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

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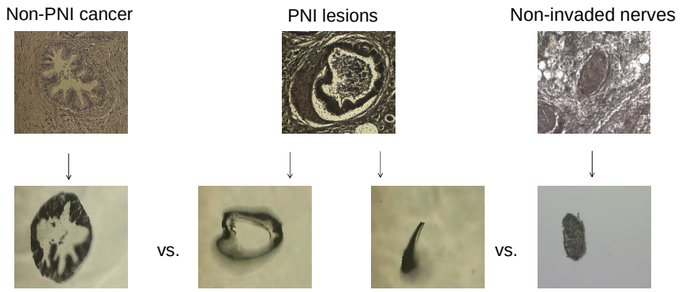
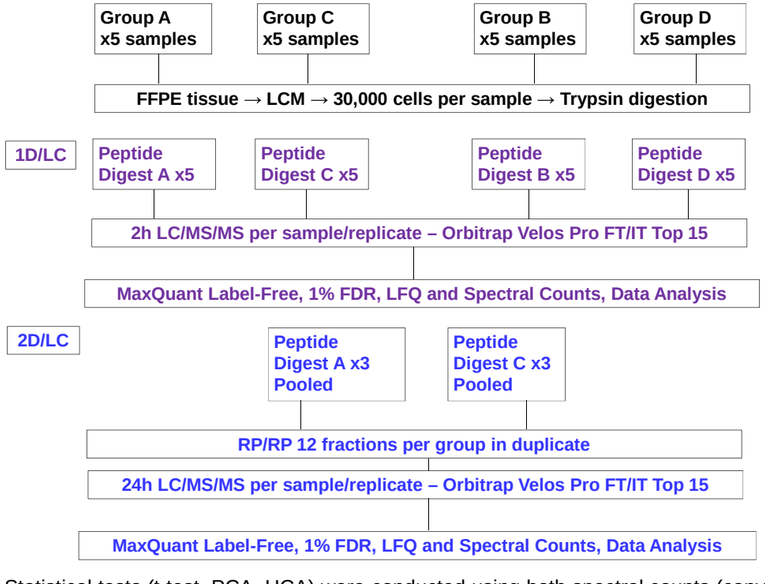
1. Introduction

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



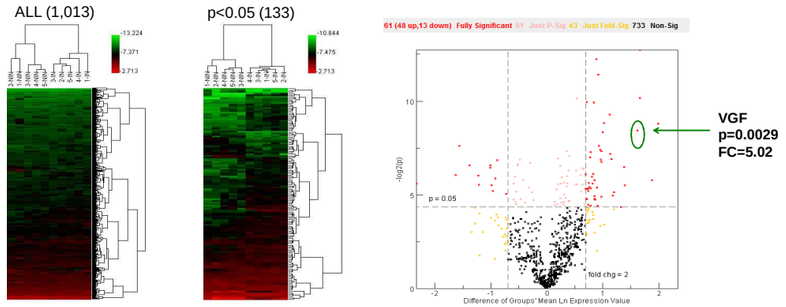
Statistical tests (t-test, PCA, HCA) were conducted using both spectral counts (converted to Normalized Spectral Abundance Factors, NSAF) and LFQ intensity values.

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

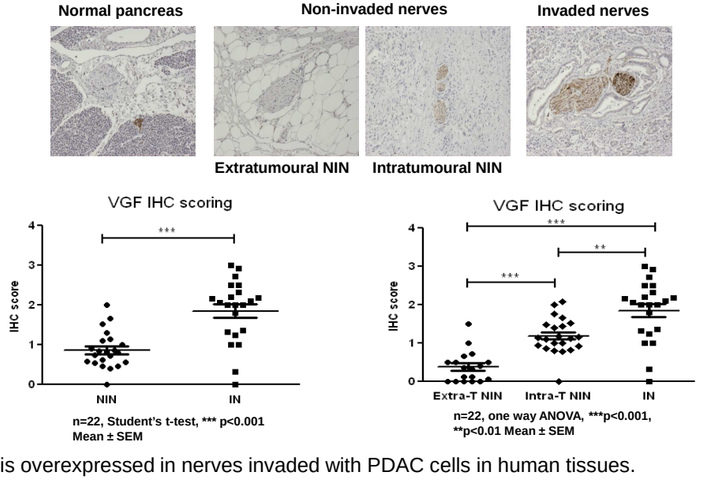
Data were searched at 1% protein and peptide FDR and requiring at least two unique peptides per protein. Match between runs was selected. Detection metrics are summarized below:

1D/LC (1,013 Total Proteins)	B1	B2	B3	B4	B5	D1	D2	D3	D4	D5
Total Proteins	875	932	917	945	939	917	863	900	920	928
Total Unique Peptides	4112	4890	4891	5015	5108	4881	4181	4834	4786	5027
Total PSMs	6155	7441	7436	7529	7558	7414	6369	7364	7216	7403

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).



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):



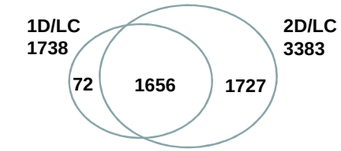
- VGF is overexpressed in nerves invaded with PDAC cells in human tissues.

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

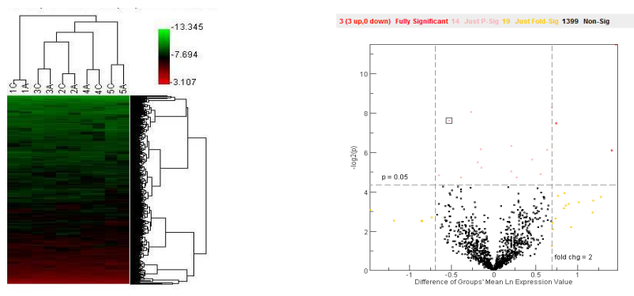
Data from the individual 1D and pooled 2D (RP/RP) analysis were searched at 1% protein and peptide FDR and requiring at least two unique peptides per protein. Match between runs was selected. Detection metrics are summarized below, 96% of the 1D proteins were observed in the 2D analysis:

1D/LC (1,738 Total Proteins)	A1	A2	A3	A4	A5	C1	C2	C3	C4	C5
Total Proteins	1366	1569	1523	1539	1648	1594	1509	1556	1610	1602
Total Unique Peptides	5518	7334	6792	6939	7847	7490	6689	7115	7484	7113
Total PSMs	7783	10575	9737	9881	11198	11037	9613	10299	10956	10274

2D/LC (3,383 Total Proteins)	A1	A2	C1	C2
Total Proteins	3351	3342	3338	3323
Total Unique Peptides	18623	18533	18642	18385
Total PSMs	63099	54088	59778	48296



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).



T-test analysis of the 1D/LC and 2D/LC LFQ intensities revealed 14 and 104 differential proteins (p<0.05, fold change >2). Half of the significant 1D proteins were also significant in the 2D data. Several candidates from the overlap of these analyses are being cross-validated using IHC in expanded cohorts.

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.