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The Proteome of Normal Pancreatic Juice

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Abstract

Objectives—The aims of this study were to characterize the proteome of normal pancreatic juice, to analyze the effect of secretin on the normal proteome, and to compare these results with published data from patients with pancreatic cancer.

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Methods—Paired pancreatic fluid specimens (before and after intravenous secretin stimulation) were obtained during endoscopic pancreatography from three patients without significant pancreatic pathology. Proteins were identified and quantified by mass spectrometry-based protein quantification technology. The human RefSeq (NCBI) database was used to compare the data in normal patient samples with published data from three pancreatic cancer patients.

Results—A total of 285 proteins were identified in normal pancreatic juice. Ninety had sufficient amino acid sequences identified to characterize the protein with a high level of confidence. All 90 proteins were present before and after secretin administration but with altered relative concentrations, usually by 1-2 folds, after stimulation. Comparison with 170 published pancreatic cancer proteins yielded an overlap of only 42 proteins.

Conclusions—Normal pancreatic juice contains multiple proteins related to many biological processes. Secretin alters the concentration but not the spectrum of these proteins. The pancreatic juice proteome of normal and pancreatic cancer patients differ markedly.

Suggested Key Words

pancreatic fluid; proteome; secretin; pancreatic adenocarcinoma

INTRODUCTION

The proteome is the entire complement of proteins expressed by a genome, cell, tissue or organism. In eukaryotic organisms, the proteome is significantly larger and more complex than the genome secondary to such biological modification processes as gene splicing and post-transcriptional modification.¹ Proteomics, the study of the proteome, may prove to be an invaluable tool in the field of oncologic research. Through methods such as gel electrophoresis and mass spectrometry, the proteome of neoplastic tissues and body fluids can be analyzed, leading to the identification of disease specific biomarkers useful in detection, staging, treatment and surveillance of several types of malignancies.²⁻²³

The proteome of human pancreatic juice has been characterized in patients with pancreatic diseases, including pancreatic cancer.²⁴⁻²⁹ However, to date, the proteome of pancreatic juice from non-diseased humans has not been reported. In addition, the influence of secretin stimulation on the human pancreatic fluid proteome is unknown. Secretin is frequently administered during endoscopic retrograde cholangiopancreatography (ERCP) to increase the yield of pancreas fluid for collection. Thus, an understanding of the effects of secretin on the proteome should be obtained prior to subsequent analysis of such samples for pertinent biomarkers of disease states. Therefore, the aims of this study were 1) to characterize the proteome of normal pancreatic fluid, 2) to analyze the effect of secretin stimulation on the normal proteome and 3) to compare these results with published data from patients with pancreatic cancer.

MATERIALS AND METHODS

Assurances

These studies have been conducted in strict compliance with the Indiana University School of Medicine Institutional Review Board

Patient Information and Specimen Collection

Three female patients, ages 29-32, underwent ERCP for clinical abdominal symptoms, but no apparent pancreatic pathology was present following diagnostic investigation. Each patient prospectively signed informed consent for collection of pancreatic ductal fluid.

Paired fluid specimens were obtained from each patient before and after administration of secretin (one ampule, intravenous administration) to increase the yield of pancreatic fluid. Specimens were placed immediately on ice after procurement and aliquoted for storage at -80°C without protease inhibitors within the Indiana University Pancreas Tissue Fluid Bank. Samples were subsequently analyzed via proteomic methods described below.

Liquid Chromatography-Mass Spectrometry (LC/MS) Analysis

One hundred microliters of each pancreatic juice sample was denatured by a buffer containing 8 M urea and 10 mM dithiothreitol (DTT). In order to take the same amount of proteins from each sample for each analysis, protein concentrations were determined by Bradford assay. The same buffer is used as the background reference for protein assay in order to obtain a relatively accurate measurement among all samples (due to the presence of urea in lysis buffer). Resulting protein extracts are subsequently reduced and alkylated with triethylphosphine and iodoethanol to block sulfhydryl groups in proteins. The volatile reagents triethylphosphine and iodoethanol were used to minimize sample preparation variations.³⁰ Protein mixtures were then digested by trypsin, and tryptic digests were filtered with 0.45 µm spin filters before applied to HPLC to avoid column clogging.

All digested samples were randomized for injection order to remove systematic bias from data acquisition. Twenty µg of the tryptic peptides from each sample were injected onto a C18 microbore column (i.d. = 1 mm, length = 5 cm, pore size = 300Å). Peptides were eluted with a linear gradient from 5 to 45% acetonitrile developed over 120 min at a flow rate of 50 µL/min, and effluent was electro-sprayed into a linear trap quadrupole (LTQ) mass spectrometer (ThermoFisher Scientific). The mass spectrometry (MS) data were collected in the data-dependent “Triple-Play” mode (MS scan, Zoom scan, and MS/MS scan). These three important experimental parameters plus chromatographic retention time determine the analytical accuracy of protein identification and protein quantification by ion intensity.³¹

Protein Identification

In this study, both SEQUEST and X! Tandem database search algorithms were used for peptide sequence identification.³²⁻³⁴ Each algorithm compares the observed peptide MS/MS spectra and theoretically derived spectra from the database to assign quality scores. These quality scores and other important predictors are combined in the algorithm that assigns an overall score to each peptide.

Identified proteins were classified according to identification (ID) quality (priority). The confidence in protein identification is increased with an increasing number of distinct amino acid sequences identified and increasing peptide ID confidence. Priority 1 proteins have the greatest likelihood of correct identification (multiple unique sequences identified) and priority 4 proteins have the least likelihood of correct identification (but still with <25% false discovery). The “peptide ID confidence” (the quality of the amino acid sequence identification) of the “best peptide” (the peptide with the highest peptide ID confidence) is used to assign the protein to a “high” (between 90% and 100% confidence), “moderate” (between 75% and 89% confidence), or “low” (less than 75% confidence) category. All “low” category proteins < 75% are eliminated from further consideration. The biologic function of priority 1 and 2 proteins was determined by the Panther Classification System.^{35, 36} The human RefSeq (NCBI) database was used to compare the data in normal patient samples with published data from three pancreatic cancer patients.

Protein Quantification

The key in our quantitative analysis is the chromatographic peak alignment.³¹ Due to the fact that large biomarker studies can produce chromatographic shifts as a result of multiple

injections of the samples onto the same HPLC column, this peak alignment is critical in order to provide the most accurate comparative data. Un-aligned peak comparison will result in larger variability and inaccuracy in peptide quantification.³¹ To be qualified for the protein quantification procedure, each aligned peak must match precursor ion (MS data), charge state (zoom scan data), fragment ions (MS/MS data) and retention time (within one-minute window). After alignment, the area-under-the-curves (AUC) for individually aligned peaks from identified peptides from each sample were computed; the AUCs were then compared for relative protein abundance. The data normalization was carried out by Quantile Normalization method.³⁷ Quantile Normalization is a method of normalization that essentially ensures that every sample has a peptide intensity histogram of the same scale, location and shape. This normalization procedure removes trends introduced by sample handling, sample preparation, total protein differences and changes in instrument sensitivity while running multiple samples.

Statistical Analysis

The number of significant changes between groups, the fold changes (FC) and the variability (Coefficient-of-Variation or CV) can be summarized for each priority level. The threshold for significance is set to control the False-Discovery-Rate (FDR) for each comparison at an investigator-desired percentile, normally 5%.³⁸ The FDR is estimated by the q -value which is an adjusted p -value. The FDR is the proportion of significant changes that are false positives. If proteins with a q -value ≤ 0.05 are declared significant, 5% of the declared changes are expected to be false positives. In the method described by Higgs, et al.³¹, the p -Value to q -Value adjustment is done separately for Priority 1, Priority 2 and the MODERATE confidence categories.

FC is computed from the means on the AUC scale (antilog) as follows:

$$\begin{aligned} \text{FC} &= \text{Mean Treated Group} / \text{Mean Control Group} \\ &\quad \text{When Mean Treated Group} \geq \text{Mean Control Group} \quad (\text{up-regulation}) \\ \text{FC} &= \text{Mean Control Group} / \text{Mean Treated Group} \\ &\quad \text{When Mean Control Group} > \text{Mean Treated Group} \quad (\text{down-regulation}) \\ \text{Absolute FC} &= |\text{FC}| = \text{absolute or positive value of the FC} \end{aligned}$$

An FC of 1 means that no change exists.

Also, the median %CV for each priority level is determined. The %CV is the standard deviation / mean on a % scale. The %CV is given both for the replicate variation as well as the combined replicate plus sample variation.

For each protein a separate analysis of variance (ANOVA) model is fit:

$$\text{Log}_2(\text{Intensity}) = \text{Overall Mean} + \text{Group Effect} + \text{Patient Effect} + \text{Residual}$$

$\text{Log}_2(\text{Intensity})$ is the protein intensity based on the weighted average of the quantile normalized log base 2 peptide intensities with the same protein identification. *Group Effect* refers to the fixed effects (not random) caused by the experimental conditions or treatments that are to be compared. *Patient Effect* refers to the fixed effects from individual patients and is NOT assumed to be random. *Residual Effect* is a mixture of patient by group interaction and pure error due to duplicate samples. This model was necessary due to the experiment only having three patients. This situation resulted in a residual with eight degrees of

freedom. Thus, this model implies that significant differences can only be attributed to these three specific patients and NOT generalized to a larger population of patients.

RESULTS

Proteome of Pancreatic Juice

Nine hundred forty-four unique amino acid sequences were identified and quantified resulting in 285 proteins. Of the 285 proteins found in normal pancreatic juice, 90 proteins were detected by the highly confident identification ($q < 0.1$) of more than one unique peptide sequence within the protein (priority 1 proteins).³⁹ Eighty-two proteins were identified by the highly confident identification of only one unique peptide sequence within the protein (priority 2 proteins). Together, the priority 1 and 2 categories resulted in 172 proteins (Table 1) that will be the focus of this study. The most commonly identified proteins were involved with proteolysis, ribonucleic acid (RNA) and deoxyribonucleic acid (DNA) function or lipid metabolism (Table 2).

Influence of Secretin on Pancreatic Juice Proteome

Importantly, all 172 proteins were present before and after secretin, suggesting that secretin does not change the spectrum of proteins but rather the relative quantity. Following secretin stimulation, 44% of proteins expressed were increased and 56% decreased, usually by 1-2 folds (Table 2).

Comparison of Pancreatic Cancer Juice Proteome with Normal Pancreatic Juice Proteome

Comparison with 170 published pancreatic cancer proteins from Goggins et al⁴⁰ yielded an overlap of only 42 proteins (Figure 1, Table 3). Putative tumor markers including azurocidin, carcinoembryonic antigen (CEA), insulin-like growth factor (ILGF) binding protein 2, lipocalin 2, mucin 1 (MUC1), pancreatitis associated protein/hepatocarcinoma-intestine-pancreas (PAP/HIP) and tumor rejection antigen were only found in cancer patients.

DISCUSSION

In summary, 285 proteins were identified in the normal pancreatic proteome and were most commonly involved with proteolysis, RNA or DNA functions or lipid metabolism. Table 2 lists the 172 priority one and two proteins according to their biological function. Several of the common proteins in the normal pancreatic proteome were those that provide the fundamental basis of the biologic and physiologic function of all cells and tissues, including basic structural cell components, cell signaling molecules and proteins involved in transcription and translation of DNA. Furthermore, as expected, several normal serum components and proteins associated with immunology and detoxification within the body also were present. However, many prominent proteins related to the specific function of the pancreas also were present.

The exocrine pancreas plays a crucial role in digestion, serving as the main source of enzymes responsible for the catabolism of proteins.⁴¹ Trypsinogen is an inactive zymogen secreted from the pancreas into the duodenum, where enteropeptidase cleaves it into its active form, trypsin, a serine protease responsible for cleaving peptide bonds and breaking proteins down into amino acids.⁴¹ Trypsin and its precursor proteins were commonly present in the normal pancreatic proteome. Other proteases were also represented, including serine proteases, chymotrypsin, elastase and carboxypeptidases.

The exocrine pancreas also contributed to several other components of digestion.⁴¹ Our studies detected the presence of pancreatic lipase, colipase and several related proteins all important for the breakdown and utilization of lipids. Amylase and enolase, both important in carbohydrate metabolism, also were well represented. Furthermore, anion exchange proteins and carbonic anhydrase were present, indicating the pancreatic production of bicarbonate ions which serve to neutralize the acidic chyme that is produced during the gastric phase of digestion.

All identified proteins of the normal pancreas were present after secretin administration, although 44% of proteins expressed were increased and 56% decreased. Such results suggest that secretin changes the relative quantity of proteins but not the overall spectrum. This observation is extremely important in clinical applications, as secretin is frequently administered during pancreatic procedures to increase the production and flow of pancreatic fluid. Our studies indicate that the use of secretin is unlikely to change the presence of biomarkers within this fluid and lend credence to studies indicating the presence and absence of such molecules. However, future scientists interested in pursuing this important and interesting area of oncologic research should bear in mind the potential for changes in protein concentrations following secretin administration.

Comparison of the 172 proteins established within the normal pancreatic proteome with 170 published pancreatic cancer proteins yielded an overlap of only 42 proteins. Again, the ubiquitous cellular and serum proteins were present. However, also highly represented in this select group were those pancreatic proteins associated with protein and lipid metabolism, as discussed above. Putative tumor markers including azurocidin, carcinoembryonic antigen (CEA), insulin-like growth factor (ILGF) binding protein 2, lipocalin 2, mucin 1 (MUC1), pancreatitis associated protein/hepatocarcinoma-intestine-pancreas (PAP/HIP) and tumor rejection antigen were only found in cancer patients.^{25, 40} Although we found the lack of these established tumor markers in the normal pancreatic proteome interesting, it must be kept in mind that the samples and proteomics methods employed in our study were different from those used in the pancreatic adenocarcinoma studies, preventing a truly accurate comparison.

In conclusion, this analysis suggests that 1) normal pancreatic juice contains multiple proteins related to many biological processes, 2) secretin alters the concentration but not the spectrum of these proteins and 3) the pancreatic juice proteome of normal and pancreatic cancer patients differ markedly.

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REFERENCES

1. Alberts, B.; Johnson, A.; Lewis, J., et al. *Molecular Biology of the Cell*. Fourth ed.. Garland Science; New York, NY: 2002.
2. Baak JP, Mohan H, Baak JPA, et al. Genomics and proteomics in neoplasia. *Indian J Pathol Microbiol.* Jan; 2007 50(1):1–5. [PubMed: 17474244]
3. Cazares LH, Diaz JI, Drake RR, et al. MALDI/SELDI protein profiling of serum for the identification of cancer biomarkers. *Methods Mol Biol.* 2008; 428:125–140. [PubMed: 18287771]
4. Cho WC, Cheng CH, Cho WCS, et al. Oncoproteomics: current trends and future perspectives. *Expert Rev Proteomics.* Jun; 2007 4(3):401–410. [PubMed: 17552924]
5. Dhamoon AS, Kohn EC, Azad NS, et al. The ongoing evolution of proteomics in malignancy. *Drug Discov Today.* Sep; 2007 12(17-18):700–708. [PubMed: 17826682]

6. Faca V, Krasnoselsky A, Hanash S, et al. Innovative proteomic approaches for cancer biomarker discovery. *Biotechniques*. Sep.2007 43(3):279. 285. [PubMed: 17907570]
7. Findeisen P, Neumaier M, Findeisen P, et al. Mass spectrometry based proteomics profiling as diagnostic tool in oncology: current status and future perspective. *Clin Chem Lab Med*. 2009; 47(6): 666–684. [PubMed: 19445650]
8. Hariharan D, Weeks ME, Crnogorac-Jurcevic T, et al. Application of proteomics in cancer gene profiling: two-dimensional difference in gel electrophoresis (2D-DIGE). *Methods Mol Biol*. 576:197–211. [PubMed: 19882264]
9. Iwate Y. Clinical proteomics in cancer research-promises and limitations of current two-dimensional gel electrophoresis. *Curr Med Chem*. 2008; 15(23):2393–2400. [PubMed: 18855668]
10. Jain KK. Innovations, challenges and future prospects of oncoproteomics. *Mol Oncol*. Aug; 2008 2(2):153–160. [PubMed: 19383334]
11. Joos TO. Advances in proteomics in cancer research. *Expert Rev Proteomics*. Jun; 2007 4(3):347–349. [PubMed: 17552917]
12. Koomen JM, Haura EB, Bepler G, et al. Proteomic contributions to personalized cancer care. *Mol Cell Proteomics*. Oct; 2008 7(10):1780–1794. [PubMed: 18664563]
13. Maurya P, Meleady P, Dowling P, et al. Proteomic approaches for serum biomarker discovery in cancer. *Anticancer Res*. May-Jun; 2007 27(3A):1247–1255. [PubMed: 17593616]
14. Rajcevic U, Niclou SP, Jimenez CR, et al. Proteomics strategies for target identification and biomarker discovery in cancer. *Front Biosci*. 2009; 14:3292–3303. [PubMed: 19273274]
15. Reymond MA, Schlegel W. Proteomics in cancer. *Adv Clin Chem*. 2007; 44:103–142. [PubMed: 17682341]
16. Simpson RJ, Bernhard OK, Greening DW, et al. Proteomics-driven cancer biomarker discovery: looking to the future. *Curr Opin Chem Biol*. Feb; 2008 12(1):72–77. [PubMed: 18295612]
17. Tainsky MA. Genomic and proteomic biomarkers for cancer: a multitude of opportunities. *Biochim Biophys Acta*. Dec; 2009 1796(2):176–193. [PubMed: 19406210]
18. Tomancova A, Sedo O, Zdrahal Z, et al. [Proteomic analysis of cancer cells]. *Klin*. 2009; 22(5): 210–217.
19. Verma M. Proteomics and cancer epidemiology. *Methods Mol Biol*. 2009; 471:197–215. [PubMed: 19109781]
20. Wang P, Whiteaker JR, Paulovich AG, et al. The evolving role of mass spectrometry in cancer biomarker discovery. *Cancer Biol Ther*. Jun; 2009 8(12):1083–1094. [PubMed: 19502776]
21. Wouters BG. Proteomics: methodologies and applications in oncology. *Semin Radiat Oncol*. Apr; 2008 18(2):115–125. [PubMed: 18314066]
22. Xue H, Lu B, Lai M, et al. The cancer secretome: a reservoir of biomarkers. *J*. 2008; 6:52.
23. Zhao Y, Lee WN, Xiao GG, et al. Quantitative proteomics and biomarker discovery in human cancer. *Expert Rev Proteomics*. Apr; 2009 6(2):115–118. [PubMed: 19385938]
24. Chen R, Pan S, Cooke K, et al. Comparison of pancreas juice proteins from cancer versus pancreatitis using quantitative proteomic analysis. *Pancreas*. January; 2007 34(1):70–79. [PubMed: 17198186]
25. Chen R, Pan S, Yi EC, et al. Quantitative proteomic profiling of pancreatic cancer juice. *Proteomics*. Jul; 2006 6(13):3871–3879. [PubMed: 16739137]
26. Chung JC, Oh MJ, Choi SH, et al. Proteomic analysis to identify biomarker proteins in pancreatic ductal adenocarcinoma. *ANZ J Surg*. Apr; 2008 78(4):245–251. [PubMed: 18366394]
27. Cui Y, Tian M, Zong M, et al. Proteomic analysis of pancreatic ductal adenocarcinoma compared with normal adjacent pancreatic tissue and pancreatic benign cystadenoma. *Pancreatol*. 2009; 9(1-2):89–98. [PubMed: 19077459]
28. Qi T, Han J, Cui Y, et al. Comparative proteomic analysis for the detection of biomarkers in pancreatic ductal adenocarcinomas. *J Clin Pathol*. Jan; 2008 61(1):49–58. [PubMed: 17412869]
29. Zhou L, Lu Z, Yang A, et al. Comparative proteomic analysis of human pancreatic juice: methodological study. *Proteomics*. Apr; 2007 7(8):1345–1355. [PubMed: 17443640]

30. Higgs RE, Knierman MD, Gelfanova V, et al. Comprehensive label-free method for the relative quantification of proteins from biological samples. *J Proteome Res.* Jul-Aug; 2005 4(4):1442–1450. [PubMed: 16083298]
31. Hale JE, Butler JP, Gelfanova V, et al. A simplified procedure for the reduction and alkylation of cysteine residues in proteins prior to proteolytic digestion and mass spectral analysis. *Anal Biochem.* Oct 1; 2004 333(1):174–181. [PubMed: 15351294]
32. Moore RE, Young MK, Lee TD. Protein identification using a quadrupole ion trap mass spectrometer and SEQUEST database matching. *Curr Protoc Protein Sci.* May.2001 Chapter 16:Unit 16.10.
33. Fitzgibbon M, Li Q, McIntosh M, et al. Modes of inference for evaluating the confidence of peptide identifications. *J Proteome Res.* Jan; 2008 7(1):35–39. [PubMed: 18067248]
34. Kandasamy K, Pandey A, Molina H, et al. Evaluation of several MS/MS search algorithms for analysis of spectra derived from electron transfer dissociation experiments. *Anal Chem.* Sep 1; 2009 81(17):7170–7180. [PubMed: 19639959]
35. Thomas PD, Campbell MJ, Kejariwal A, et al. PANTHER: a library of protein families and subfamilies indexed by function. *Genome Res.* Sep; 2003 13(9):2129–2141. [PubMed: 12952881]
36. Thomas PD, Kejariwal A, Campbell MJ, et al. PANTHER: a browsable database of gene products organized by biological function, using curated protein family and subfamily classification. *Nucleic Acids Res.* Jan 1; 2003 31(1):334–341. [PubMed: 12520017] [Erratum appears in *Nucleic Acids Res.* 2003 Apr 1;31(7):2024].
37. Bolstad BM, Irizarry RA, Astrand M, et al. A comparison of normalization methods for high density oligonucleotide array data based on variance and bias. *Bioinformatics.* Jan 22; 2003 19(2):185–193. [PubMed: 12538238]
38. Reiner A, Yekutieli D, Benjamini Y, et al. Identifying differentially expressed genes using false discovery rate controlling procedures. *Bioinformatics.* Feb 12; 2003 19(3):368–375. [PubMed: 12584122]
39. Higgs RE, Knierman MD, Freeman AB, et al. Estimating the statistical significance of peptide identifications from shotgun proteomics experiments. *J Proteome Res.* May; 2007 6(5):1758–1767. [PubMed: 17397207]
40. Gronborg M, Bunkenborg J, Kristiansen TZ, et al. Comprehensive proteomic analysis of human pancreatic juice. *J Proteome Res.* Sep-Oct; 2004 3(5):1042–1055. [PubMed: 15473694]
41. O'Leary, JP., editor. *The Physiologic Basis of Surgery.* Third ed.. Lippincott, Williams & Wilkins; Philadelphia, PA: 2002.

Figure 1. Comparison to Proteome of Pancreatic Adenocarcinoma

Pancreatic juice proteome of normal patients (172 proteins, white) compared with the pancreatic juice proteome of published patients with adenocarcinoma (170 patients, black). 42 proteins (grey) were found in both normal and in adenocarcinoma pancreatic juice.

Table 1
Number of Proteins Identified at Each Priority Level

A priority level of one indicates that multiple peptide sequences within the protein were identified with high confidence ($q < 0.1$). A priority level of two indicates that only one peptide sequence was identified but with high confidence ($q < 0.1$). A priority level of three indicates that multiple peptide sequences within the protein were identified with moderate confidence ($0.1 < q < 0.25$). A priority level of four indicates that only one peptide sequence was identified with moderate confidence ($0.1 < q < 0.25$). Priority one and two proteins were discussed in this article.

Protein Priority	Peptide ID Confidence	Multiple Sequences	Median Number of Sequences	Number of Proteins
1	HIGH	YES	8	90
2	HIGH	NO	1	82
3	MODERATE	YES	2	2
4	MODERATE	NO	1	111
<i>Overall</i>			<i>1</i>	285

ID = Identification

Table 2

Pancreatic Juice Proteome

Of the 172 proteins identified with high confidence (Priority 1 and 2), 117 had known names and functions listed in the Panther DataBase and are listed below according to biologic function. Proteins listed in bold demonstrated significant changes. A negative fold change indicates a decrease in the protein following secretin administration, while a positive fold change indicates an increase in the protein following secretin administration.

Priority	Immunology and Detoxification	Fold Change	% ID Confidence	# Peptides ID	Best Sequence (with highest ID confidence)	Protein ID
1	α -2-Glycoprotein 1, Zinc-Binding (AZGP1)	-1.1	99.9	2	HVEDVPAFQALGSLNDLQFFR	IP100166729.2
1	Complement Component 3 (C3)	1.3	100.0	5	VQLSNDFFEYIMAEQTIK	IP100164623.3
1	Glutathione S-Transferase P1 (GSTP1)	-1.6	100.0	3	FQDGDLTLYQSNITLR	IP100219757.8
1	Glyceraldehyde-3-Phosphate Dehydrogenase (GAPDH)	-1.2	100.0	2	VVDLMAHMASKE	IP100219018.1
1	Orosomucoid 1 (ORM1)	1.4	100.0	5	EQLGEFYEALDCLR	IP100022429.1
1	Orosomucoid 2 (ORM2)	1.4	100.0	3	WFYIASAFR	IP100020091.1
2	Defensin, α 1 (DEFA1)	-1.3	99.8	1	IPACIAGERR	IP100005721.1
2	Peroxiredoxin 3 (PRDX3)	-1.5	93.2	1	FKDLSLDDFK	IP100024919.1

Priority	Metabolism	Fold Change	% ID Confidence	# Peptides ID	Best Sequence (with highest ID confidence)	Protein ID
General						
2	aarF Domain Containing Kinase 5 (ADCK5)	-2.2	92.7	1	QPIAAASLAQVHR	IP100418366.2
2	Thioredoxin (TXN)	-1.6	99.7	1	TAFQEALDAAGDKLVVDFSATWCGPCK	IP100216298.2
Carbohydrate						
1	Amylase α 1A, salivary (AMY1A)	1.1	100.0	79	IAEYMNHLIDIGVAGFR	IP100300786.1
1	Amylase α 2A, pancreatic (AMY2A)	1.1	100.0	103	IAEYMNHLIDIGVAGFR	IP100025476.1
1	Amylase α 2B, pancreatic (AMY2B)	1.1	100.0	99	IAEYMNHLIDIGVAGFR	IP100021447.1
1	Enolase 1 α (ENO1)	-1.3	100.0	2	YISPDQLADLYK	IP100465248.1
2	Carbonic Anhydrase I (CA1)	1.7	100.0	1	HDTSLKPISVSYPATAK	IP100215983.1
2	Carbonic Anhydrase II (CA2)	-1.6	100.0	1	AVQQPDGLAVLGFLK	IP100218414.1
2	Glucose Phosphate Isomerase (GPI)	-1.6	96.3	1	TFTTQETITNAETAK	IP100027497.3
Lipid						
1	Annexin A4 (ANXA4)	-1.6	100.0	8	AASGFNAMEDAQTLR	IP100221225.1
1	Annexin A5 (ANXA5)	-2.0	100.0	2	GLGTDEESILTLLTSR	IP100329801.6
1	Carboxyl Ester Lipase (Bile Salt-Stimulated Lipase) (CEL)	-1.7	100.0	50	TTFDVYTESWAQDPSQENK	IP100099670.1

Priority	Metabolism	Fold Change	%ID Confidence	# Peptides ID	Best Sequence (with highest ID confidence)	Protein ID
1	Colipase, pancreatic (CLPS)	-1.2	100.0	37	GHIINLENGELCMNSAQCK	IP100022232.1
1	Pancreatic Lipase (PNLIP)	1.1	100.0	162	LGCFSDDSPWSGITER	IP100027720.1
1	Pancreatic Lipase-Related Protein 1 (PNLIPRP1)	1.4	100.0	4	FIHGFIDK	IP100005923.1
1	Pancreatic Lipase-Related Protein 2 (PNLIPRP2)	-1.2	100.0	15	AIDVDFNVGK	IP100005924.1
1	Phospholipase A2, Group IB, pancreas (PLA2G1B)	-1.1	100.0	12	SYSCSGSAITCSSK	IP100021792.1
Protein						
1	α 2-Macroglobulin (A2M)	-1.2	100.0	5	LLIYAVLPTGDVIGDSAK	IP100478003.1
1	Carboxypeptidase A1, pancreatic (CPA1)	-1.1	100.0	53	EWVTQASGVWFAK	IP100009823.1
1	Carboxypeptidase A2, pancreatic (CPA2)	-1.0	100.0	30	LDDFDELSEVAQK	IP100296930.1
1	Carboxypeptidase B1, tissue (CPB1)	-1.0	100.0	56	WETIEAWTQQVATENPALISR	IP100009826.2
1	Chymotrypsin C (Caldecrin) (CTRC)	1.1	100.0	26	LQQLQPVVDHATCSR	IP100018553.1
1	Chymotrypsinogen B1 (CTRB1)	1.2	100.0	49	LQQAALPLLSNAECK	IP100015133.1
1	Elastase 2A, unassigned	1.2	100.0	26	LQTNGAVPDVLQQGR	IP100027722.1
1	Elastase 3A, pancreatic (ELA3A)	-1.0	100.0	42	SCVACGNDIALIK	IP100295663.1
1	Elastase 3B, pancreatic (ELA3B)	1.1	100.0	33	SCVACGNDIALIK	IP100307485.3
1	Peptidylprolyl Isomerase A (Cyclophilin A) (PPIA)	-1.4	100.0	2	VSFELFADKVPK	IP100419585.3
1	Serine Peptidase Inhibitor, Kazal Type 1 (SPINK1)	-1.2	100.0	5	IYDPVCGTDGNTYPNECVLCFENR	IP100020687.1
1	Serine Protease 1 (Trypsin 1) (PRSS1)	-1.2	100.0	41	VSTISLPTAPPATGTK	IP100011694.1
1	Serine Protease 2 (Trypsin 2) (PRSS2)	-1.5	100.0	28	ITNNMFCVGFLEGGK	IP100011695.2
2	Carboxypeptidase A4 (CPA4)	1.0	94.2	1	QIPTAEETWLGLK	IP100008894.1
2	Heat shock 70kDa Protein 8 (HSPA8)	-1.9	100.0	1	TTPSYVAFTDTER	IP100003865.1
2	Peptidase Inhibitor 3, skin-derived (SKALP) (PI3)	-1.2	93.9	1	VPFNGQDPVK	IP100021434.1
2	Peptidylprolyl Isomerase A (Cyclophilin A)-Like 4 (PPIAL4)	-1.2	100.0	1	IIPGFMCQGGDFTR	IP100030144.1
2	Ribosomal Protein L4 (RPL4)	1.1	93.7	1	VLKKNPL	IP100003918.2

Priority	Normal Serum Components	Fold Change	%ID Confidence	# Peptides ID	Best Sequence (with highest ID confidence)	Protein ID
1	Fibrinogen β Chain (FGB)	1.1	100.0	4	TPCTVSCNIPVVSQK	IP100298497.3
1	Fibrinogen γ Chain (FGG)	1.1	100.0	2	VAQLEAQCEPCKDTVQIHDITGK	IP100021891.5
1	Haptoglobin-Related Protein (HPR)	1.6	100.0	7	DIAPTLTLVYGK	IP100477597.1
1	Hemoglobin α 1 (HBA1)	1.9	100.0	21	VGAHAGEYGAELER	IP100410714.1
1	Hemoglobin δ (HBD)	1.7	100.0	20	VLGAFSDGLAHLNLIK	IP100473011.1

Priority	Normal Serum Components	Fold Change	%ID Confidence	# Peptides ID	Best Sequence (with highest ID confidence)	Protein ID
1	Hemoglobin γ A (HBG1)	1.7	100.0	3	LLVYPWTQR	IP100220706.1
1	Hemopexin (HPX)	1.3	100.0	4	EVGTPHGILDSVDAAFICPGSSR	IP100022488.1
1	Kallikrein 1 (KLK1)	-1.5	100.0	4	LTEPADTITDAVK	IP100304808.1
1	Transferrin (TF)	1.4	100.0	15	SAGWNIPIGLLCYCDLPEPR	IP100022463.1
2	Fibrinogen α Chain (FGA)	-1.9	99.9	1	VQHIQLLQK	IP100021885.1
2	Hemoglobin ϵ 1 (HBE1)	2.1	100.0	2	LLVYPWTQR	IP100217471.1
2	Hemoglobin zeta (HBZ)	2.6	95.5	2	LRVDPVNFK	IP100217473.1
2	Transferrin (Prealbumin, Amyloidosis Type D) (TTR)	-1.0	99.8	1	TSESGELHGLTTEEEFVEGIYKVEIDTK	IP100022432.1

Priority	Nucleic Acids	Fold Change	%ID Confidence	# Peptides ID	Best Sequence (with highest ID confidence)	Protein ID
1	H2A Histone Family, Member V (H2AFV)	-3.0	100.0	2	HLQLAIR	IP100018278.1
1	Histone Cluster 1, H1e (HIST1H1E)	-1.6	100.0	2	KASGPPVSELITK	IP100217467.1
1	Histone Cluster 1, H4j (HIST1H4J)	-1.9	100.0	3	ISGLIYEETR	IP100453473.1
1	Histone Cluster 2, H2be (HIST2H2BE)	-2.0	100.0	2	AMGIMNSFVNDIFER	IP100003935.2
1	Ribonuclease, RNase A Family, 1, pancreatic (RNASE1)	-1.3	100.0	3	CKPVNTFVHEPLVDVQNVCFQEK	IP100014048.1
1	S100 Calcium Binding Protein A11 (S100A11)	-1.9	100.0	2	TEFLSFMTNELAAFTK	IP100013895.1
2	Basonuclin 2 (BNC2)	1.6	95.4	1	MQFGTR	IP100465099.1
2	B-Cell CLL/Lymphoma 6, Member B (Zinc Finger Protein) (BCL6B)	6.6	98.0	1	LSPTAATVQFK	IP100166071.1
2	Caudal Type Homeobox Transcription Factor 1 (CDX1)	1.3	95.7	1	RSVAAGGGGGS	IP100026827.1
2	Centrosomal Protein 350kDa (CEP350)	2.1	95.6	1	THISDAVVASGAPL	IP100103595.1
2	Chromosome 20 Open Reading Frame 20 (C20orf20)	2.6	99.5	1	NSSDLGCKEGADK	IP100019451.1
2	Cleavage and Polyadenylation Factor 1 Subunit, Homolog (S. cerevisiae) (CLP1)	1.4	90.4	1	GSGYQALVHAASAFEVDVVVVLDQER	IP100024381.1
2	H3 Histone, Family 3B (H3.3B) (H3F3B)	2.2	99.9	1	EIAQDFKTDLR	IP100219038.3
2	Heterogeneous Nuclear Ribonucleoprotein A2/B1 (HNRPA2B1)	-2.4	100.0	1	GFQFVTFDDHDPVDKIVLQK	IP100396378.2
2	Heterogeneous Nuclear Ribonucleoprotein C-Like 1 (HNRPC1)	1.7	90.6	1	MDPHSMNSR	IP100027569.1
2	Histone Cluster 1, H1c (HIST1H1C)	-1.3	100.0	1	KASGPPVSELITK	IP100217465.1
2	Histone Cluster 2, H3a (HIST2H3A)	2.2	99.9	1	EIAQDFKTDLR	IP100171611.3
2	HIV-1 Tat Interacting Protein, 60kDa (HTATIP)	1.1	91.3	1	GPPVADPGAALSPQGEIIEGCR	IP100394657.1

Priority	Nucleic Acids	Fold Change	%ID Confidence	# Peptides ID	Best Sequence (with highest ID confidence)	Protein ID
2	Hydroxypyruvate Isomerase Homolog (E. coli) (HYI)	1.2	100.0	1	IHLMAGR	IP100465271.1
2	Nuclear Receptor Subfamily 5, Group A, Member 1 (NR5A1)	-1.5	95.5	1	FILFSLDLK	IP100012446.1
2	Phosphodiesterase 6A, cGMP-Specific, Rod, α (PDE6A)	1.1	91.6	1	VLQQNPI	IP100218730.3
2	Purine-Rich Element Binding Protein B (PURB)	-1.8	91.5	1	FLKIAEVGAGGSKSR	IP100045051.1
2	PWWP Domain Containing 2 (PWWP2)	-1.4	99.2	1	LSNRDPGR	IP100103812.4
2	TAF6 RNA polymerase II, TATA Box Binding Protein (TBP)-Associated Factor, 80kDa (TAF6)	1.2	94.2	1	DFAARLVAQICK	IP100028579.1
2	THO Complex 2 (THOC2)	-1.3	92.6	1	SQCGVKA VNK	IP100158615.4
2	Transcription Factor 15 (Basic Helix-Loop-Helix) (TCF15)	1.1	90.3	1	AFALLRPVGAHVLPDVR	IP100010762.5
2	Transcription Factor 3 (E2A Immunoglobulin Enhancer Binding Factors E12/E47) (TCF3)	-1.0	92.7	1	ARTSPDEDEDDLPPPEQKAER	IP100013929.1

Priority	Receptors (Signal Transduction)	Fold Change	%ID Confidence	# Peptides ID	Best Sequence (with highest ID confidence)	Protein ID
2	Adrenergic α -2B-Receptor (ADRA2B)	-2.7	94.2	1	EEGETPEDTGTR	IP100297315.1
2	Ankyrin Repeat and Sterile α Motif Domain Containing 1A (ANKS1A)	-1.1	93.5	1	DIGISDPQHRR	IP100395663.1
2	Nuclear Receptor Subfamily 5, Group A, Member 1 (NR5A1)	-1.5	95.5	1	FILFSLDLK	IP100012446.1
2	Olfactomedin-Like 1 (OLFML1)	-2.0	99.8	1	VYLLIGSR	IP100394820.1
2	Olfactory Receptor, Family 2, Subfamily AG, Member 1 (OR2AG1)	1.2	100.0	1	ALADFLR	IP100022324.1
2	Olfactory Receptor, Family 8, Subfamily D, Member 4 (OR8D4)	-2.9	93.1	1	MLSGFLCRDR	IP100169064.1
2	Phosphatidylethanolamine Binding Protein 1 (PEBP1)	-1.3	95.3	1	WSGPLSLQEVDEQPHPLHVTYAGAAVDELGK	IP100219446.1

Priority	Signaling Molecules	Fold Change	%ID Confidence	# Peptides ID	Best Sequence (with highest ID confidence)	Protein ID
1	Regenerating Islet-Derived 1 α (Pancreatic Stone Protein) (REG1A)	-1.2	100.0	25	SWGIGAPSSVNPNGYCVSLTSSTGFQK	IP100009027.1
1	Regenerating Islet-Derived 1 β (Pancreatic Stone Protein) (REG1B)	-1.1	100.0	10	LVSVLTAEGAFVASLIK	IP100009197.1
1	Regenerating Islet-Derived 3 α (REG3A)	-3.1	100.0	7	SWTDADLACQK	IP100029039.1
2	Dual Adaptor of Phosphotyrosine and 3-Phosphoinositides (DAPPI)	1.2	94.1	1	MSTQDPDSLWSR	IP100004301.1

Priority	Structural Proteins	Fold Change	%ID Confidence	# Peptides ID	Best Sequence (with highest ID confidence)	Protein ID
1	Actin β (ACTB)	-2.1	100.0	8	SYELPDGQVITIGNER	IP100021439.1
1	Actin α 1, skeletal muscle (ACTA1)	-2.4	100.0	4	SYELPDGQVITIGNER	IP100021428.1
1	Actin, α 2, smooth muscle, aorta (ACTA2)	-2.6	100.0	3	SYELPDGQVITIGNER	IP100008603.1
1	Annexin A2 (ANXA2)	-1.1	100.0	2	GVDEVITVNIL.TNR	IP100418169.1
1	Glycoprotein 2 (Zymogen Granule Membrane) (GP2)	1.5	100.0	14	CLLGGLGLGEEVIA.YLR	IP100470823.1
1	Glycoprotein 2 (Zymogen Granule Membrane) (GP2)	1.4	100.0	8	CLLGGLGLGEEVIA.YLR	IP100299429.2
2	ATP-Binding Cassette, Sub-Family G (WHITE), Member 5 (Sterol 1) (ABCG5)	-1.7	90.3	1	NKLAVITR	IP100007839.1
2	Calsynenin 1 (CLSTN1)	1.8	97.9	1	YISNEFK	IP100007257.4
2	Collagen, Type XXI, α 1 (COL21A1)	1.1	99.7	1	IKGYEVTSK	IP100102435.1
2	Lectin, Galactoside-Binding, Soluble, 4 (Galectin 4) (LGALS4)	-1.6	100.0	1	FFVNFVVGQDPGSDVAFHNPR	IP100009750.1
2	Talin 1 (TLN1)	1.2	100.0	1	AIADMLR	IP100298994.2
2	Tectorin α (TECTA)	-1.3	97.2	1	VPLHK	IP100023051.1
2	Tropomyosin 4 (TPM4)	-1.2	99.7	1	LATALQKLEAEKAADESER	IP100010779.1

Priority	Miscellaneous or Unknown Functions	Fold Change	%ID Confidence	# Peptides ID	Best Sequence (with highest ID confidence)	Protein ID
1	Anterior Gradient 2 Homolog (Xenopus laevis) (AGR2)	-2.0	100.0	3	IMFVDPSLTVR	IP100007427.1
1	CUB and Zona Pellucida-Like Domains 1 (CUZD1)	1.5	100.0	12	VFDGTSSNGPLLGQVCSK	IP100249672.1
2	Chromosome X Open Reading Frame 58 (CXorf58)	-1.4	90.8	1	KVAPLEAKLIK	IP100065057.1
2	Family with Sequence Similarity 129, Member B (FAM129B)	1.1	93.9	1	EVGLP	IP100456750.1
2	Loss of Heterozygosity, 11, Chromosomal Region 2, Gene A (LOH11CR2A)	-1.6	97.9	1	YTQQTMEEALGR	IP100005609.2
2	Proline Rich Gla (G-Carboxyglutamic Acid) 2 (PRRG2)	1.2	93.2	1	GVHDAPPPPYTSLR	IP100000460.1
2	TRAF3 Interacting Protein 2 (TRAF3IP2)	1.3	92.7	1	TVMIIVAISPK	IP100013883.1
2	Ubiquitin-Fold Modifier Conjugating Enzyme 1 (UF1C1)	-1.5	92.4	1	RVVSEIPVLKTNAGPR	IP100294495.1
2	Uridine Monophosphate Synthetase (UMPS)	1.1	93.9	1	EVGLP	IP100003923.1

Table 3
Proteins Identified in Normal Pancreatic Fluid and Pancreatic Fluid in Patients with Pancreatic Adenocarcinoma

The following represents a list of those 42 proteins present in the pancreatic fluid proteome of patients without pancreatic disease and those with pancreatic adenocarcinoma.

α -2-glycoprotein 1 (zinc-binding) (AZGP1)
α -2-macroglobulin (A2M)
Actin β (ACTB)
Amylase, α 2A (pancreatic) (AMY2A)
Amylase, α 2B (pancreatic) (AMY2B)
Annexin A4 (ANXA4)
Carboxypeptidase A1 (pancreatic) (CPA1)
Carboxypeptidase A2 (pancreatic) (CPA2)
Carboxypeptidase B1 (tissue) (CPB1)
Chymotrypsin C (Caldecrin) (CTRC)
Chymotrypsinogen B1 (CTRB1)
Colipase (pancreatic) (CLPS)
Complement Component 3 (C3)
CUB and Zona Pellucida-Like Domains 1 (CUZD1)
Elastase 2A
Elastase 3A, pancreatic (ELA3A)
Elastase 3B, pancreatic (ELA3B)
Enolase 1 α (ENO1)
Fibrinogen α Chain (FGA)
Glyceraldehyde-3-Phosphate Dehydrogenase (GAPDH)
Glycoprotein 2 (Zymogen Granule Membrane) (GP2)
H3 Histone, Family 3B (H3F3B)
Hemoglobin δ (HBD)
Hemoglobin, α 1 (HBA1)
Hemoglobin, γ A (HBG1)
Hemopexin (HPX)
Histone Cluster 1 (HIST1H4J)
Histone Cluster 2, H2be (HIST2H2BE)
Interferon Induced Transmembrane Protein 1 (9-27) (IFITM1)
Kallikrein 1 (KLK1)
Orosomucoid 2 (ORM2)
Pancreatic Lipase (PNLIP)
Pancreatic Lipase-Related Protein 1 (PNLIPRP1)
Peptidylprolyl Isomerase A (Cyclophilin A) (PPIA)
Phospholipase A2, Group IB (pancreas) (PLA2G1B)

Protease, Serine, 1 (Trypsin 1) (PRSS1)
Protease, Serine, 2 (Trypsin 2) (PRSS2)
Regenerating Islet-Derived 1 β (Pancreatic Stone Protein, Pancreatic Thread Protein) (REG1B)
Regenerating Islet-Derived 3 α (REG3A)
Ribonuclease, RNase A family, 1 (pancreatic) (RNASE1)
Transferrin (TF)
Transthyretin (Prealbumin, Amyloidosis type I) (TTR)