Published in final edited form as:

Pancreas. 2015 May ; 44(4): 574–582. doi:10.1097/MPA.0000000000000304.

The Proteome of Postsurgical Pancreatic Juice

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Abstract

Objective—This study aimed to evaluate the proteome of the pancreatic juice after pancreatectomy.

Methods—Pancreatic juice samples were obtained during surgery and the postoperative period. Proteins were identified by mass spectrometry–based protein quantification technology and compared with published data of the nonoperated pancreas. Subgroup analyses were done in patients with pancreatic ductal adenocarcinoma (PDAC) receiving neoadjuvant chemotherapy and in smokers.

Results—Five hundred eighteen proteins were identified in the postoperative pancreatic juice, encompassing all of the main organ functions. Sixty-seven of these were also present in the published data of the nonoperated pancreas and 7 of these had significant variation of concentration after surgery. Growth factors that have been described in postsurgical regeneration of the liver were not found to be overexpressed, whereas clusterin did, confirming the finding of previous experimental studies on pancreatic regeneration. Several proteins involved in immunomodulation and organ functions were differently expressed, depending on PDAC, neoadjuvant therapy, and smoking.

Conclusions—The proteome of the pancreas after surgical resection contains factors related to all main organ functions, changes over time, and is different in patients with PDAC receiving neoadjuvant therapy and in smokers. The pancreas reacts to the surgical trauma by producing proteins that protect the organ and stimulate the restoration of its function.

Keywords

proteome postsurgical pancreatic juice; proteomics; pancreas; surgery; regeneration; Whipple

The study of the proteome, or the protein content of a cell, tissue, or fluid at a given time under specific conditions, is being increasingly used, particularly in oncology research.¹

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The authors declare no conflict of interest.

Supplemental digital contents are available for this article. Direct URL citations appear in the printed text and are provided in the HTML and PDF versions of this article on the journal's Web site (www.pancreasjournal.com).

Proteomics can provide key information in the analysis of neoplasm onset, tumor biomarkers, and even chemotherapy effect.² The proteome of the normal human pancreatic juice (PJ) has been recently assessed, revealing the presence of multiple proteins related to a diversity of biological processes.³ However, no studies have been performed on the proteomics of the PJ after partial resection of the organ. In other organs, such as the liver and kidney, analysis of the spectrum of proteins that are produced after surgery has led to the clarification of many processes related to their regeneration.^{4–6}

The aims of this study were to characterize the proteome of PJ after pancreatic resection and to compare it with the normal juice. Moreover, we sought to describe the evolution of the pancreatic proteome over time after surgery. Finally, we aimed to identify differences in the proteome related to factors that might impact protein production, such as neoadjuvant chemotherapy, the presence of pancreatic ductal adenocarcinoma (PDAC), and a history of smoking.

MATERIALS AND METHODS

Specimen Collection

After approval of the institutional review board of the Massachusetts General Hospital, patients scheduled for pancreaticoduodenectomy (Whipple procedure) were prospectively recruited. The PJ was collected at 3 time points. The first was during the surgical procedure, at the time of the transection of the neck of the pancreas. A sterile syringe with a cannula was used to aspirate the PJ directly from inside the main pancreatic duct. Pancreaticojejunal anastomosis at our institution is routinely done over a stent that is exteriorized. This allows PJ to drain directly to the outside in a sterile collection bag. Additional samples of PJ were harvested on postoperative days (PODs) 5 and 21. After the collection at POD 21, the catheter was removed by simply pulling it out in the office.

A total of 19 samples from 11 patients were used for the study analysis (Table 1). The first 12 samples were chosen with the requirement that the protein content was of sufficient amount to perform mass spectrometry and for which we had 3 time points for each patients. The remaining samples were taken from time point 0 for which a sufficient amount of protein for mass spectrometric analysis could be obtained. Samples with obvious blood contamination were excluded.

Trypsin sequencing grade (V5113) was from Thermo Scientific (Rockford Ill). LysC was purchased from Waco Chemicals USA (Richmond Va). Water and organic solvents were from J.T. Baker (Center Valley, Pa).

Liquid Chromatography and Tandem Mass Spectrometry

Mass spectrometry data were collected using an Orbitrap Elite mass spectrometer (Thermo Fisher Scientific, San Jose, Calif) coupled with a Proxeon EASY-nLC II liquid chromatography pump (Thermo Fisher Scientific). The general mass spectrometry workflow is shown in Figure 1. Peptides were separated on a 100- μ m inner diameter microcapillary column packed with approximately 0.5 cm of Magic C4 resin (5 μ m, 10 nm beads; Michrom Bioresources) followed by approximately 20 cm of Accucore 150 resin (2.6 μ m, 150 Å;

Thermo Fisher Scientific). For each analysis, we loaded approximately 1 μ g onto the column.

Peptides were separated using a 1.5-hour gradient of 6% to 30% acetonitrile in 0.125% formic acid with a flow rate of approximately 300 nL/min. The scan sequence began with an MS¹ spectrum (Orbitrap analysis: resolution, 60,000; 300–1500 Thomson; automatic gain control target, 1×10^6 ; maximum injection time, 150 milliseconds). The top 10 precursors were then selected for MS² analysis. MS² analysis consisted of collision-induced dissociation, quadrupole ion trap analysis, automatic gain control of 2×10^3 , normalized collision energy of 35, q value of 0.25, and maximum injection time of 100 milliseconds.

Data Analysis

Mass spectra were processed using a Sequest-based in-house software pipeline. The spectra were converted to mzXML using a modified version of ReAdW.exe. Database searching included all entries from the mouse UniProt database (August 10, 2011), which was concatenated with a reverse database composed of all protein sequences in reversed order. Searches were performed using a 50-ppm precursor ion tolerance. Product ion tolerance was set to 1 d. Carbamidomethylation of cysteine residues (+57.022 d), deamidation of glutamine and glutamate (+0.984 d), as well as oxidation of methionine, histidine, and tryptophan residues (+15.995 d) were set as a variable modification.

Peptide spectral matches were filtered to a 1% false discovery rate (FDR).^{7,8} Peptide spectral match filtering was performed using linear discriminant analysis, as described previously,⁹ while considering the following parameters: XCorr, Cn, missed cleavages, peptide length, charge state, and precursor mass accuracy. For protein-level comparisons, peptide spectral matches were identified, quantified, and collapsed to a 1% FDR and then further collapsed to a final protein-level FDR of 1%. Furthermore, protein assembly was guided by principles of parsimony to produce the smallest set of proteins necessary to account for all observed peptides.

RESULTS

The Proteome of Postsurgical PJ

A total of 518 proteins were identified using mass spectrometry-based techniques in the PJ of the pancreas undergoing surgical resection (Supplemental Table 1, at <http://links.lww.com/MPA/A354>). Using data from the PANTHER^{10,11} database, we determined that the most commonly detected proteins were involved in catalytic biological processes (Fig. 2A) and metabolic molecular functions (Fig. 2B). As expected, pancreatic enzymes, such as trypsin, chymotrypsin, lipase, elastase, as well as amino and carboxypeptidase, were identified. Moreover, proteins from the serpin, 14-3-3, mucin, apolipoprotein, peroxiredoxin, immunoglobulins, and complement cascades were well represented in the proteome. Our search for growth factor known to be involved in the postsurgical liver proliferation and function regeneration, such as hepatocyte growth factor, epidermal growth factor receptor, tumor necrosis factor, interleukin 6, and met proto-oncogene, found them to

be present at very low concentrations in PJ, falling beyond the analytical depth of our mass spectrometric analysis.

With regard to factors involved in the pancreatic regeneration process in vitro and in animal model,¹² the isoform 1 of clusterin was identified in the PJ at significant concentrations.

The comparison between the proteins identified in the surgical PJ and in the recently published “normal” PJ demonstrated overlap of 67 proteins (Fig. 3A).³ Of note, only “high-confidence” proteins in reference to the “normal” PJ were considered for this analysis. The comparison between the proteome of the postsurgical PJ and a nonredundant list of proteins culled from 6 recent proteomics studies (5 studies focusing on patients with cancer and the aforementioned study of the normal PJ) showed overlap of 134 proteins (Fig. 3B).^{2,3,13–16} All of the proteins found in the PJ of our pancreatic cancer (PDAC) cohort were present in the published series of PJ proteome in patients with cancer.

Proteins With Significant Difference in Concentrations During Time Points

The analysis of the protein concentrations along the 3 time points (PODs 0, 5, and 21) identified 7 proteins having a significant concentration change over time (Fig. 4). Three of these proteins showed a decreasing trend from day of surgery to POD 5 but, then, a subsequent increase from POD 5 to POD 21. They were β -2-glycoprotein 1 (APOH), complement factor I (CFI), and Ig heavy chain V-I region V35 (HV103). APOH has been implicated in a variety of physiologic pathways including lipoprotein metabolism and coagulation.¹⁷ CFI activation has been shown to have a role in pancreatitis.^{18–20} HV103 is a heavy chain immunoglobulin that has a role in the immune system.²¹

The other 4 proteins had a trend of progressive decrease over time. Carbonic anhydrase 1 (CA1) is a zinc metalloenzyme that catalyzes the reversible hydration of carbon dioxide. Elevated levels of CAI have been identified in patients with chronic pancreatitis²² and pancreatic cancer.²³ Hemoglobin subunit α (HBA1) and hemoglobin subunit δ (HBD) are the subunits of hemoglobin, which is a secreted iron-containing oxygen-transporting metalloprotein that is found abundantly in blood.²⁴ Their presence due to contamination from blood of the samples at the time of surgery cannot be excluded. Polyubiquitin-C (UBC) is associated with protein degradation, DNA repair, and cell cycle regulation among other cell signaling pathways.²⁵

Proteome in Patients With PDAC Versus Patients Without PDAC

Of the 11 patients, 9 underwent surgery for PDAC, whereas 2 patients underwent surgery for nonmalignant neoplasms. The comparison between the proteome of the 2 cohorts produced 5 proteins with statistically significant differences in concentration (Fig. 5). Three of these 5 proteins were significantly more expressed by the patients without PDAC. They were carboxypeptidase A5 (CPA5), inactive pancreatic lipase–related protein 1 (PNLIPRP1), and kallikrein 1 (KLK1).

CPA5 is a common pancreatic fluid enzyme involved in the breakdown of peptides. Serum levels of carboxypeptidase are significantly higher in patients with acute and chronic pancreatitis compared with healthy controls and are even higher in patients with PDAC.²⁶

PNLIPRP1 is involved in hydrolyses of fat molecules. Plasmatic KLK1 liberates kinins (bradykinin and kallidin) from the kininogens peptides responsible for the regulation of blood pressure and activation of inflammation.²⁷

The 2 other proteins significantly differing between cohorts were HBD and transthyretin (TTR). Of note, these proteins were expressed only in patients undergoing resection for PDAC, whereas they were not detected in the nonmalignant neoplasm cohort. HBD has been discussed previously as a subunit of hemoglobin. TTR is a protein commonly detected in serum as well as cerebrospinal fluid and is a carrier of the hormone thyroxine and retinol-binding protein. The expression of TTR has been linked to endocrine tumors in the pancreas^{28,29} and diabetes.³⁰

Proteome after Neoadjuvant Therapy Versus Up-front Surgery

Six of the 11 patients underwent neoadjuvant chemotherapy, whereas 5 patients did not. The comparison between the proteome of the 2 groups is shown in Figure 6. Nine proteins were found to significantly differ between the 2 groups. Five of these 9 proteins were associated with up-front surgery: pancreatic triacylglycerol lipase (PNLIP), inactive pancreatic lipase-related protein 1 (PNLIPRP1), pancreatic lipase-related protein 2 (PNLIPRP2), chymotrypsinlike elastase family member 3B (CELA3B), and carboxypeptidase A1 (CPA1). PNLIP, PNLIPRP1, and PNLIPRP2 are abundant proteins in pancreatic fluid that hydrolyze fat molecules.^{31,32} CELA3B is a member of a subfamily of serine proteases that hydrolyze proteins and is commonly secreted from the pancreas. Of note, elastase is regularly used as test for the status of pancreatic exocrine function.³³ CPA1 is an abundant enzyme in pancreatic fluid in which variants are associated with pancreatic disease.^{34,35}

The remaining 4 differentially expressed proteins were associated with neoadjuvant chemotherapy. Apolipoprotein B (APOB) is responsible for carrying cholesterol to tissues.³⁶ Fibronectin (FN1) has been shown to be regulated in tumorigenesis and proliferation related to pancreatic cancer.^{37,38} α -2-HS-glycoprotein (AHSG), also known as fetuin-A, is a secreted serum protein produced by the liver that is involved in several functions including endocytosis.³⁹ With regard to pancreatic disease, changes in fetuin-A levels represent a potential marker of acute pancreatitis.⁴⁰ Finally, inter- α -trypsin inhibitor heavy chain H1 (ITIH1) is the heavy chain of a serine protease inhibitor, which is found in plasma, and has been associated with various human tumors.^{41,42}

Proteome in Smokers Versus Nonsmokers

Of the 11 patients analyzed, 4 were active smokers at the time of surgery. Figure 7 shows the 7 proteins whose concentrations were significantly different in the 2 cohorts. Of note, all of the 7 proteins were positively associated with smoking and, of these, 5 proteins were not detected at all in nonsmokers. These 5 proteins were aminopeptidase N (ANPEP), Ig γ -3 chain C region (IGHG3), Ig κ chain V-III region HIC (KV313), mucin 1 (MUC1), and attractin (ATR). The remaining 2 proteins were galectin-3-binding protein (LGALS3BP) and mucin 5B (MUC5B).

ANPEP, also known as alanine aminopeptidase, is an enzyme used as a biomarker to detect kidney damage and may assist in diagnosing certain kidney disorders.⁴³ In addition, ATRN

is involved in the immune cell clustering during inflammatory responses and may regulate the chemotactic activity of chemokines.⁴⁴ Similarly, IGHG3 and KV313 are immunoglobulins with a crucial role in the immune system.⁴⁵ LGALS3BP is a member of the β -galactoside-binding protein family that is implicated in modulating cell-to-cell and cell-to-matrix interactions.⁴⁶ LGALS3BP, along with MUC5AC, has been associated with anomalous expression in patients with pancreatic cancer in a previous glycoproteomic study.⁴⁷ Mucins are highly glycosylated, secreted proteins that are present in many malignant tumors.⁴⁸ They have been studied extensively in the bronchial secretions with their production being stimulated by irritating factors such as cigarette smoke.^{49,50} MUC1 expression has been examined in normal pancreas, in benign/borderline neoplasms, as well as in PDAC.⁵¹ MUC5B has been found at increased levels in patients with PDAC.⁵²

DISCUSSION

Through the analysis of the proteins secreted in PJ, the present study represents a picture of the evolving process of readjustment by the human pancreas after surgical resection. The postsurgical pancreas reveals an active secreting activity, and all the main functions of the organ remain operative, with most of the factors produced being involved in metabolic processes and catalytic molecular functions. In particular, many of the proteins secreted in the postoperative period are involved in immunologic response and modulation, as well as in processes of localization and response to the stimulus. Of these, immunoglobulins and complement cascade factors were the most represented. Other secreted proteins play a role in preventing the cell damage, such as the peroxiredoxins. Therefore, even in the lack of a reliable quantitative comparison with “standard” secretion conditions, one may speculate that surgical trauma plays a major role in triggering a “defensive” proteomic activity.

Unlike the liver, the pancreas has no proper mass-regenerative properties after partial resection.⁵³ However, one of the main points of the study was to investigate whether the production of growth factors involved in liver proliferation takes place in the postsurgical pancreas as well. Our results showed that factors such as hepatocyte growth factor, epidermal growth factor receptor, tumor necrosis factor, interleukin 6, and met proto-oncogene were generally present at very low concentrations in PJ. This indicates that their potential role in the postoperative processes is likely secondary. Greater sample fractionation to reduce the abundant peptides in the samples or targeted mass spectrometric techniques may be applied in future studies in the aim to detect these scarce proteins.

Several studies during the last decade have isolated factors involved in the pancreatic regeneration process in vitro and in animal models.^{12,54,55} Of these, we have been able to identify in the postsurgical PJ the isoform 1 clusterin, which is a secreted chaperone ubiquitously expressed in different tissues. This factor experimentally protects cells against apoptosis and cytolysis by complement, resulting in restoration of pancreatic function after injury.⁵⁶ The finding is relevant because it indicates that the human pancreatic remnant is able to produce factors promoting both exocrine and endocrine functional enhancements.

The dynamic analysis of the proteome at different time points allowed us to identify several proteins whose expression varies significantly. Two different trends were identified. In both

trends, the protein concentration initially decreases, but for some proteins involved in immunologic response, it upturns again at 3 weeks after surgery. This finding may fit with the clinical evidence that patients undergoing partial pancreatic resection can experience adjustment in pancreatic functions up to several weeks after surgery.⁵⁷

Finally, the presence of PDAC in the resected specimen, neoadjuvant chemotherapy, and a history of smoking seemed to be associated with different patterns of protein expression. The spectrum of proteins that the pancreas produces after resection of a malignant tumor may be important because, potentially, it has implications in the postoperative delivery of targeted chemotherapy and radiotherapy.¹ On the other hand, neoadjuvant treatment induces an alteration of the pancreatic proteome. Not surprisingly, nontreated organs seem to have a more thriving production of enzymes implicated in their physiologic function, such as lipase and elastase. Neoadjuvant therapies are believed to decrease the secretions of the pancreas, as indirectly shown by the lower rate of postoperative pancreatic fistula in patients treated with fluorouracil, oxaliplatin, irinotecan, and leucovorin (FOLFIRINOX) versus undergoing up-front surgery.⁵⁸

The dramatic multiorgan impact of cigarette smoke has been widely investigated. Our results show an increase in factors related to inflammation and immunologic response in the resected pancreas of smokers. In particular, we found mucins to be elevated, which is similar to what occurs in the respiratory tract as a defensive response to the smoke insult.

In conclusion, the proteome of the pancreas after surgical resection has been defined revealing numerous metabolic, immunologic, and regulatory functions that evolve over time. Factors such as PDAC, neoadjuvant therapy, and smoke influence the proteome. Unlike the liver, a proper regeneration of the pancreatic parenchyma neither takes place nor are the same factors involved. However, the pancreas reacts to the surgical trauma by producing proteins that stimulate a defensive reaction and restoration of pancreatic function.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

The authors thank the members of the Gygi Lab, particularly Dr. Steven Gygi and Ross Tomaino for the use of the mass spectrometer and associated software of the Taplin Facility at Harvard Medical School.

This study was supported in part by an National Institutes of Health/National Institute of Diabetes and Digestive and Kidney Diseases grant K01 DK098285 (J.P.).

References

1. Cecconi D, Palmieri M, Donadelli M. Proteomics in pancreatic cancer research. *Proteomics*. 2011; 11:816–828. [PubMed: 21229586]
2. Chen R, Brentnall TA, Pan S, et al. Quantitative proteomics analysis reveals that proteins differentially expressed in chronic pancreatitis are also frequently involved in pancreatic cancer. *Mol Cell Proteomics*. 2007; 6:1331–1342. [PubMed: 17496331]
3. Doyle CJ, Yancey K, Pitt HA, et al. The proteome of normal pancreatic juice. *Pancreas*. 2012; 41:186–194. [PubMed: 22129531]

4. Michalopoulos GK. Liver regeneration after partial hepatectomy: critical analysis of mechanistic dilemmas. *Am J Pathol.* 2010; 176:2–13. [PubMed: 20019184]
5. Taub R. Liver regeneration: from myth to mechanism. *Nat Rev Mol Cell Biol.* 2004; 5:836–847. [PubMed: 15459664]
6. Franquesa M, Flaquer M, Cruzado JM, et al. Kidney regeneration and repair after transplantation. *Curr Opin Organ Transplant.* 2013; 18:191–196. [PubMed: 23425788]
7. Elias JE, Gygi SP. Target-decoy search strategy for mass spectrometry-based proteomics. *Methods Mol Biol.* 2010; 604:55–71. [PubMed: 20013364]
8. Elias JE, Gygi SP. Target-decoy search strategy for increased confidence in large-scale protein identifications by mass spectrometry. *Nat Methods.* 2007; 4:207–214. [PubMed: 17327847]
9. Huttlin EL, Jedrychowski MP, Elias JE, et al. A tissue-specific atlas of mouse protein phosphorylation and expression. *Cell.* 2010; 143:1174–1189. [PubMed: 21183079]
10. Mi H, Lazareva-Ulitsky B, Loo R, et al. The PANTHER database of protein families, subfamilies, functions and pathways. *Nucleic Acids Res.* 2005; 33:8. (Database issue).
11. Thomas P, Campbell M, Kejariwal A, et al. PANTHER: a library of protein families and subfamilies indexed by function. *Genome Res.* 2003; 13:2129–2141. [PubMed: 12952881]
12. Lee S, Hong SW, Min BH, et al. Essential role of clusterin in pancreas regeneration. *Dev Dyn.* 2011; 240:605–615. [PubMed: 21290478]
13. Chen R, Pan S, Cooke K, et al. Comparison of pancreas juice proteins from cancer versus pancreatitis using quantitative proteomic analysis. *Pancreas.* 2007; 34:70–79. [PubMed: 17198186]
14. Gronborg M, Bunkenborg J, Kristiansen TZ, et al. Comprehensive proteomic analysis of human pancreatic juice. *J Proteome Res.* 2004; 3:1042–1055. [PubMed: 15473694]
15. Park JY, Kim SA, Chung JW, et al. Proteomic analysis of pancreatic juice for the identification of biomarkers of pancreatic cancer. *J Cancer Res Clin Oncol.* 2011; 137:1229–1238. [PubMed: 21691750]
16. Zhou L, Lu Z, Yang A, et al. Comparative proteomic analysis of human pancreatic juice: methodological study. *Proteomics.* 2007; 7:1345–1355. [PubMed: 17443640]
17. Nimpf J, Wurm H, Kostner GM. Interaction of beta 2-glycoprotein-I with human blood platelets: influence upon the ADP-induced aggregation. *Thromb Haemost.* 1985; 54:397–401. [PubMed: 4082080]
18. Tjernberg J, Ekdahl KN, Lambris JD, et al. Acute antibody-mediated complement activation mediates lysis of pancreatic islets cells and may cause tissue loss in clinical islet transplantation. *Transplantation.* 2008; 85:1193–1199. [PubMed: 18431241]
19. Hartwig W, Klafs M, Kirschfink M, et al. Interaction of complement and leukocytes in severe acute pancreatitis: potential for therapeutic intervention. *Am J Physiol Gastrointest Liver Physiol.* 2006; 291:G844–G850. [PubMed: 17030899]
20. Muraki T, Hamano H, Ochi Y, et al. Autoimmune pancreatitis and complement activation system. *Pancreas.* 2006; 32:16–21. [PubMed: 16340739]
21. Mallery DL, McEwan WA, Bidgood SR, et al. Antibodies mediate intracellular immunity through tripartite motif-containing 21 (TRIM21). *Proc Natl Acad Sci U S A.* 2010; 107:19985–19990. [PubMed: 21045130]
22. Frulloni L, Bovo P, Brunelli S, et al. Elevated serum levels of antibodies to carbonic anhydrase I and II in patients with chronic pancreatitis. *Pancreas.* 2000; 20:382–388. [PubMed: 10824693]
23. Sheng WW, Dong M, Zhou JP, et al. Clinicopathological significance of the expression of carbonic anhydrase I and II in human pancreatic cancer. *Zhonghua Yi Xue Za Zhi.* 2012; 92:3407–3411. [PubMed: 23327700]
24. Weed RI, Reed CF, Berg G. Is hemoglobin an essential structural component of human erythrocyte membranes? *J Clin Invest.* 1963; 42:581–588. [PubMed: 13999462]
25. Komander D. The emerging complexity of protein ubiquitination. *Biochem Soc Trans.* 2009; 37(Pt 5):937–953. [PubMed: 19754430]
26. Kemik O, Kemik AS, Sumer A, et al. Serum procarboxypeptidase A and carboxypeptidase A levels in pancreatic disease. *Hum Exp Toxicol.* 2012; 31:447–451. [PubMed: 21502183]

27. Bhoola KD, Figueroa CD, Worthy K. Bioregulation of kinins: kallikreins, kininogens, and kininases. *Pharmacol Rev.* 1992; 44:1–80. [PubMed: 1313585]
28. Jacobsson B, Carlstrom A, Platz A, et al. Transthyretin messenger ribonucleic acid expression in the pancreas and in endocrine tumors of the pancreas and gut. *J Clin Endocrinol Metab.* 1990; 71:875–880. [PubMed: 2401717]
29. Jacobsson B. In situ localization of transthyretin-mRNA in the adult human liver, choroid plexus and pancreatic islets and in endocrine tumours of the pancreas and gut. *Histochemistry.* 1989; 91:299–304. [PubMed: 2659558]
30. Itoh N, Hanafusa T, Miyagawa J, et al. Transthyretin (prealbumin) in the pancreas and sera of newly diagnosed type I (insulin-dependent) diabetic patients. *J Clin Endocrinol Metab.* 1992; 74:1372–1377. [PubMed: 1592883]
31. Lowe ME. Pancreatic triglyceride lipase and colipase: insights into dietary fat digestion. *Gastroenterology.* 1994; 107:1524–1536. [PubMed: 7926517]
32. Antonov VK, Dyakov VL, Mishin AA, et al. Catalytic activity and association of pancreatic lipase. *Biochimie.* 1988; 70:1235–1244. [PubMed: 3147716]
33. Molinari I, Souare K, Lamireau T, et al. Fecal chymotrypsin and elastase-1 determination on one single stool collected at random: diagnostic value for exocrine pancreatic status. *Clin Biochem.* 2004; 37:758–763. [PubMed: 15329313]
34. Witt H, Beer S, Rosendahl J, et al. Variants in CPA1 are strongly associated with early onset chronic pancreatitis. *Nat Genet.* 2013; 45:1216–1220. [PubMed: 23955596]
35. Laethem RM, Blumenkopf TA, Cory M, et al. Expression and characterization of human pancreatic preprocarboxypeptidase A1 and preprocarboxypeptidase A2. *Arch Biochem Biophys.* 1996; 332:8–18. [PubMed: 8806703]
36. Contois JH, Warnick GR, Sniderman AD. Reliability of low-density lipoprotein cholesterol, non-high-density lipoprotein cholesterol, and apolipoprotein B measurement. *J Clin Lipidol.* 2011; 5:264–272. [PubMed: 21784371]
37. Jagadeeshan S, Krishnamoorthy YR, Singhal M, et al. Transcriptional regulation of fibronectin by p21-activated kinase-1 modulates pancreatic tumorigenesis. *Oncogene.* 2015; 34:455–464. [PubMed: 24561527]
38. Schwarz RE, Awasthi N, Konduri S, et al. Antitumor effects of EMAP II against pancreatic cancer through inhibition of fibronectin-dependent proliferation. *Cancer Biol Ther.* 2010; 9:632–639. [PubMed: 20212356]
39. Heiss A, Eckert T, Aretz A, et al. Hierarchical role of fetuin-A and acidic serum proteins in the formation and stabilization of calcium phosphate particles. *J Biol Chem.* 2008; 283:14815–14825. [PubMed: 18364352]
40. Kusnierz-Cabala B, Gurda-Duda A, Panek J, et al. Serum fetuin A concentrations in patients with acute pancreatitis. *Clin Lab.* 2010; 56:191–195. [PubMed: 20575466]
41. Hull RL, Johnson PY, Braun KR, et al. Hyaluronan and hyaluronan binding proteins are normal components of mouse pancreatic islets and are differentially expressed by islet endocrine cell types. *J Histochem Cytochem.* 2012; 60:749–760. [PubMed: 22821669]
42. Hamm A, Veeck J, Bektas N, et al. Frequent expression loss of inter-alpha-trypsin inhibitor heavy chain (ITIH) genes in multiple human solid tumors: a systematic expression analysis. *BMC Cancer.* 2008; 8:25. [PubMed: 18226209]
43. Look AT, Ashmun RA, Shapiro LH, et al. Human myeloid plasma membrane glycoprotein CD13 (gp150) is identical to aminopeptidase N. *J Clin Invest.* 1989; 83:1299–1307. [PubMed: 2564851]
44. Duke-Cohan JS, Gu J, McLaughlin DF, et al. Attractin (DPPT-L), a member of the CUB family of cell adhesion and guidance proteins, is secreted by activated human T lymphocytes and modulates immune cell interactions. *Proc Natl Acad Sci U S A.* 1998; 95:11336–11341. [PubMed: 9736737]
45. Mian IS, Bradwell AR, Olson AJ. Structure, function and properties of antibody binding sites. *J Mol Biol.* 1991; 217:133–151. [PubMed: 1988675]
46. Calabrese G, Sures I, Pompetti F, et al. The gene (*LGALS3BP*) encoding the serum protein 90 K, associated with cancer and infection by the human immunodeficiency virus, maps at 17q25. *Cytogenet Cell Genet.* 1995; 69:223–225. [PubMed: 7698018]

47. Pan S, Chen R, Tamura Y, et al. Quantitative glycoproteomics analysis reveals changes in *N*-glycosylation level associated with pancreatic ductal adenocarcinoma. *J Proteome Res*. 2014; 13:1293–1306. [PubMed: 24471499]
48. Singh AP, Chauhan SC, Bafna S, et al. Aberrant expression of transmembrane mucins, MUC1 and MUC4, in human prostate carcinomas. *Prostate*. 2006; 66:421–429. [PubMed: 16302265]
49. Montalbano AM, Albano GD, Anzalone G, et al. Cigarette smoke alters non-neuronal cholinergic system components inducing MUC5AC production in the H292 cell line. *Eur J Pharmacol*. 2014; 736C:35–43. [PubMed: 24797786]
50. Di YP, Zhao J, Harper R. Cigarette smoke induces MUC5AC protein expression through the activation of Sp1. *J Biol Chem*. 2012; 287:27948–27958. [PubMed: 22700966]
51. Masaki Y, Oka M, Ogura Y, et al. Sialylated MUC1 mucin expression in normal pancreas, benign pancreatic lesions, and pancreatic ductal adenocarcinoma. *Hepatogastroenterology*. 1999; 46:2240–2245. [PubMed: 10521973]
52. Balague C, Gambus G, Carrato C, et al. Altered expression of MUC2, MUC4, and MUC5 mucin genes in pancreas tissues and cancer cell lines. *Gastroenterology*. 1994; 106:1054–1061. [PubMed: 8143972]
53. Tsiotos GG, Barry MK, Johnson CD, et al. Pancreas regeneration after resection: does it occur in humans? *Pancreas*. 1999; 19:310–313. [PubMed: 10505763]
54. Morisset J. Intervention of GI neuropeptides in pancreatic growth and regeneration: comparison with cholecystokinin. *J Physiol Pharmacol*. 2003; 54(suppl 4):127–141. [PubMed: 15075455]
55. Yang M, Liu W, Wang CY, et al. Proteomic analysis of differential protein expression in early process of pancreatic regeneration in pancreatectomized rats. *Acta Pharmacol Sin*. 2006; 27:568–578. [PubMed: 16626512]
56. Xie MJ, Motoo Y, Su SB, et al. Expression of clusterin in human pancreatic cancer. *Pancreas*. 2002; 25:234–238. [PubMed: 12370533]
57. Maeda H, Hanazaki K. Pancreatogenic diabetes after pancreatic resection. *Pancreatology*. 2011; 11:268–276. [PubMed: 21734430]
58. Ferrone CR, Marchegiani G, Hong TS, et al. Radiological and surgical implications of neoadjuvant treatment with FOLFIRINOX for locally advanced and borderline resectable pancreatic cancer. *Ann Surg*. 2015; 261:12–17. [PubMed: 25599322]

FIGURE 1.
General mass spectrometry workflow.

FIGURE 2. Panther database analysis grouping proteins identified in our proteomic analysis by molecular function (A) and biological process (B).

FIGURE 3.

Venn diagrams comparing proteins identified in the following: (A) this study versus a recent study analyzing normal PJ (high-confidence proteins only)³ and (B) this study versus 6 recent PJ studies (5 cancer studies and 1 normal PJ study).^{2,3,13–16}

FIGURE 4.

Histograms illustrating statistically significant proteins in the time course study. Error bars represent standard error of the mean (n = 4 patients). POD indicates postoperative day.

FIGURE 5. Histograms illustrating statistically significant proteins when comparing cohorts with and without PDAC. Error bars represent standard error of the mean (n = 11 patients).

FIGURE 6.

Histograms illustrating statistically significant proteins when comparing cohorts of up-front surgery (none) versus adjuvant treatment. Error bars represent standard error of the mean (n = 11 patients).

FIGURE 7. Histograms illustrating statistically significant proteins when comparing cohorts of smokers versus nonsmokers. Error bars represent standard error of the mean (n = 11 patients).

TABLE 1

Patient Characteristics

Patient	Time Point	Smoker	Neoadjuvant	PDAC	Proteins Identified
A	0	Yes	Yes	Yes	385
A	5	Yes	Yes	Yes	284
A	21	Yes	Yes	Yes	184
B	0	No	Yes	Yes	74
B	5	No	Yes	Yes	83
B	21	No	Yes	Yes	86
C	0	Yes	Yes	Yes	262
C	5	Yes	Yes	Yes	235
C	21	No	Yes	Yes	243
D	0	No	Yes	Yes	230
D	5	No	Yes	Yes	137
D	21	No	Yes	Yes	50
E	0	No	No	No	73
F	0	No	No	Yes	52
G	0	No	Yes	Yes	48
H	0	No	No	Yes	71
I	0	No	No	No	68
J	0	Yes	No	Yes	98
K	0	Yes	Yes	Yes	277