DEEP, UNBIASED MULTI-OMICS APPROACH FOR THE IDENTIFICATION OF PANCREATIC CANCER BIOMARKERS FROM BLOOD

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ABSTRACT

- Pancreatic cancer is the seventh leading cause of cancer related death worldwide and the third leading cause of cancer related death in the USA.[1]
- The low survival rate of pancreatic cancer is due to the challenges in early detection of disease, highlighting the need for early diagnostic test development.[2]
- In this case-control study of 196 subjects, we measured the plasma proteome, metabolome, and lipidome of 92 pancreatic cancer subjects and 104 healthy subjects (3 subjects were excluded from the analysis due to failed quality control metrics). Subject samples were collected post diagnosis, but pre-treatment for cancer subjects versus non-cancer controls. Sample collection and handling was the same for all samples. Of the most significantly changed analytes, both previously identified and novel biomarkers were discovered.
- Individual proteomics, metabolomics and lipidomics data demonstrated statistically significant markers for the detection of pancreatic cancer. This data demonstrates the promise of non-invasive liquid biopsies as a tool for early detection of pancreatic cancer.

INTRODUCTION

- Pancreatic cancer is a major contributor of cancer-associated mortality resulting from poor disease prognosis at diagnosis.
- Symptoms during the early-stage development of pancreatic cancer are non-specific to the disease.[2]
- Proximity of the developing tumor tissue to major blood vessels leads to these advanced tumors being nonresectable and contributes to metastatic disease state later in development.[3]
- Patients usually do not exhibit symptoms until the cancer has progressed to catastrophic levels where treatment is unable to overcome the disease.[1]
- Currently, early diagnosis of pancreatic cancer at curable stages is not possible due to the lack of identified markers that are sensitive and specific to the disease.[4]
- Recently, several 'omics technological breakthroughs have facilitated the deeper understanding of complex biology behind disease occurrence and progression. Combining these technologies to probe multiple levels within systems biology is key for development of new early disease screening tests.
- Here we utilize several mass-spectrometry-based approaches to characterize the proteome, metabolome and lipidome of healthy and diseased patients that have various stages of pancreatic cancer.

MULTI-OMICS WORKFLOW

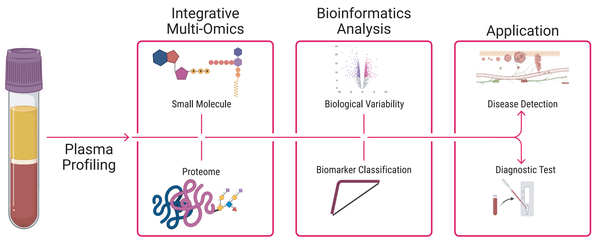


FIGURE 1

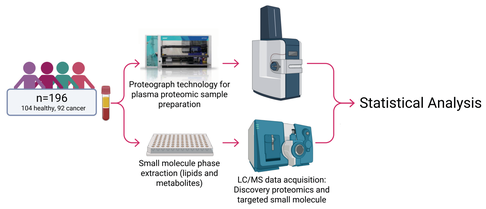
EXPERIMENTAL METHOD

The samples were obtained from pancreatic cancer patients and healthy controls from sites participating in the study "A Prospective Blood Sample Collection Study to Evaluate a Panel of Protein-based Biomarkers". The study was approved at all sites by an institutional review board and all patients provided written informed consent before samples were collected.

For the proteomics workflow, plasma samples were processed through the Proteograph (Seer, Redwood City, CA) using the standard five nanoparticle panel and three process controls following the manufacturer's protocol. Reconstituted peptides were loaded onto Evotips (Evosep, Denmark) packed with C18 resin following the manufacturer's protocol and separated on the Evosep One LC system (Evosep, Denmark). Samples were subjected to LC-MS/MS analysis on a timsTOF Pro II (Bruker, Germany) using Data Independent Acquisition mode with Parallel Accumulation-Serial Fragmentation (diaPASEF). All data were analyzed by Seer Proteograph Analysis Software (PAS).

For the lipidomics and metabolomics workflows, lipids and metabolites were extracted from plasma samples using separate single phase organic extraction methods. All extracted lipids underwent two chromatographic separations one each for positive and negative modes. Extracted metabolites underwent one chromatographic separation for both positive and negative mode. Data were collected for both workflows using multiple reaction monitoring (MRM) mode on a SCIEX7500 triple quadrupole mass spectrometer. All MRM mode data were processed using SCIEX OS Analytics (SCIEX, Redwood City, CA) software.

METHODS



Study design and LC-MS based multi-omics workflow for pancreatic cancer multiomics analysis.

FIGURE 2

RESULTS: PROTEINS DETECTED ACROSS SAMPLES

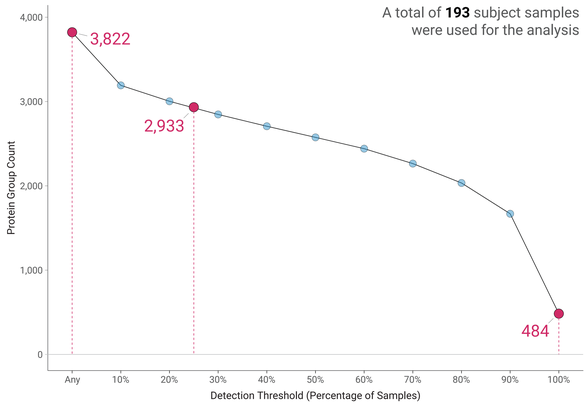


FIGURE 3

In our proteomics workflow, we detected 3,822 proteins groups across all 5 nanoparticles for the 193 pancreatic cancer and healthy subject samples. The 2933 protein groups were identified in at least 25% of the patient samples.

PROTEINS DETECTED IN PANCREATIC CANCER STUDY MATCHED TO HPPP DATABASE

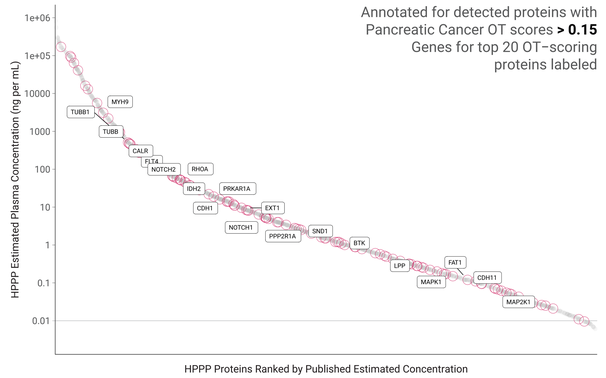
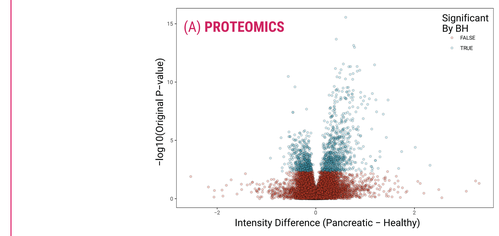


FIGURE 4

The 3,822 detected protein groups were mapped to the HPPP database. Identified proteins had concentrations ranging 8 orders of magnitude. All identified proteins are displayed above (grey) with proteins having significant pancreatic cancer OT scores > 0.15 highlighted (red circle).

VOLCANO PLOTS



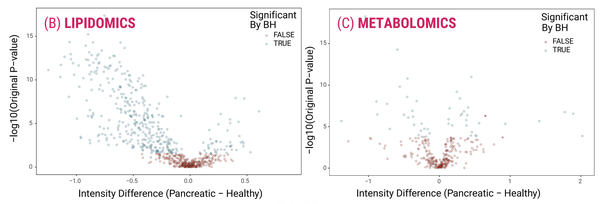


FIGURE 5

A statistically significant signal for the individual `omics platforms was detected and is illustrated in the volcano plots for proteomics (5a), lipidomics (positive mode) (5b) metabolomics (negative mode) (5c). 124 of 3,381 detected proteins were shown to be statistically significant. Additionally, 200 of 678 total lipids and 49 of 299 metabolites present in all samples were statistically significant. All analytes in each platform with a p-value < 0.5 when utilizing a Bonferroni multiple testing correction are colored in blue and only non-imputed data with at least 3 measures per class were used in the analysis. The Log2 fold change for each analyte is demonstarted on the x-axis.

BOX AND WHISKER PLOTS OF MOST SIGNIFICANTLY DIFFERENT ANALYTES PER OMICS WORKFLOW

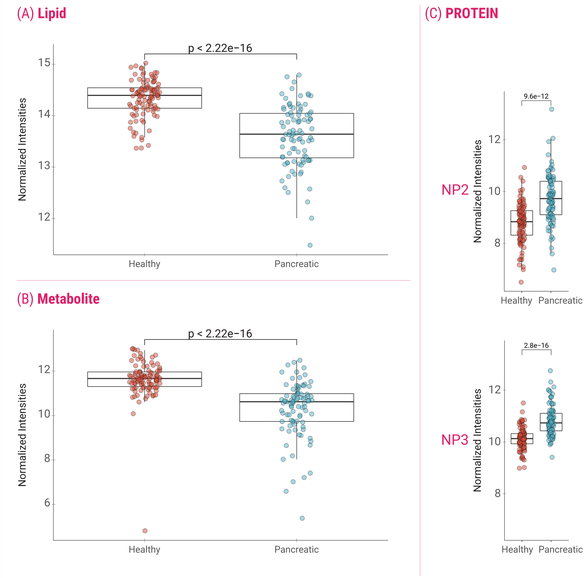


FIGURE 6

Figure 6 highlights the most statistically significant analyte for lipids (6a), metabolites (6b) and proteins in Proteograph data (6c) as individual box and whisker plots. The most statistically significant protein in Proteograph data (6c) was significant in 2 of the 5 nanoparticle samples. Unique surface chemistry on each of the nanoparticles maximizes protein identifications and diversity across all 5 nanoparticles.

CONCLUSIONS

- In this proof-of-concept study, we demonstrate the promise of a broad phenotypic unbiased multi-omics approach to identify previously known and, more importantly, novel biomarker candidates that were significantly different between healthy and pancreatic cancer patients.
- Incorporation of the phenotypic multi-omics data into classifiers that distinguish cancer patients from healthy controls are under development.
- This initial study is limited by sample size, and additional studies are required to demonstrate the genaralizability of the markers in a larger population.
- Ongoing work will incorporate methylation, mRNA, and miRNA data from all subjects to further improve the sensitivity and specificity for the early detection of pancreatic cancer.

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