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A Proteomic Comparison of Formalin-Fixed Paraffin-Embedded Pancreatic Tissue from Autoimmune Pancreatitis, Chronic Pancreatitis, and Pancreatic Cancer

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Abstract

Context—Formalin-fixed paraffin-embedded (FFPE) tissue is a standard for specimen preservation, and as such FFPE tissue banks are an untapped resource of histologically-characterized specimens for retrospective biomarker investigation for pancreatic disease.

Objectives—We use liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS) to compare FFPE specimens from three different diseases of the exocrine pancreas.

Design—We investigated the proteomic profile of FFPE pancreatic tissue from 9 archived specimens that were histologically classified as: autoimmune pancreatitis (n=3), chronic pancreatitis (n=3), and pancreatic cancer (n=3), using LC-MS/MS.

Setting—This is a proteomic analysis experiment of FFPE pancreatic tissue in an academic center.

Patients—FFPE tissue specimens were provided by Dana-Farber/Harvard Cancer Center (Boston, MA, USA).

Interventions—FFPE tissue specimens were collected via routine surgical resection procedures.

Main outcome measures—We compared proteins identified from chronic pancreatitis, autoimmune pancreatitis, and pancreatic cancer FFPE pancreatic tissue.

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Results—We identified 386 non-redundant proteins from 9 specimens. Following our filtering criteria, 73, 29, and 53 proteins were identified exclusively in autoimmune pancreatitis, chronic pancreatitis, and pancreatic cancer specimens, respectively.

Conclusions—We report that differentially-expressed proteins can be identified among FFPE tissues specimens originating from individuals with different histological diagnoses. These proteins merit further confirmation with a greater number of specimens and orthogonal validation, such as immunohistochemistry. The mass spectrometry-based methodology used herein has the potential to enhance diagnostic biomarker and therapeutic target discovery, further advancing pancreatic research.

Keywords

Autoimmune Diseases; Biological Markers; Pancreas; Pancreatic Neoplasms; Pancreatitis; Chronic

INTRODUCTION

Diseases of the pancreas affect more than 1 million individuals in the United States annually, resulting in nearly $3 billion in direct and indirect medical costs [1]. Clarification of the biomolecular mechanisms of pancreatic diseases, such as pancreatitis and pancreatic cancer, is vital in identifying diagnostic biomarkers of early disease and developing targeted treatments to modify and/or retard disease progression.

Autoimmune pancreatitis is a benign idiopathic inflammatory disease of the pancreas resulting in masses and duct strictures. A recent study from Japan determined the prevalence of this rare disease to be 0.8/100,000 [2]. Another study estimated that 5–6% of patients classified previously as having idiopathic chronic pancreatitis have had diagnoses reclassified as autoimmune pancreatitis [3, 4]. Autoimmune pancreatitis may closely resemble pancreatic carcinoma, both clinically and radiographically, and thus the two pancreatic diseases can be difficult to differentiate [5, 6]. Current laboratory tests and biopsies by fine needle aspiration are limited in distinguishing the two diseases and cannot definitively rule out malignancy. In fact, over 10% of patients undergoing surgery for suspected pancreatic cancer, instead have autoimmune pancreatitis [7]. Therefore, improved diagnostic methods are needed to distinguish the two diseases. To that end, we propose a mass spectrometry-based proteomic study of formalin-fixed paraffin-embedded (FFPE) tissue for the discovery of biomarker candidates. We have applied previously similar techniques to differentiate late stage chronic pancreatitis from pancreatic cancer [8]; however, we now present the use of this technique in a more clinically relevant context that is the unmet need of distinguishing between autoimmune pancreatitis and pancreatic cancer.

Formalin fixing and paraffin embedding tissue is the standard technique for preserving specimens in hospital pathology departments. FFPE tissue banks are a rich resource for retrospective protein biomarker investigation. These types of specimens have historically been used to investigate the cellular localization of specific proteins via antibody-based immunohistochemistry [9]. Immunohistochemistry requires *a priori* knowledge of the proteins to be specifically targeted making it unsuitable for large-scale protein identification.

State-of-the-art proteomics techniques offer an unbiased exploratory approach, which if applied to tissue repositories, may enhance our understanding of pancreatic disease pathogenesis and pathophysiology, potentially identifying novel targets for immunohistochemistry analysis.

Mass spectrometry-based proteomics is quickly becoming the model strategy for unbiased large-scale protein investigation [10]. Archived FFPE pancreatic tissue specimens offer a robust sample set with which to investigate altered biochemical pathways and uncover potential biomarkers of pancreatic disease. Analogous techniques have been applied previously to FFPE tissues from various organs [11]. The major objectives of this proteomic investigation are: 1) to identify proteins present in FFPE pancreatic tissue using liquid chromatography-coupled with tandem mass spectrometry (LC-MS/MS), and 2) to compare the proteomic profiles of autoimmune pancreatitis, chronic pancreatitis, and pancreatic cancer FFPE pancreas tissue specimens. To our knowledge, we report the first comparison of FFPE pancreas tissue from autoimmune pancreatitis, chronic pancreatitis, and pancreatic cancer.

METHODS

Study Design and Setting

Proteomic analysis of archived formalin-fixed paraffin-embedded (FFPE) pancreas tissue in an academic center.

MATERIALS

FFPE tissue specimens were provided by Dana-Farber/Harvard Cancer Center (Boston, MA, USA). Heptane (product #51750) was from Sigma-Aldrich (St. Louis, MO, USA). Pep-clean C18 spin columns (product #89870) were from Thermo Scientific (Waltham, MA, USA). Sequencing-grade modified trypsin (V5111) was obtained from Promega (Madison, WI, USA). Other reagents and solvents were purchased from Sigma-Aldrich (St. Louis, MO, USA) and Burdick & Jackson (Morristown, NJ, USA), respectively.

Formalin Fixing and Paraffin Embedded of Pancreatic Tissue

FFPE tissue specimens were fixed in 10% neutral-buffered formalin for 48 h (Dana-Farber/Harvard Cancer Center, Research Pathology Core Laboratory). Routine paraffin treatment was performed as follows: specimens were treated twice with 70% ethanol for 45 minutes, twice with 80% ethanol for 60 minutes, twice with 95% ethanol for 60 minutes, thrice with 100% ethanol for 60 minutes, twice with #83 xylene substitute for 60 minutes, and twice with paraffin for 60 minutes. Tissue sections of 5 μm thickness were cut from the FFPE whole-mount pancreatic tissue block, mounted on standard glass slides, and heated for 60 min at 60°C. Slides were stored at room temperature until use.

Experimental Workflow

The experimental analysis is outline in Figure 1: 1) FFPE tissue specimens were obtained from the Dana-Farber/Harvard Cancer Center core facility; 2) a 1.5 cm x 1 cm x 5 μm slice

of the FFPE tissue was scraped from the slide into a microcentrifuge tube; 3) paraffin was removed with heptane; 4) disulfide bonds were reduced with dithiotreitol (DTT) and alkylated with iodoacetamide; 5) sample was digested overnight with trypsin; 6) peptides were isolated with C18 spin columns; 7) LC-MS/MS analysis was performed; and 8) database searching and bioinformatics processing was performed.

Sample Preparation for Mass Spectrometry Analysis

The sample preparation protocol for mass spectrometry analysis was assembled from several sources [11, 12, 13, 14, 15]. After removing the excess paraffin from the tissue slice, the FFPE pancreatic tissue specimen from each glass slide was scraped, using a clean razor blade, into a 2 mL microcentrifuge tube. To remove the remaining paraffin, 0.5 mL of heptane was added to each sample, followed by vigorous vortexing for 10 seconds and incubation at room temperature for 1 h. Twenty-five microliters of methanol was then added to each tube, followed by vigorous vortexing for 10 seconds and centrifugation (20,000 *g* for 2 min at 4°C). Immediately following centrifugation, the upper (heptane) layer was discarded and the lower layer was allowed to evaporate. Proteins were then extracted by resuspending the dried material in 250 μL of 6 M guanidine-HCl/50 mM ammonium bicarbonate/20 mM DTT, pH 8.5, briefly sonicating, and incubating at 70°C for 1 h. After cooling to room temperature, iodoacetamide was added to a final concentration of 40 mM and the sample was incubated in the dark for 1 h. The alkylation reaction was quenched by adding 3 μL of 2 M DTT.

In preparation for tryptic digestion, the sample was diluted 1:6 with 50 mM ammonium bicarbonate (pH 8.1) to reduce the concentration of guanidine-HCl to 1 M. Each sample was incubated with 2.5 μg of trypsin. Following the incubation, the reaction was acidified with formic acid to a final concentration of 0.1% and evaporated via vacuum centrifugation. To remove substances which may interfere with mass spectrometry, peptides were isolated with C18 spin columns according to the manufacturer's instructions. Samples were again vacuum centrifuged until dry and stored at −80°C until analysis. Immediately prior to analysis, the peptides were resuspended in sample loading buffer (5% formic acid, 5% acetonitrile, 90% water).

Mass Spectrometry

Mass spectrometry analysis was performed at the Proteomics Center at Children's Hospital Boston, MA, USA. The resuspended peptides were fractionated using reversed-phase high pressure liquid chromatography (HPLC; Thermo Scientific, Waltham, MA, USA) and the gradient-eluted peptides were analyzed using an LTQ FT Ultra mass spectrometer (Thermo Scientific, Waltham, MA, USA). The liquid chromatography columns (15 cm x 100 μm inner diameter) were packed in-house with Magic C18 (5 μm, 100 Å; Michrom BioResources, Auburn, CA, USA), into PicoTips (New Objective, Woburn, MA, USA). Samples were analyzed with a 90 minute linear gradient (0–35% acetonitrile with 0.2% formic acid) and data were acquired in a datadependent manner, in which MS/MS fragmentation was performed on the six most intense peaks of every full MS scan.

ETHICS

The study was approved by our Institutional Review Committee. This protocol was approved by the Institutional Review Board at Brigham and Women's Hospital and Children's Hospital Boston (IRB # 2007-P-002480/1).

Written informed consent was obtained from each patient and the study protocol conforms to the ethical guidelines of the "World Medical Association (WMA) Declaration of Helsinki - Ethical Principles for Medical Research Involving Human Subjects" adopted by the 18th WMA General Assembly, Helsinki, Finland, June 1964 and amended by the 59th WMA General Assembly, Seoul, South Korea, October 2008.

STATISTICS

Bioinformatics and Data Analysis

Raw files were converted to mascot generic files (mgf) using MSconvert [16] for downstream database searching in ProteinPilot (v.4; AB SCIEX, Foster City, CA, USA). All data generated were searched against the UniProt human database (downloaded November 11, 2011) using the Paragon algorithm [17], which is integrated into the ProteinPilot search engine. Search parameters were set as follows: 1) sample type: identification; 2) Cys alkylation: iodoacetamide; 3) instrument: Orbitrap/FT (1–3 ppm) or LTQ FT Ultra mass spectrometer (Thermo Scientific, Waltham, MA, USA) MS/MS; 4) special factors: none; 5) identification focus: none; 6) database: UniProt human; and 7) search effort: thorough identification. A 1% false discovery rate (FDR) for protein identifications was determined using the Posterior Error Probability algorithm integrated into ProteinPilot. Relative protein quantification was performed using label-free spectral counting, which compared the number of identified tandem mass spectra for the same protein across multiple data sets [18, 19].

RESULTS

Proteomic Analysis of FFPE Tissue Identified Several Hundred Proteins Using Mass Spectrometry-Based Proteomic Techniques

FFPE tissue from nine histologically-classified archived specimens (autoimmune pancreatitis, n=3; chronic pancreatitis, n=3; and pancreatic cancer, n=3) were subjected to mass spectrometry-based proteomic analysis, as depicted in Figure 1. The analysis of each specimen, was performed using a single 1.5 cm x 1 cm x 5 μm slice of FFPE tissue. Mass spectrometric analysis of all 9 FFPE specimens identified a total of 386 non-redundant proteins, which we list in Supplementary Table 1. A nonredundant protein is one that has been counted only once, regardless the number of specimens in which it has been identified.

Non-Redundant Proteins Were Exclusive to Each Pancreatic Disease Cohort

We summarize the number of non-redundant proteins identified in this study in Table 1. Considering each specimen individually, we identified 131, 141, and 103 non-redundant proteins in each autoimmune pancreatitis (AIP) specimen; 188, 138, and 153 non-redundant proteins in each chronic pancreatitis (CP) specimen; and 150, 190, and 140 non-redundant

proteins in each pancreatic cancer (PC) specimen. We then merged the three lists of proteins identified in each cohort to determine the number of non-redundant proteins identified within each cohort, as listed in Table 1. The numbers of non-redundant proteins identified in each cohort were: 211 in autoimmune pancreatitis, 265 in chronic pancreatitis, and 280 in pancreatic cancer.

We then compared proteins among cohorts to identify those exclusive to each cohort and those common to the three cohorts (Figure 2). We identified 29 proteins exclusive to the autoimmune pancreatitis cohort (Table 2), 53 proteins exclusive to the chronic pancreatitis cohort (Table 3), and 73 proteins exclusive to the pancreatic cancer cohort (Table 4). Proteins identified exclusively in the autoimmune pancreatitis specimens include immunoglobulins and histocompatibility antigens. Epiplakin, protein disulfide-isomerase, and mucin 2 were among the proteins that were identified exclusively in the pancreatic cancer specimens. Hornerin and several ribosomal subunits were identified exclusively in the chronic pancreatitis specimens. Our analysis also identified 139 non-redundant proteins common to all three cohorts, 24 common to only autoimmune pancreatitis and chronic pancreatitis (Supplementary Table 2), 19 common to only autoimmune pancreatitis and pancreatic cancer (Supplementary Table 3) and 49 common to only pancreatic cancer and chronic pancreatitis (Supplementary Table 4).

DISCUSSION

We have successfully applied LC-MS/MS analysis to FFPE tissue of autoimmune pancreatitis, chronic pancreatitis, and pancreatic cancer specimens. In total, we have identified 386 non-redundant proteins among all 9 specimens. By comparing these proteins, we have identified those that were found exclusively in autoimmune pancreatitis (29 proteins), chronic pancreatitis (53 proteins), and pancreatic cancer (73 proteins). A previously published study analyzed pancreatic cancer FFPE tissue using a Liquid Tissue (Expression Pathology, Rockville, MD, USA) workflow for a global proteomics analysis [20]. FFPE tissue processing techniques and mass spectrometry strategies analogous to those which we performed have been used previously to study tissues of other organ systems, including prostate [21], cochlea [14], liver [13, 22], glioblastoma [23], renal carcinoma [24], kidney glomeruli [25], mesenchymal tissue [12], and B-cell lymphoma cells [26], as well as our own recent study comparing pancreatic cancer and chronic pancreatitis [8]. We, however, present the first comparative LC-MS/MS analysis of FFPE pancreas tissue from individuals with autoimmune pancreatitis, chronic pancreatitis, and pancreatic cancer.

Our mass spectrometry-based analysis revealed several proteins as exclusive to specimens from particular cohorts and which may merit further investigation. Genetic and immunologic factors impact the pathogenesis of certain pancreatic diseases [27], and several proteins identified exclusively in a particular disease cohort may directly reflect the presence of that disease process. Histocompatibility antigen (HLA) DRB1-1 beta chain (HLA-DRB1 beta chain) may be correlated to autoimmune pancreatitis. Studies have demonstrated that HLA class II DRB1-1 beta chain allele (HLA-DRB1*0401) may have a protective role in autoimmune pancreatitis [28], while others have investigated allelic polymorphisms in the genes of the major histocompatibility complex (MHC) region, in particular HLA-

DRB1*0401, suggest this allele is a susceptibility factor in patients with chronic pancreatitis [27]. Also identified exclusively in autoimmune pancreatitis, Ig gamma-1 chain (IGHG1) has been shown to down-regulate the cytotoxic activity of natural killer cells through inhibition of antibody-dependent cellular cytotoxicity [29].

Proteins identified exclusively in pancreatic cancer samples included: epiplakin, mucin2 (MUC2), and protein disulfide-isomerase A3. In a mouse model study, epiplakin was found in centroacinar cells and duct cells in the adult pancreas. Its presence has been observed in pancreatic intraepithelial neoplasia, previously identified as pancreatic ductal adenocarcinoma precursor lesions [30]. Mucins are large multifunctional glycoproteins that play an important role in the ductal structures within the pancreas. MUC2 has been shown to be expressed mainly in intraductal papillar mucinous neoplasms (IPMNs), but is occasionally also detected in aggressive pancreatic tumors [31]. Protein disulfide-isomerase A3 (ERp57) is a member of the protein disulfide-isomerase (PDI) family that is involved in the cleavage of disulfide bonds between cysteine residues. This enzyme has been implicated mainly in Alzheimer's disease, but also has been associated with the pathogenesis of cancers, including pancreatic cancer [32]. Hornerin and several ribosomal subunits were identified exclusively in the chronic pancreatitis specimens; however, there is currently no other evidence that these proteins have a role in the development or progression of chronic pancreatitis. Following orthogonal validation, the proteins described above maybe potential diagnostic biomarkers of their respective diseases, as well as targets for directed investigations into disease pathogenesis and progression. Further biological and biochemical studies (e.g., immunohistochemistry using larger cohorts), must be performed to analyze further these selected proteins.

A comparison of frozen verses FFPE tissue would reveal further insight into the utility of FFPE specimens in pancreatic research. However, standardized conditions for frozen tissue are not well established. Such conditions include the initial freezing state (e.g., flash-freezing in liquid nitrogen or dry ice) or procedures for long-term storage (e.g., storage in liquid nitrogen or −80°C). In contrast, FFPE specimens are typically processed with standardized methodologies and are inherently stable at room temperature or below. Studies have also indicated that fresh-frozen tissue do not properly maintain cellular morphology [33, 34]. Moreover, recent studies of follicular lymphoma [11], renal carcinoma [24], ear canal [14], breast cancer [35], and liver [15], have identified similar numbers of proteins using either FFPE tissue or frozen tissue. However, no such comparison has been performed using pancreatic tissue. A systematic study of a statistically significant number of FFPE and frozen pancreatic tissue may provide further evidence as to the benefits of one method over the other for mass spectrometry-based proteomic analyses.

Diagnostic specificity and relatively standardized specimen preservation renders FFPE tissue analysis superior to other organ-specific biomarker discovery strategies that typically analyze body fluids, such as blood derivatives, urine, or proximal fluids [36, 37, 38, 39]. As such, recent efforts have attempted to further standardize collection, handling, and storage of various body fluid specimens [36, 37, 40, 41, 42, 43, 44, 45, 46]. Nevertheless, meaningful inter-laboratory data comparisons have historically been limited due to differences in specimen handling. Moreover, the availability of vast archives of patient samples with

clearly defined clinical histories, diagnoses, and outcomes enhances the robustness and utility of FFPE-based investigations. The value of tissuebased analysis is not limited to biomarker discovery; thorough analysis may also identify therapeutic targets and improve understanding of the molecular basis of pathogenesis, pathophysiology and clinical course. In addition, miRNA and/or mRNA studies can also be performed on FFPE tissue [47, 48, 49, 50]. Data from these studies can be compared to proteomics-based FFPE analysis, providing insight into organ- or diseasespecific translational regulation. However, to achieve the maximum analytical depth, more efficient protein (or peptide) extraction protocols must be developed and potential specimen preparation artifacts, such as amino acid residue modifications, must be reduced or eliminated.

The identification of a greater number of proteins than presented herein would be possible by overcoming procedural limitations. For example, harsh FFPE tissue preparation conditions can limit protein identification. More specifically, irreversible formalin-induced intra- and intermolecular crosslinking in FFPE specimens often hinders the solubility of proteins, complicating the extraction of these proteins and, potentially, larger peptides [51]. During tissue fixation, formalin adds a methylene hydrate group to the side chain of certain amino acids resulting in a crosslinking methylene bridge (both inter- and intra-peptide) which will likely prevent peptide identification [52, 53]. Moreover, chemical modifications of side chain moieties of lysyl, arginyl, tyrosyl, histidyl, and seryl are common during FFPE preparation [33], and further limit protein identifications. In addition, in nucleic acid extraction from FFPE tissue, extensive periods of fixation significantly reduces RNA extraction [54] and typically results in DNA fragmentation [55], and by extension, such effects may also be prevalent in proteins. As FFPE sections generally consist of different cell types, sample heterogeneity may be an issue. If a more homogeneous sample is needed, laser-capture microdissection (LCM) and serial sectioning may be implemented for such analyses [25, 56, 57]. Cellular microdissection decreases sample complexity thereby increasing analytical depth. Improvements in sample fixation and analysis may overcome these obstacles, and greatly increase the depth of the proteomic analysis of wellpreserved FFPE specimens. In addition, future MSbased studies will achieve greater depth of quantitative proteomic analysis by exploiting the higher resolution, high mass accuracy and higher scanning speeds possible with emerging mass spectrometry platforms, such as the Orbitrap Elite [58] and the Q-Exactive [59] (Thermo Scientific, Waltham, MA, USA).

CONCLUSION

We have shown that differentially-expressed proteins can be identified among FFPE tissues specimens originating from individuals with autoimmune pancreatitis, chronic pancreatitis, and pancreatic cancer. With improvements in experimental methods, LC-MS/MS-based proteomic analysis of FFPE pancreatic tissue specimens may provide novel therapeutic targets and/or a means of high-throughput validation of current diagnostic biomarkers. With further investigation, this knowledge may result in methods enabling the discrimination between autoimmune pancreatitis and pancreatic cancer, which represents an unmet medical need. Given the plethora of FFPE specimen archives, large databases of disease-specific proteins may be assembled for validation via orthogonal methods, such as immunohistochemistry studies. The methodology described herein, in addition to the

proteins which we have identified as differentially expressed, may offer a scaffold upon which to build further FFPE-based studies of pancreatic diseases. The work which we present demonstrates the potential of using LCMS/MS for the analysis of archived FFPE pancreatic tissue, particularly to differentiate autoimmune pancreatitis and pancreatic cancer, and provides a basis upon which biomarker studies can be developed further.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Abbreviations

AIP	autoimmune pancreatitis
CP	chronic pancreatitis
DTT	dithiotreitol
FFPE	formalin-fixed paraffin-embedded
LC-MS/MS	liquid chromatography coupled with tandem mass spectrometry
PC	pancreatic cancer

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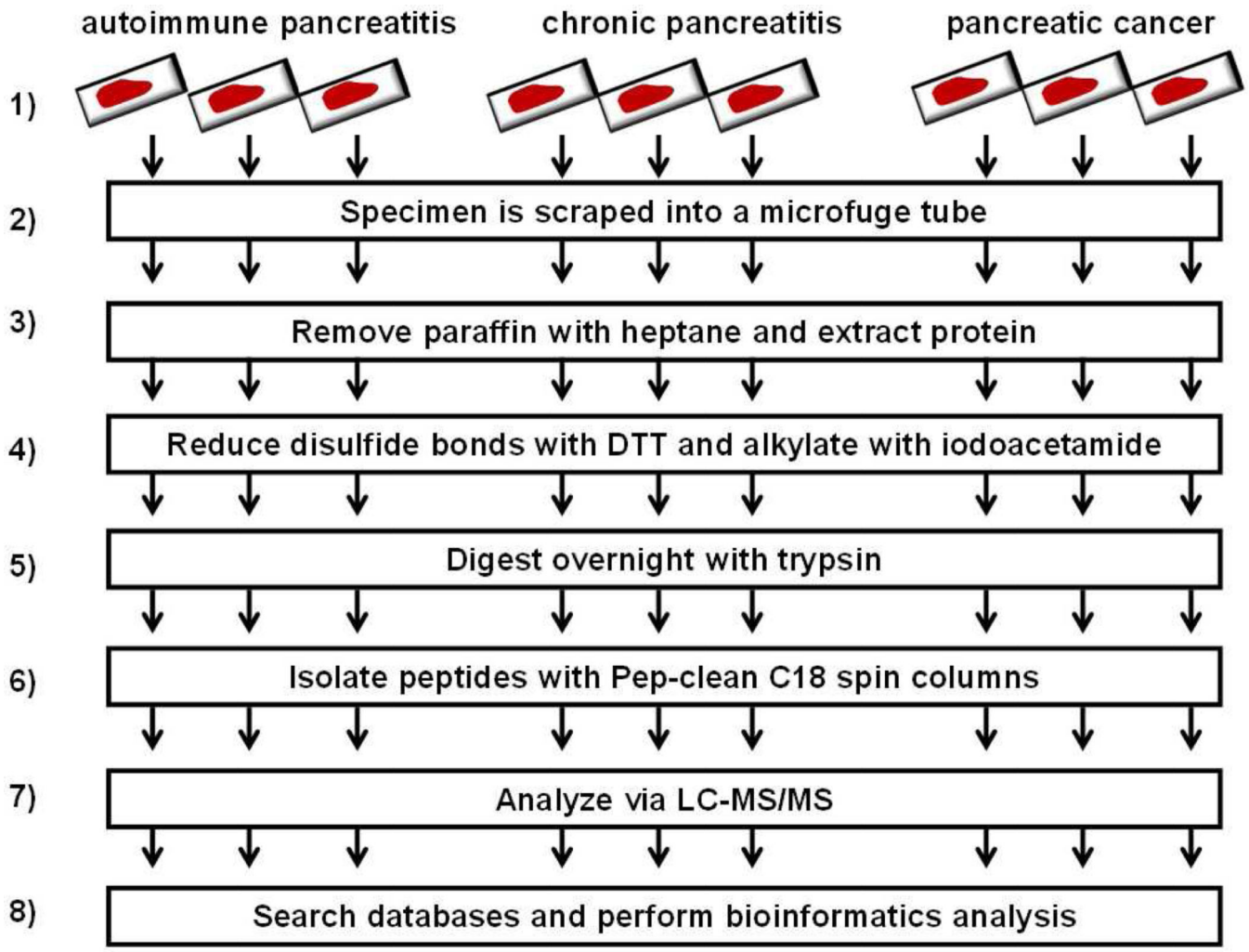

Figure 1.

Experimental workflow. 1) FFPE tissue specimens were obtained; 2) a 1.5 cm 1.5 × 1 cm x 5 μm slice of the specimen was scraped into a microcentrifuge tube; 3) paraffin was removed with heptane; 4) disulfide bonds were reduced with dithiotreitol (DTT) and alkylated with iodoacetamide; 5) specimens were digested overnight with trypsin; 6) peptides were isolated; 7) peptides were analyzed via LC-MS/MS; and 8) bioinformatics analysis was performed.

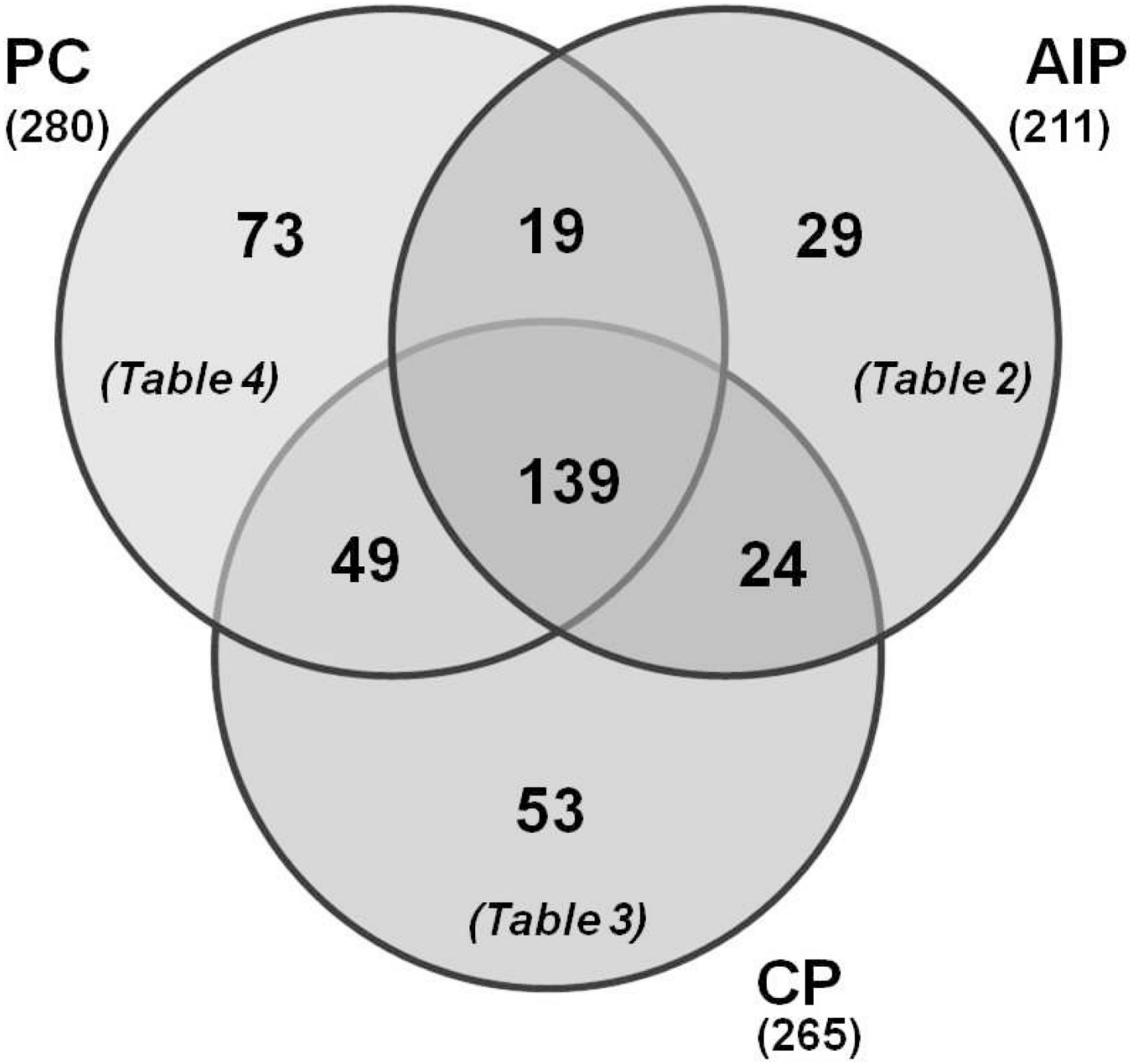

Figure 2.

Distribution of proteins among the three cohorts. Venn diagrams showing unique and overlapping proteins among the three cohorts. Also indicated in the figure are references to the tables in which these sets of proteins are listed.

AIP: autoimmune pancreatitis; CP: chronic pancreatitis; PC: pancreatic cancer

Table 1

Summary of the number of proteins identified from FFPE tissue specimens by cohort. A total of 386 non-redundant proteins were identified in the study.

Cohort	Specimen	No. of non-redundant identified proteins		
		In each specimen	Within each cohort	Exclusive to each cohort
AIP	**#1**	131	211	29
	#2	141		
	#3	103		
CP	**#1**	188	265	53
	#2	138		
	#3	153		
PC	**#1**	150	280	73
	#2	190		
	#3	140		

AIP: autoimmune pancreatitis; CP: chronic pancreatitis; PC: pancreatic cancer

Table 2

Twenty-nine proteins identified exclusively in autoimmune pancreatitis and not in the other two cohorts, as illustrated in the Venn diagram in Figure 2.

Name	UniProt ID	Spectral counts				No. of specimens in which a protein was identified
		AIP#1	AIP#2	AIP#3	Mean	
40S ribosomal protein S16	P62249	0	20	0	7	1
Actin-related protein 2/3 complex subunit 4	P59998	22	0	0	7	1
Adenylate kinase 2, mitochondrial	P54819	15	0	32	16	2
Band 3 anion transport protein	P02730	0	32	0	11	1
BCL2/adenovirus E1B 19 kDa protein-interacting protein 2	Q12982	0	6	0	2	1
Beta-1,4-galactosyltransferase 3	O60512	27	0	0	9	1
Clathrin light chain A	P09496	0	14	0	5	1
Coronin-1A	P31146	15	0	0	5	1
Coronin-1C	Q9ULV4	0	0	41	14	1
Eosinophil peroxidase	P11678	99	0	0	33	1
Heat shock protein beta-6	O14558	0	0	35	12	1
Histone H2A type 1	P0C0S8	0	109	0	36	1
Histone H2A type 1-J	Q99878	96	0	73	56	2
Histone H2B type 1-C/E/F/G/I	P62807	156	0	0	52	1
HLA class I histocompatibility antigen, A-30 alpha chain	P16188	27	0	0	9	1
HLA class II histocompatibility antigen, DRB1-1 beta chain	P04229	32	0	0	11	1
HLA class II histocompatibility antigen, DRB1-11 beta chain	P20039	20	0	0	7	1
Ig gamma-1 chain C region	P01857	0	16	0	5	1
Ig heavy chain V-III region BRO	P01766	0	12	0	4	1
Ig heavy chain V-III region KOL	P01772	0	14	0	5	1
Inner centromere protein	Q9NQS7	67	0	0	22	1
Keratin, type I cuticular Ha3-I	O76009	20	0	0	7	1
Keratin, type II cuticular Hb6	O43790	42	0	0	14	1
Laminin subunit alpha-5	O15230	0	99	0	33	1
Myosin-11	P35749	0	0	185	62	1
Synemin	O15061	0	0	147	49	1
Tropomyosin beta chain	P07951	0	0	88	29	1

Name	UniProt ID	Spectral counts				No. of specimens in which a protein was identified
		AIP#1	AIP#2	AIP#3	Mean	
Tubulin alpha-1B chain	P68363	0	0	32	11	1
Ubiquitin-like modifier-activating enzyme 1	P22314	45	0	0	15	1

AIP: autoimmune pancreatitis

Table 3

Fifty-three proteins identified exclusively in chronic pancreatitis specimens and not in the other two cohorts, as illustrated in the Venn diagram in Figure 2.

Name	UniProt ID	Spectral counts				No. of specimens in which a protein was identified
		CP#1	CP#2	CP#3	Mean	
28S ribosomal protein S29, mitochondrial	P51398	0	0	21	7	1
40S ribosomal protein S26	P62854	0	0	18	6	1
60S ribosomal protein L10	P27635	23	43	0	22	2
60S ribosomal protein L38	P63173	0	0	11	4	1
60S ribosomal protein L5	P46777	29	0	0	10	1
60S ribosomal protein L6	Q02878	61	0	62	41	2
60S ribosomal protein L9	P32969	21	43	14	26	3
AF4/FMR2 family member 4	Q9UHB7	0	0	80	27	1
Annexin A6	P08133	40	0	0	13	1
Antigen peptide transporter 1	Q03518	44	0	0	15	1
Arf-GAP domain and FG repeats-containing protein 1	P52594	11	0	16	9	2
Beta-2-microglobulin	P61769	6	0	0	2	1
BTB/POZ domain-containing protein KCTD12	Q96CX2	15	0	0	5	1
Cathepsin B	P07858	8	0	0	3	1
Coagulation factor XIII A chain	P00488	25	0	0	8	1
Complement C4-B	P0C0L5	0	0	80	27	1
Cytosol aminopeptidase	P28838	27	0	0	9	1
F-actin-capping protein subunit alpha-1	P52907	32	0	0	11	1
F-actin-capping protein subunit alpha-2	P47755	23	0	0	8	1
Glucosidase 2 subunit beta	P14314	48	0	0	16	1
Heat shock-related 70 kDa protein 2	P54652	0	0	48	16	1
Histone H1.0	P07305	0	0	43	14	1
Histone H2A.J	Q9BTM1	0	89	0	30	1
Histone H2B type 1-L	Q99880	0	0	160	53	1
HLA class II histocompatibility antigen, DRB1-15 beta chain	P01911	21	0	0	7	1
HLA class II histocompatibility antigen, DRB1-7 beta chain	P13761	23	0	0	8	1

Name	UniProt ID	Spectral counts				No. of specimens in which a protein was identified
		CP#1	CP#2	CP#3	Mean	
Homerin	Q86YZ3	0	77	68	48	2
Ig heavy chain V-I region EU	P01742	19	0	0	6	1
Ig kappa chain V-I region HK101	P01601	25	0	0	8	1
Ig kappa chain V-III region POM	P01624	15	0	0	5	1
Insulin	P01308	0	22	0	7	1
Junction plakoglobin	P14923	0	43	0	14	1
Lithostathine-1-beta	P48304	0	0	5	2	1
Lysyl oxidase homolog 1	Q08397	0	48	0	16	1
Mimecan	P20774	0	36	0	12	1
Moesin	P26038	29	0	0	10	1
Neurosecretory protein VGF	O15240	0	31	0	10	1
Non-secretory ribonuclease	P10153	0	0	7	2	1
Pancreatic alpha-amylase	P04746	0	29	0	10	1
Pancreatic triacylglycerol lipase	P16233	0	0	27	9	1
Peroxiredoxin-5, mitochondrial	P30044	11	0	0	4	1
Phosphoglycerate mutase 1	P18669	13	0	0	4	1
Probable ATP-dependent RNA helicase DDX17	Q92841	0	0	55	18	1
Protein canopy homolog 2	Q9Y2B0	6	0	0	2	1
Protein disulfide-isomerase A6	Q15084	25	0	0	8	1
Putative 40S ribosomal protein S26-like 1	Q5JNZ5	11	0	0	4	1
Putative 60S ribosomal protein L39-like 5	Q59GN2	8	0	0	3	1
Serum amyloid A protein	P02735	0	0	16	5	1
Serum amyloid P-component	P02743	0	14	0	5	1
Sulfatase-modifying factor 2	Q8NBJ7	0	19	16	12	2
Thioredoxin domain-containing protein 5	Q8NBS9	23	0	0	8	1
Tryptophan-tRNA ligase, cytoplasmic	P23381	13	0	0	4	1
Tubulin alpha-1A chain	Q71U36	0	38	0	13	1

CP: chronic pancreatitis

Table 4

Seventy-tree proteins identified exclusively in pancreatic cancer specimens and not in the other two cohorts, as illustrated in the Venn diagram in Figure 2.

Name	UniProt ID	Spectral counts				No. of specimens in which a protein was identified
		PC#1	PC#2	PC#3	Mean	
14-3-3 protein epsilon	P62258	0	31	0	10	1
40S ribosomal protein S18	P62269	0	27	0	9	1
40S ribosomal protein S24	P62847	15	49	0	21	2
40S ribosomal protein S3	P23396	0	27	0	9	1
40S ribosomal protein SA	P08865	20	25	0	15	2
60S acidic ribosomal protein P0	P05388	0	40	0	13	1
60S acidic ribosomal protein P2	P05387	0	4	0	1	1
60S ribosomal protein L37a	P61513	24	0	0	8	1
Acyl-coenzyme A synthetase ACSM6, mitochondrial	Q6P461	18	0	0	6	1
Aldehyde dehydrogenase family 16 member A1	Q8IZ83	24	0	0	8	1
Aldehyde dehydrogenase, mitochondrial	P05091	22	0	0	7	1
Alpha-1-acid glycoprotein 1	P02763	0	0	12	4	1
Alpha-1-antichymotrypsin	P01011	0	0	16	5	1
Anterior gradient protein 2 homolog	O95994	16	0	0	5	1
Anterior gradient protein 3 homolog	Q8TD06	0	0	5	2	1
Apolipoprotein E	P02649	0	0	28	9	1
Azurocidin	P20160	27	29	0	19	2
Band 4.1-like protein 2	O43491	31	0	0	10	1
Carcinoembryonic antigen-related cell adhesion molecule 5	P06731	0	0	16	5	1
Catalase	P04040	13	0	0	4	1
Catenin alpha-2	P26232	67	0	0	22	1
Chymotrypsin-C	Q99895	0	13	0	4	1
Complement C4-A	P0C0L4	0	0	56	19	1
Complement component C9	P02748	0	0	21	7	1
Dolichyl-diphosphooligosaccharide-protein glycosyltransferase 1	P04843	0	47	0	16	1
ELAV-like protein 1	Q15717	24	0	0	8	1

Name	UniProt ID	Spectral counts				No. of specimens in which a protein was identified
		PC#1	PC#2	PC#3	Mean	
Epiplakin	P58107	165	0	171	112	2
Erythrocyte band 7 integral membrane protein	P27105	0	0	28	9	1
Fatty acid synthase	P49327	45	0	0	15	1
Fatty acid-binding protein, liver	P07148	13	0	0	4	1
Filamin-B	O75369	51	0	0	17	1
Heat shock protein HSP 90-beta	P08238	62	0	0	21	1
Heterogeneous nuclear ribonucleoprotein Q	O60506	0	51	0	17	1
Heterogeneous nuclear ribonucleoproteins C1/C2	P07910	29	0	0	10	1
Histone H2A type 2-A	Q6FI13	0	87	0	29	1
Histone H2A.Z	P0C0S5	34	0	0	11	1
Histone H2B type 1-O	P23527	140	0	0	47	1
Histone H2B type 2-F	Q5QNW6	0	0	185	62	1
HLA class I histocompatibility antigen, Cw-7 alpha chain	P10321	22	0	0	7	1
HLA class II histocompatibility antigen, DRB1-9 beta chain	Q9TQE0	15	0	0	5	1
IgGFc-binding protein	Q9Y6R7	42	0	0	14	1
Importin subunit beta-1	Q14974	22	0	0	7	1
Integrin alpha-M	P11215	0	0	68	23	1
Keratin, type I cuticular Ha6	O76013	31	0	0	10	1
Keratin, type I cytoskeletal 16	P08779	53	0	0	18	1
Keratin, type II cuticular Hb5	P78386	33	0	0	11	1
Keratin, type II cytoskeletal 7	P08729	83	60	105	83	3
Lactotransferrin	P02788	22	0	37	20	2
L-lactate dehydrogenase A chain	P00338	25	0	30	18	2
Mucin-2	Q02817	34	0	0	11	1
Mucin-5B	Q9HC84	0	0	91	30	1
Myeloblastin	P24158	18	0	9	9	2
Myosin-14	Q7Z406	123	0	0	41	1
Nicotinate phosphoribosyltransferase	Q6XQN6	7	0	0	2	1
Outer dense fiber protein 3	Q96PU9	0	0	14	5	1
PDZ and LIM domain protein 1	O00151	18	0	0	6	1

Name	UniProt ID	Spectral counts				No. of specimens in which a protein was identified
		PC#1	PC#2	PC#3	Mean	
Phenylalanine-tRNA ligase alpha chain	Q9Y285	0	22	0	7	1
Protein disulfide-isomerase A3	P30101	31	47	0	26	2
Protein DJ-1	Q99497	0	27	0	9	1
Protein S100-A8	P05109	0	0	9	3	1
Proteolipid protein 2	Q04941	0	4	0	1	1
Putative trypsin-6	Q8NHM4	0	0	47	16	1
Retinal dehydrogenase 1	P00352	0	31	0	10	1
Solute carrier family 12 member 2	P55011	51	0	0	17	1
Spectrin beta chain, brain 1	Q01082	71	0	0	24	1
Staphylococcal nuclease domain-containing protein 1	Q7KZF4	0	65	0	22	1
Superoxide dismutase	P00441	9	0	0	3	1
Translocon-associated protein subunit delta	P51571	0	9	0	3	1
Transthyretin	P02766	0	0	9	3	1
Tripeptidyl-peptidase 1	O14773	11	0	19	10	2
Trypsin-3	P35030	0	63	0	21	1
Tubulin beta-4B chain	P68371	45	25	0	23	2
Tubulin-specific chaperone A	O75347	9	0	0	3	1

PC: pancreatic cancer