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Proteomic and Statistical Analysis of Neuroendocrine Neoplasm Markers

Introduction: From 1973 to 2012, the incidence of Neuroendocrine Tumors (NET) in the USA increased 6.4 times. In Norway, from 1993 to 2021, the incidence increased more than 2.6 times. Moreover, the rise in incidence has been seen in various countries across Europe, Asia and North America. The two most common types that account for around 50% of incidence are small bowel (sb) and pancreatic (p) NET. Although these cancer types come from the same family, the median overall survival of sbNET is around 14 years, which is almost 4 times higher than pNET, with overall survival of only 3.6 years. Since 1990, various markers, such as serum Chromogranin-A, have been found to distinguish NETs from other diseases. Yet the gold-standard diagnostic tool in NEN typing remains to be histopathology.

As our primary objective, we search for the existence of serum protein markers with the potential to differentiate between the two most common subtypes of NET without the need for an invasive procedure such as histopathology.

Methods: Twenty serum samples, 10 sbNET and 10 pNET, were prepared following a bottom-up proteomics workflow, which consisted of denaturation, reduction, alkylation, trypsin digestion, and desalination. LC-MS timsTOF coupling method was used for sample analysis. This state-of-the-art method allows for 4-D analysis of proteins with high sensitivity.
Using MaxQuant and Perseus software, we performed a series of statistical analyses with varying parameters to compare different methods of data analysis. Using Ingenuity Pathway Analysis (IPA) software for pathway enrichment analysis, multiple canonical pathways were differentially enriched across the two groups.

Result: Overall, 330 different proteins were identified in our samples. T-test between sbNET and pNET identified 18 statistically significant proteins (p-value <0.05). IPA showed that the top-ranked enriched pathways are the Complement Cascade, Binding and Uptake of Ligands by Scavenger Receptors, and multiple pathways involved with B-cell signalling because of the dysregulation in the B-cell receptor component between two NET groups.

Conclusion: Even though the nature of both sbNET and pNET is neuroendocrine, the overall survival of sbNET is almost 4 times higher. This 20-sample NET+ serum proteomics workflow introduced a set of 18 significantly regulated proteins and their associated enriched pathways that would benefit from future targeted research. Most notably, Mannose-Binding-Lectin 2 and its impact in pancreatic cancer.