red spots in volcano plot).

Neurosecretory protein VGF was one of the top up-regulated proteins in IN compared to NIN. VGF is a protein previously shown to play a role in nociceptive processing and neuropathic pain in peripheral nerve injury, and was therefore selected for follow-up and cross-validated in an expanded patient cohort (n=22) using immunohistochemistry (IHC):

- VGF is overexpressed in nerves invaded with PDAC cells in human tissues.

4. PNI Cancer vs. Non-PNI Cancer (A vs. C)

Data from the individual 1D and pooled 2D (RP/RP) analysis were searched at 1% protein and peptide FDR and requiring at least two unique peptides per protein. Match between runs was selected. Detection metrics are summarized below, 96% of the 1D proteins were observed in the 2D analysis.

<table>
<thead>
<tr>
<th>Total Proteins</th>
<th>A1</th>
<th>A2</th>
<th>A3</th>
<th>A4</th>
<th>A5</th>
<th>C1</th>
<th>C2</th>
<th>C3</th>
<th>C4</th>
<th>C5</th>
</tr>
</thead>
<tbody>
<tr>
<td>1D/LC (1,738 Total Proteins)</td>
<td>1366</td>
<td>1969</td>
<td>1523</td>
<td>1539</td>
<td>1648</td>
<td>1594</td>
<td>1598</td>
<td>1556</td>
<td>1610</td>
<td>1602</td>
</tr>
<tr>
<td>Total Unique Peptides</td>
<td>5518</td>
<td>7334</td>
<td>6792</td>
<td>6939</td>
<td>7847</td>
<td>7490</td>
<td>7115</td>
<td>7484</td>
<td>7113</td>
<td></td>
</tr>
<tr>
<td>Total PSMs</td>
<td>6155</td>
<td>7441</td>
<td>7436</td>
<td>7529</td>
<td>7558</td>
<td>7414</td>
<td>6369</td>
<td>7364</td>
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<td></td>
</tr>
</tbody>
</table>

Hierarchical Cluster Analysis (HCA) using Ln NSAF of 1D analysis showed individual patients clustering and not sample groups. This is reflected by only 3 proteins (0.4%) having p<0.05 and fold change >2 (red spots in volcano plot).

5. Summary

- Combination of laser microdissection and mass spectrometry successfully identified thousands of proteins from the microscopic lesions of PNi.
- Whilst large number of proteins were differentially regulated in invaded compared to non-invaded nerves, the comparison of PNi and non-PNi cancer revealed only small number of deregulated proteins.
- The up-regulation of Neurosecretory protein VGF in IN was validated using IHC.
- Parallel 1D and 2D/LC analytical approaches allow for greater confidence in deciding candidate biomarkers.
- Parallel data processing using both NSAF and LFQ intensities allows for the advantages of both approaches to be exploited.