Proteomic and Statistical Analysis of Neuroendocrine Neoplasm Markers

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Introduction

- The incidence of Neuroendocrine Tumors (NET) has increased 6.4 times in the USA in the last 4 decades.
- Increased incidence has been measured across multiple countries from different continents.
- Combined, around 50% of NET incidence consists of pancreatic (p)NET and small bowel (sb)NET.
- pNET's (3.6 years) median Overall Survival is 4 times lower than sbNET's (14 years).

Objective

- The aim of this 20-sample serum proteomic analysis of pNET and sb-NET is to identify a protein marker molecule that could serve as a foundation in future targeted research.

Methods

- To prepare the samples we performed a bottom-up proteomic workflow which consist of denaturation reduction alkylation and digestion.
  - MS-grade trypsin was used for digestion.
- Data was acquired by the LC-MS timsTOF coupling method.
- MaxQuant and Perseus software was used to filter and process the data.
- Student's T-test was used to analyse the data.
- **I**ngenuity **P**athway **A**nalysis software was used to map the significant proteins on the cellular pathways

	Sample distribution	Age	Sex	Race
sbNET	10x	Range = 36 - 69 Mean = 56	6x female 4x male	9x Caucasian 1x African-American
pNET	10x	Range 41 - 63 Mean = 55	2x female 8x male	8x Caucasian 1x African-American 1x Unknown

Statistical analysis

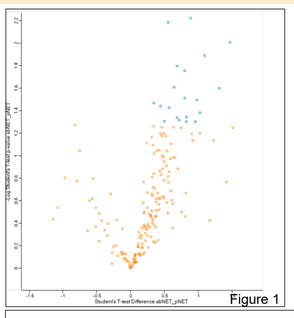


Figure 1

Protein ID	P-Value	Protein Name
P11226	20,1753	MBL-2
P35557	17,5794	HK-4
A0A075B7D8	2,22023	IGHV3OR15
P03952	2,18526	KLKB1
Q15468	2,0084	SCLp
P78563	1,88828	RED-1
P01860	1,79529	unknown
P04196	1,75583	HRG
P49908	1,60573	SELENOP
P40197	1,60147	GP5

Figure 2

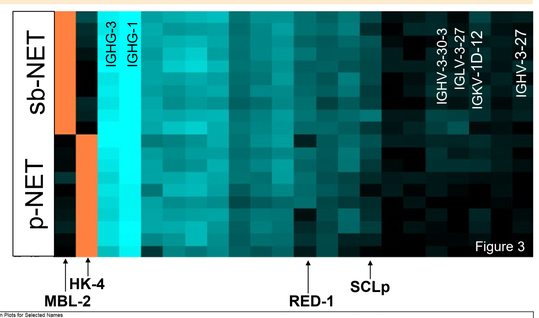


Figure 3

Figure 1,2,3 show the distribution of expression in significant proteins.
• The expression in pNET is more dispersed across samples
• The overall expression in pNET is lower than that of sbNET
Figure 1 - **Non-significant/significant proteins**
Figure 2 - shows the top 10 proteins with the highest P-Value after statistical processing
Figure 3 - **0, 0.1,14, 24 intensity**
Figure 4 - **sb-NET/pNET**

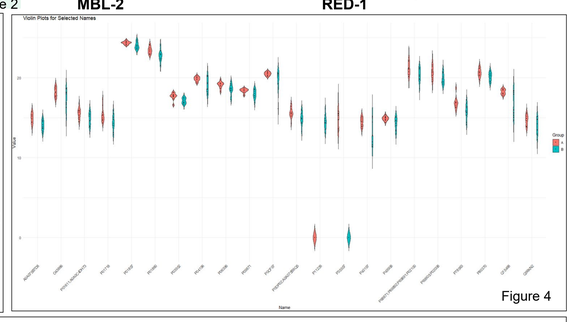
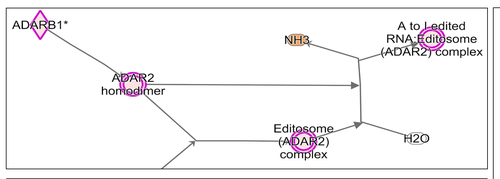


Figure 4

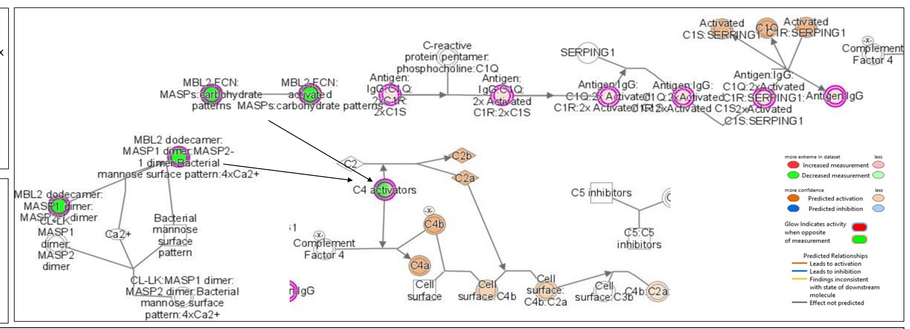
- Data was filtered, processed, then statistically analysed with student's T-test
- 22 proteins were evaluated as significant with P-value over 0,5
- Students T-test showed **MBL-2**, **HK-4**, RED-1, SCLp and IGHV-3-15 to be the most differentially expressed proteins between sbNET and pNET
- MBL-2 was the only protein overexpressed in p-NET
- 6 Imunoglobulin (Ig) proteins had been significantly overexpressed in sbNET

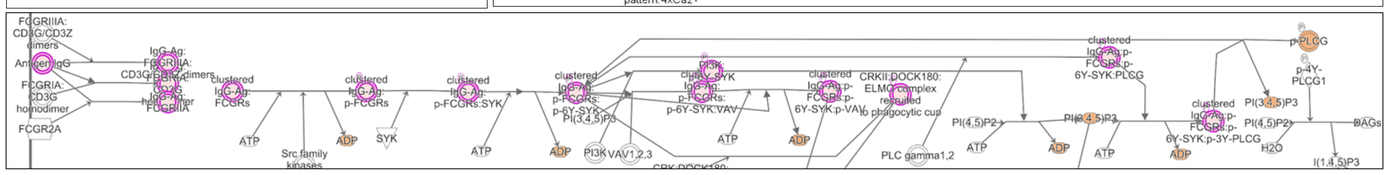
Pathway enrichment

- **HK-4 -** Hexokinase 4 belongs to a family of phosphorylating enzymes that are crucial for glucose metabolism. HK-4 was the only protein from the HK family in our samples and it was the only significantly expressed sugar regulator.
- **MBL-2** mannose-binding-lectin-2 was mapped as a lectin complement pathway activator.
- **RED-1 -** Double-stranded-RNA editase-1 (ADARB1) was mapped in IPA as a editosome regulating molecule



Among the main affected pathways are:
- mRNA editing pathway (fig. 5)
- Complement Cascade (fig. 6
- IgG mediated phagocytosis (fig.7)





Discussion

- Energy metabolism, particularly glucose utilization pathways in cancer is very often dysregulated
- Out of the hexokinase family in cancer HK-2 and HK-1 are most likely to be dysregulated, moreover it has been observed that one of the earliest adaptation of cancers expressing HK-4 is a "switch-over" to higher affinity HK-2 and to lesser extend HK-1 which could explain lower HK-4 in "more dangerous" pNET.
- Hexokinase enzymes are however tissue specific so it is possible the difference in expression is coincidental

Different studies have shown that:
- Species of fungi *Malassezia spp. p*romotes pancreatic oncogenesis in Pancreatic ductal Adenocarcinoma (PDAC) in mice while furthermore oncogenesis does not occur with MBL-2 or C3aR knockout
- The role of pancreatic microbiota in PDAC is probable and it can help determining the OS.
- One of the ways how certain microbiota modulates pancreatic cancer is inducing differentiation of intertumoral immunosuppressive myeloid cells where there might be correlation with Ig underproduction seen in our study.

Conclusion

- In this proteomic workflow we analyzed 20 samples equally distributed between pNET and sbNET
- Out of 22 significant proteins we found across our samples, these had the lowest p-value: MBL-2, HK-4, ADARB-1,SCLp and IGLV-3-27
- Molecules MBL-2 and HK-4 have potential of being significant in cancer marker research.

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