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Method optimization for the identification of proteins associated with pancreatic ductal adenocarcinoma in plasma using mass spectrometr y

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ABSTRACT

Background: Pancreatic ductal adenocarcinoma (PDAC) is a leading cause of cancer-related deaths. The poor survival rate for PDAC is mostly due to the lack of effective biomarkers for early diagnosis. This study aimed to develop a standard operating procedure for a high-throughput mass spectrometry-based workflow that can be used for plasma-based biomarker discovery studies.

Methods: Total protein was extracted from a master pool consisting of 28 PDAC samples (M211158). SDS-PAGE was conducted to visualize the dynamic range of the plasma proteins. Three sample preparation methods were compared, Method 1: plasma diluted 25X with buffer (50mM Tris-HCl pH 8 with 2% SDS), Method 2: plasma diluted 5X with 50mM TEAB buffer, and Method 3: precipitation (using 80% and 100% acetone) followed by resuspension in 50 mM Tris-HCl, 2% SDS. The Hydrophilic Interaction Liquid Chromatography (HILIC) and protein aggregation capture (PAC) based protocols were compared for clean-up and digestion of the proteins. Low pH reverse phase chromatography was applied to separate the peptides and SWATH-MS analysis was conducted for label-free quantification. The methods were compared based on reproducibility, ease of automation and the number of peptides identified. A pilot study including 6 PDAC, 6 Chronic pancreatitis, and 6 healthy individuals was conducted to test the selected optimized method.

Results: The combination of the 25-fold dilution of Method 1 and the 2 hrs HILIC bead-based method (with a Trypsin and LysC combination) were effective in protein sample clean-up and digestion, yielding the highest number of peptides (mean: 2856). The combination of Trypsin and LysC enzymes contributed to the improvements in technical variation and peptide quantitation. The preliminary analysis identified 25 differentially expressed proteins (22 downregulated and 3 upregulated) between the healthy and PDAC patients. Most of these proteins are associated with tumorigenesis.

Conclusion: This study developed a standard operating procedure for processing plasma samples and can be applied in future studies for the identification of potential markers for PDAC.