Laser Capture Microdissection of Pancreatic Acinar Cells to Identify Proteomic Alterations in a Murine Model of Caerulein-Induced Pancreatitis

John P. Shapiro, MS[1,8], Hannah M. Komar, BS[2,8], Baris Hancioglu, PhD[3], Lianbo Yu, PhD[4], Ming Jin, MD, PhD[5], Yuko Ogata, PhD[6], Phil A. Hart, MD[1], Zobeida Cruz-Monserrate, PhD[1,2], Gregory B. Lesinski, PhD, MPH[7] and Darwin L. Conwell, MD, MS[1]

OBJECTIVES: Chronic pancreatitis (CP) is characterized by inflammation and fibrosis of the pancreas, leading to pain, parenchymal damage, and loss of exocrine and endocrine function. There are currently no curative therapies; diagnosis remains difficult and aspects of pathogenesis remain unclear. Thus, there is a need to identify novel biomarkers to improve diagnosis and understand pathophysiology. We hypothesize that pancreatic acinar regions contain proteomic signatures relevant to disease processes, including secreted proteins that could be detected in biofluids.
METHODS: Acini from pancreata of mice injected with or without caerulein were collected using laser capture microdissection followed by mass spectrometry analysis. This protocol enabled high-throughput analysis that captured altered protein expression throughout the stages of CP.
RESULTS: Over 2,900 proteins were identified, whereas 331 were significantly changed $\geq$ 2-fold by mass spectrometry spectral count analysis. Consistent with pathogenesis, we observed increases in proteins related to fibrosis (e.g., collagen, $P < 0.001$), several proteases (e.g., trypsin 1, $P < 0.001$), and altered expression of proteins associated with diminished pancreas function (e.g., lipase, amylase, $P < 0.05$). In comparison with proteomic data from a public data set of CP patients, a significant correlation was observed between proteomic changes in tissue from both the caerulein model and CP patients ($r = 0.725$, $P < 0.001$).
CONCLUSIONS: This study illustrates the ability to characterize proteome changes of acinar cells isolated from pancreata of caerulein-treated mice and demonstrates a relationship between signatures from murine and human CP.
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INTRODUCTION

Chronic pancreatitis (CP) is a debilitating condition characterized by chronic inflammation and progressive scarring, leading to abdominal pain, irreversible damage to the pancreas, and the loss of its exocrine and endocrine function.[1] To date, there are no curative therapies or approaches to dampen or reverse the inflammatory response associated with CP. Disease management for these patients is focused primarily on screening for and managing disease-related complications, including abdominal pain, diabetes mellitus (due to endocrine failure), malnutrition (due to exocrine failure), and metabolic bone disease.[2]

Although CP can be easily diagnosed at an advanced stage of disease, the damage to the organ and loss of function are irreversible at this stage.[3] Rather, there is a clinical need to diagnose the disease in an early stage and modify risk factors to prevent disease progression. Unfortunately, there are currently no accurate disease biomarkers or morphologic features of disease for the early stage of CP. Similarly, little is known about the molecular changes that take place during the onset and early stages of CP. As such, coupling in vivo models with analytic methods such as mass spectrometry may be useful to not only understand the disease pathogenesis but also to identify potential biomarkers for early CP. Once characterized, these biomarkers may allow for early diagnosis and monitoring of medical/surgical intervention(s) in CP patients. Mass spectrometry has been used in several studies to investigate the molecular physiology underlying pancreatic diseases. For instance, several studies have examined clinical biopsies collected from patients with pancreatic ductal adenocarcinoma (PDAC) using mass spectrometry, and characterized the proteome changes that occur with this disease. This includes studies that examined whole tissue extracts of normal vs. PDAC[4–7] as well as those that used a more sophisticated approach, by combining laser capture microdissection and mass spectrometry.[8–11] These studies offer insight to the underlying mechanism of disease

[1]Department of Internal Medicine, Division of Gastroenterology, Hepatology, and Nutrition, Wexner Medical Center, The Ohio State University, Columbus, Ohio, USA; [2]Comprehensive Cancer Center, The Arthur G. James Cancer Hospital and Richard J. Solove Research Institute, Columbus, Ohio, USA; [3]Department of Biomedical Informatics, Columbus, Ohio, USA; [4]Center for Biostatistics, Wexner Medical Center, The Ohio State University, Columbus, Ohio, USA; [5]Department of Pathology, Wexner Medical Center, The Ohio State University, Columbus, Ohio, USA; [6]The Fred Hutchinson Cancer Research Center Proteomics Core, Seattle, Washington, USA and [7]Department of Hematology and Medical Oncology, Winship Cancer Institute of Emory University, Atlanta, Georgia, USA
Correspondence: Darwin L. Conwell, MD, MS, Department of Internal Medicine, Division of Gastroenterology, Hepatology and Nutrition, Wexner Medical Center, The Ohio State University, 410 West 10th Avenue, Room 288A, Faculty Office Tower, Columbus, Ohio 43210, USA. E-mail: Darwin.Conwell@osumc.edu
[8]These authors contributed equally to this work.
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development and identified several candidate proteins that may serve as useful early indicators of PDAC.

Mass spectrometry has also been used to characterize changes in protein expression that are associated with pancreatitis. Several reports have examined proteome changes in animal models of (acute) pancreatitis following administration of caerulein. These include studies that examined proteins from whole tissue extracts[12,13] and those characterizing effects of caerulein on proteome changes in isolated rough endoplasmic reticulum.[14] Similar studies have used mass spectrometry to examine pancreatitis in human biopsies.[5,6,15] Although these reports identified many changes and offered a broad portrait of proteomic alterations during the generation of this disease, our understanding of precise mechanisms leading to CP remain unclear.

Several *in vivo* models of CP have been established. Each model has strengths and weaknesses related to their ability to approximate the etiology and pathogenesis of the human condition. Some models use exogenous sensitizing and inflammatory agents, such as ethanol feeding, combined with administration of lipopolyssacharide. Other models require injection of pancreatic toxins, such as L-arginine or dibutyltin dichloride.[16] However, these agents induce pancreatitis via unknown mechanisms of action and produce abnormal pathophysiology accompanied by nonspecific damage outside the pancreas organ site. Genetically modified mice carrying deletions or mutations in clinically relevant genes including *CFTR*, *SPINK3*, and *PRSS1* have also been integrated to approximate hereditary pancreatitis. However, these models often require additional inflammatory stimuli and do not accurately recapitulate human pathology.[17]

To date, caerulein-induced CP remains the most commonly used murine model with a well-characterized pathology that approximates inflammation, acinar loss, and fibrosis in human disease. The major limitation of this model is its reversible nature upon cessation of caerulein administration. However, this model is useful in understanding pancreatic changes that occur in acinar, islet, and fibrotic regions of the damaged pancreas. Repeated administration of supramaximal doses of the cholecystokinin analog caerulein causes overproduction and accumulation of digestive enzymes within pancreatic acinar cells. These enzymes autoactivate, leading to tissue autodigestion with accompanying inflammation and fibrosis similar to human CP.[16,18] As such, this model has been well accepted for studying basic inflammatory changes in the pancreas in the context of acute and CP, as well as in the acceleration of pancreatic carcinogenesis in genetically predisposed PDAC models.

In the present study, pancreata from mice with caerulein-induced pancreatitis were isolated and subjected to laser capture microdissection (LCM) to identify proteomic alterations in the acini. Our focus was on the acinar cell compartment given the secretory nature of these cells, which could increase the probability of identifying potential biomarkers of a diseased pancreas that could be validated in subsequent studies. We hypothesized that this methodology would be useful to identify secreted proteins in the acinar cells; identified proteins would be potential targets for future studies to assess diagnostic utility using pancreatitis biological specimens from humans.

METHODS

Caerulein-induced model of pancreatitis. Female C57BL/6 mice aged 4–6 weeks were injected hourly with phosphate-buffered saline (PBS) (control) or 50 μg/kg caerulein (Sigma-Aldrich, St Louis, MO) 6 times per day, 3 days per week for 1, 3, or 6 weeks ($n = 3$ per group). At the study end point for each group, mice were killed 3 days after the final caerulein injection. Whole blood was collected for the analysis of serum amylase and lipase. Pancreas tissue was formalin-fixed overnight and paraffin-embedded for histopathologic analyses. All specimens were batch processed to minimize variability.

Immunohistochemical analysis. Formalin-fixed paraffin-embedded (FFPE) pancreas tissue from mice was analyzed by immunohistochemistry using hematoxylin and eosin and Mason's trichrome stains according to standard laboratory protocols. All specimens were batch processed to minimize variability.

Analysis of blood. Serum was collected following centrifugation of freshly isolated from whole blood of mice and immediately subjected to biochemical analysis by the Alfa Wassermann VetAce analyzer (Alfa Wassermann Diagnostics, West Caldwell, NJ) to measure levels of circulating amylase and lipase. This analysis was performed in the Comparative Pathology and Mouse Phenotyping Shared Resource veterinary biosciences clinical laboratory.[19]

Sample preparation for LCM. Sections from paraffin blocks were cut at 10 μm and wet-mounted on Zeiss PEN membrane slides in a protease-free container with mass spectrometry grade H_2O at 42 °C. Sections on slides were completely dried for 1 week in a desiccant containing desiccator under vacuum to help avoid sections releasing from slides during depariffinization. Dried sections were deparaffinized and stained in a LockMailer microscope mailer and staining jars (Ted Pella, Redding, CA) using the following procedure keeping slides wet at all times: (1) three changes of octane, followed by three changes of 200 proof ethanol, (2) single changes in 90 and 70% ethanol (made with mass spectrometry grade H_2O), and then finally two changes of mass spectrometry grade H_2O—all steps performed 2 min each. Sections were briefly stained with hematoxylin (Vector Laboratories, Burlingam, CA) for 3 s, followed by immediate washes in two changes of mass spec grade H_2O. Sections were then treated with 2 min changes each in 70%, then 90% ethanol, followed by two each 2 min changes in 100% ethanol. Slides were removed from ethanol and air dried for laser capture microdissection.

Laser capture microdissection. LCM was performed using the laser microdissection system from PALM Technologies (Carl Zeiss Micro Imaging GmbH, Munchen, Germany) containing a PALM MicroBeam and RoboStage for high-throughput sample collection and a PALM RoboMover (PALM Robo software, version 2.2, Carl Zeiss, Microimaging GmbH, Munchen, Germany). Typical settings used for laser cutting were UV-Energy of 75–85 and UV-Focus of 52. Sections

were cut and captured under a ×10 ocular lens. Cut elements were catapulted into 25 μl of 0.5% Rapigest (Waters Corporation, Milford, MA) resuspended in 50 mM NH_3HCO_3. Total number of cells captured was determined using the conversion factor of 50,000 $\mu m^2 = 200$ cells for acinar cells or by assuming an average cell diameter of 11 μm for acinar cells. Upon completion of microdissection, the captured material was spun down into a 0.2 ml PCR tube and held at −80 °C until protein retrieval.

Protein extraction and digestion. Samples that had been stored at −80 °C were thawed briefly and boiled (in the original PCR tube) for 20 min, followed by a 2 h incubation in a 60 °C water bath for protein recovery.[20] Trypsin was added in a ratio of 1:30 trypsin:protein (no. V511A; Promega, Madison, WI) assuming ∼2 μg retrieved protein/10,000 isolated cells. After overnight incubation at 37 °C, formic acid was added to a final concentration of 30–40% until the solution clouded after ∼1 min room temperature incubation. This suspension was incubated for 30 min at 37 °C to completely precipitate the Rapigest. The samples were centrifuged 3× at 15,000 r.p.m. at 4 °C for 10 min to eliminate cellular debris and the precipitated Rapigest from the sample. The extracts were dried in a speedvac to dryness and the dried peptides were resuspended in 20 μl solution of 2% acetonitrile with 0.1% formic acid and then sonicated for 1–2 min in a water bath sonicator at 4 °C to ensure peptide solubilization. Final peptide concentrations were controlled by measurement of 280 nm absorbance to obtain peptide concentration in the tryptic digest on a Nanodrop ND-1000 spectrometer (Thermo Fisher, Waltham, MA).

Mass spectrometry and data analysis. Liquid chromatography tandem-mass spectrometry (LC-MS/MS) analysis was performed with an Easy-nLC 1000 coupled to an Orbitrap Fusion mass spectrometer (Thermo Scientific, Waltham, MA). The LC system configured in a vented format[21] consisted of a fused-silica nanospray needle (PicoTip emitter, 75 μm inner diameter) (New Objective, Woburn, MA) packed in-house with 25 cm Magic C18 AQ 100 Å reverse-phase media (Michrom Bioresources, Auburn, CA) and a trap (IntegraFrit Capillary, 100 μm inner diameter) (New Objective, Woburn, MA) containing Magic C18 AQ 200 Å (2 cm). A measure of 1.5 μg of peptides were loaded onto the column from each sample and separated using a two-mobile-phase system consisting of 0.1% formic acid in water (A) and 0.1% formic acid in acetonitrile (B). The chromatographic separation was achieved over a 163-min gradient from 2 to 50% B (5–30% B for 150 min, 30–50% B for 10 min, and 50% B for 3 min) at a flow rate of 300 nl/min. The mass spectrometer was operated in a data-dependent MS/MS mode over the *m/z* range of 400–1,500. The mass resolution was set to 120,000. The automatic gain control target for the orbitrap was set to 2×10^5 with an injection time of 50 ms. For MS^2, the quadrupole was used for isolation with a window of 1.6 *m/z*. The cycle time was set to 3 s, and the most abundant ions from the precursor scan with a charge state between 2 and 6 were selected for MS/MS analysis using 27% normalized HCD collision energy and analyzed with an ion trap. The automatic

gain control target for the ion trap was set to 1×10^4 with an injection time of 90 ms. Selected ions were dynamically excluded for 20 s.

Data analysis was performed using Proteome Discoverer 2.1 (Thermo Scientific). The data were searched against Mus musculus protein database downloaded on 14 March 2014 from Uniprot (http://www.uniprot.org/), which included additional common contaminants. Trypsin was set as the enzyme with maximum missed cleavages set to 2. The precursor ion tolerance was set to 10 p.p.m., and the fragment ion tolerance was set to 0.6 Da. Variable modifications were set to carbamidomethyl on cysteine residues, and oxidation of methionine residues. SEQUEST[22] search results were run through Percolator[23] for scoring. Identified peptides were filtered by a 1% peptide-level false discovery rate using *q* value of 0.01 from Percolator. In addition, false discovery rate at the protein level was calculated, and only the proteins with false discovery rate ≤1% are reported.

Comparison of caerulein mouse vs. human CP proteome changes. We compared our data with those from a previously published MS study using archived FFPE human biopsies from mild ($n = 5$) and severe ($n = 5$) CP and normal subjects ($n = 5$).[6] The method used 15-μm-thick (macrodissected) pancreatic sections for LC-MS/MSMS-generated peptide ion intensities to produce the ratio of normal vs. mild or severe pancreatitis protein expression. For the comparison described here, the correlation of the log(2) of the ratio (normal vs. diseased) between mouse and human data was assessed by the Pearson's correlation coefficient. A simple linear regression line was fitted to the data points.

Statistical analysis. Five groups of three mice (non-injected controls, 6-week PBS-injected controls, 1 week with caerulein, 3 weeks with caerulein, and 6 weeks with caerulein) were studied and compared for difference in protein spectral (peptide) counts obtained using LC-MS/MS technology. Proteins with <3 spectral counts for at least two mice of each group were filtered out, and normalization was applied to the observed spectral counts toward the common library size. It is assumed that the spectral count data follows negative binomial distribution.[24] To improve the estimate of overdispersion and statistical test for differential expression, R package DESeq2 was used to estimate the smoothed overdispersion parameters and calculate *P* values with Wald test for each group pair comparison under a generalized linear model.[25] A linear trend test was also performed over the three time points (1, 3, and 6 weeks) with caerulein treatment. The *P* value cutoffs were determined by controlling the mean number of false positives.

RESULTS

Caerulein-induced CP recapitulates many histologic and physiologic features of human disease. Mice treated with supramaximal doses of caerulein exhibit a progressive inflammatory and fibrotic disease similar to the natural course of human CP (Figure 1a–d). Analysis of pancreatic tissue demonstrated inflammatory cell infiltrates and loss of acinar
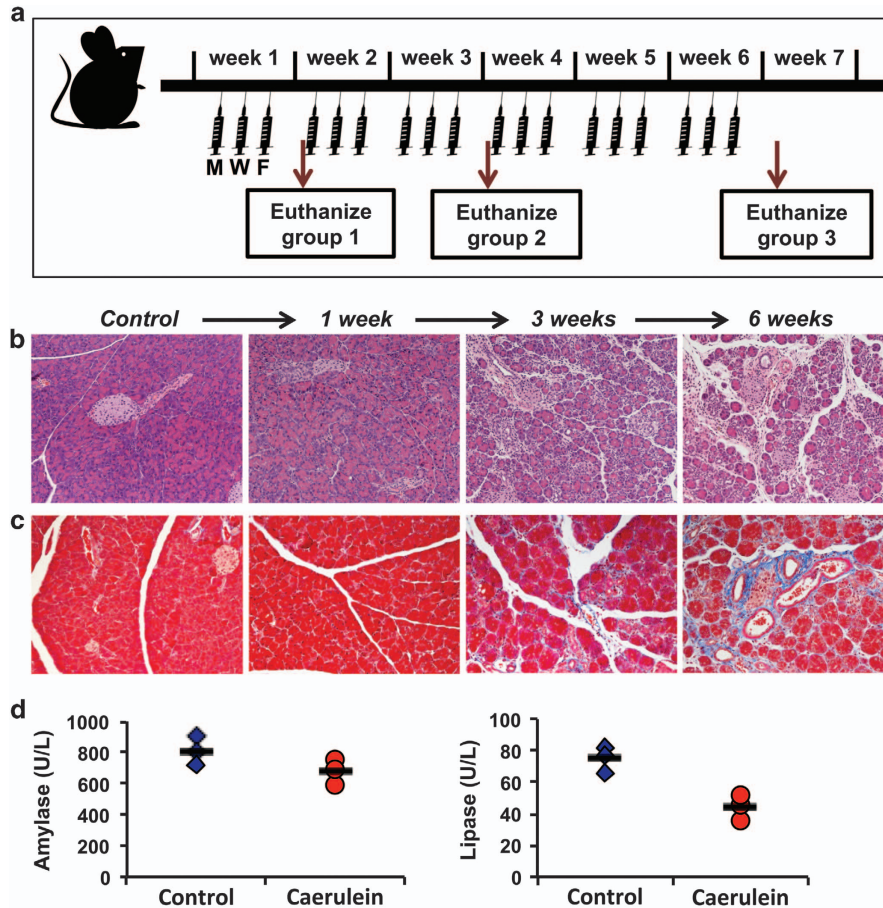
Figure 1 (**a**) Regimen for induction of pancreatitis via caerulein injection. (**b**) Pancreata of caerulein or phosphate-buffered saline (PBS)-injected (control) mice from each time-point were stained with hematoxylin and eosin (H&E) or (**c**) Masson's trichrome and imaged at ×20 magnification. (**d**) Serum amylase and lipase are reduced in 6-week caerulein-treated mice compared with control ($n = 3$). Bars represent mean of each group.
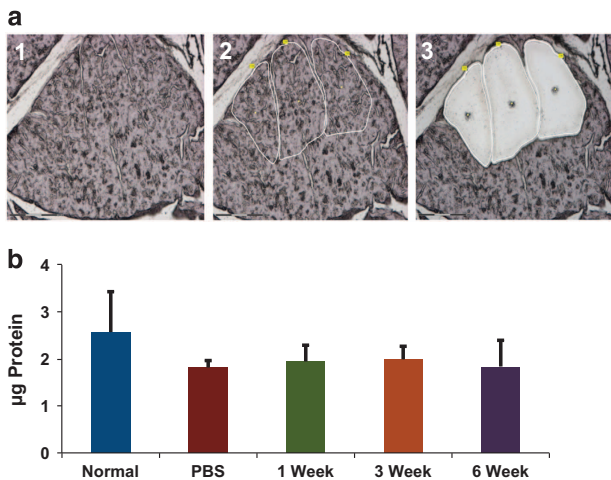
Figure 2 (**a**) Images demonstrating collection of pancreas acinar cells by laser capture microdissection. (1) Precollection, (2) outline of area to be collected and (3) post laser capture microdissection showing outline of area of acinar cells collected. (**b**) Average ± s.d. of micrograms of protein recovered from 10,000 acinar cells. Protein concentration was determined by Nanodrop 280 absorbance (NanoDrop, Wilmington, DE). Data shown from LCM acinar cells isolated from $n = 3$ each of untreated normal, PBS control, and 1-, 3-, and 6-week animals presumes acinar cell diameter = 11 μm.

cells, which progress over time with continued administration of caerulein (Figure 1b). The presence of collagen, indicated by blue Mason's Trichrome staining, demonstrated an increase in fibrosis over time (Figure 1c). Furthermore, serum levels of the pancreatic enzymes amylase and lipase were reduced in caerulein-treated mice compared with control, as is observed in human CP (Figure 1d).

Efficient recovery of protein from LCM FFPE pancreatic tissue. To better understand the changes occurring during caerulein treatment, we characterized acinar cells isolated from formalin-fixed pancreas biopsies using a combination of LCM and LC-MS/MS. The application of these methods for characterizing the proteome of acinar cells in the caerulein mouse model of pancreatitis is a novel approach to understanding the underlying molecular physiology of this model. Using this method, the ability to efficiently recover protein from the FFPE biopsies is critical for reliability and reproducibility in proteomic studies using tissue fixed with formalin. The precision of the LCM excision of acinar regions from pancreas tissue is demonstrated in Figure 2a. The amount of protein recovered from acinar cells isolated using LCM equal to 1 mm² (~10,000 cells presuming an acinar cell radius of 11 μm) was on average 2.03 μg. In general, tryptic digestion

of proteins from 10^6 cells yields ~200–300 μg protein (or 2–3 μg/10,000 cells).[26] Therefore, we achieved ~66–100% efficiency of recovery of available protein from the LCM isolates. Figure 2b shows averages (μg) recovered s.d. for $n=3$ normal, PBS and 1, 3, and 6 weeks mice.

In terms of assay performance, the LC-MS/MS analysis of the acinar cell LCM isolates consistently detected on average over 21,000 peptides for each sample run. Supplementary Table 1 describes details of the peptides that were detected by the mass spectrometer from all analyzed samples. Overall, 2,995 proteins were identified in this study (false discovery rate <1%) using LC-MS/MS to analyze the LCM isolated pancreas tissue. Statistical analysis of the spectral count data of the LC-MS/MS for identification of candidate proteins that are differentially regulated between caerulein and control groups resulted in 134, 303, and 331 proteins that were significantly changed by >2-fold in the 1-, 3-, and 6-week-treated animals, respectively (P value <0.05). Supplementary Table 2 shows protein spectral count data from all proteins identified by the mass spectrometer for each of the analyzed samples.

Characterization of proteins identified by LC-MS/MS of pancreatic acinar cells. Consistent with the pathophysiology of the caerulein model, examination of the MS data indicated that several proteins typically associated with the presence of fibrosis were significantly increased in LCM isolated acinar sections from caerulein-treated mice. Among these were seven isotypes of collagen that were increased ($P<0.001$ for all seven isotypes at 6 weeks and significantly increased at the majority of other time points) in the caerulein-treated mice compared with vehicle controls (Figure 3a). The mass spectrometer identified 18, 10, 7, 8, 15, 14, and 39 unique peptides for the collagen isotypes col6a1, col1a2, col1a1, col12a1, col6a2, col6a6, and col14a1, respectively. These data were consistent with the immunohistochemistry collagen staining completed on these same tissues (Figure 1c). Particular isotypes such as collagen 14 α1 were increased 28-fold after 6 weeks of caerulein treatment. Additionally, several other proteins associated with fibrosis, including the four different laminin isotypes Lamc1, Lama2, Lamb2, and Lama5 ($P<0.05$) as well as fibronectin ($P<0.001$ at 3 and 6 weeks and <0.05 at 1 week) were significantly increased in tissues from caerulein-treated mice (Figure 3b). The mass spectrometer identified 29, 49, 22, and 6 unique peptides for the laminin isotypes Lamc1, Lama2, Lamb2, and Lama5, respectively.

Another hallmark of CP is the diminished function of the acinar cells during progression of the disease. Consistent with this, reduced lipase and amylase were noted in the blood of caerulein-treated mice (Figure 1d). LC-MS/MS peptide profiles also indicated significant reduction of these proteins in the pancreas of caerulein-treated mice ($P<0.05$; Figure 4a).

Although the role of proteases in the development of CP is not firmly established, many studies suggest their participation in the generation of disease pathophysiology. This effect results from excess trypsin activity, either from overexpressed trypsin or underexpressed trypsin inhibitors leading to cellular autodigestion and subsequent inflammation.[27] Analysis of the LC-MS/MS data demonstrated three proteases (trypsin 1; chymotrypsin B; trypsinogen 4) that were expressed at
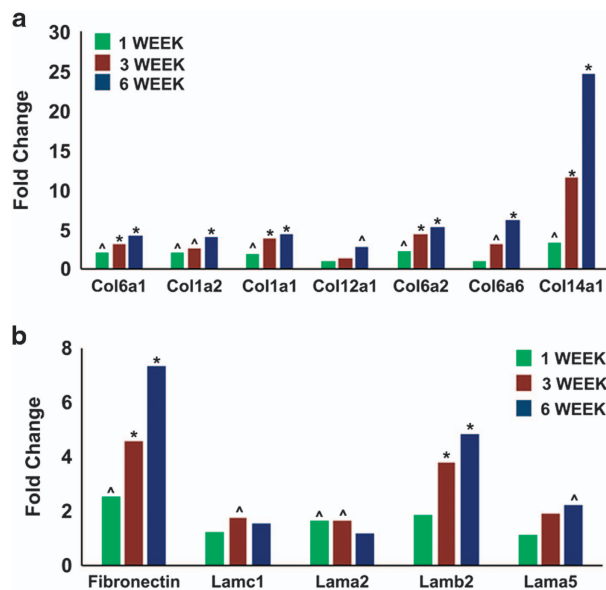
Figure 3 (**a**) Fold changes of collagen isotypes following 1, 3, and 6 weeks of caerulein injections vs. phosphate-buffered saline (PBS) control provided by quantitative analysis of spectral counts. (**b**) Fold changes of fibronectin and laminin isotypes following 1, 3, and 6 weeks of caerulein injections vs. PBS control provided by quantitative analysis of spectral counts. (*$P<0.001$ and ^$P<0.05$).
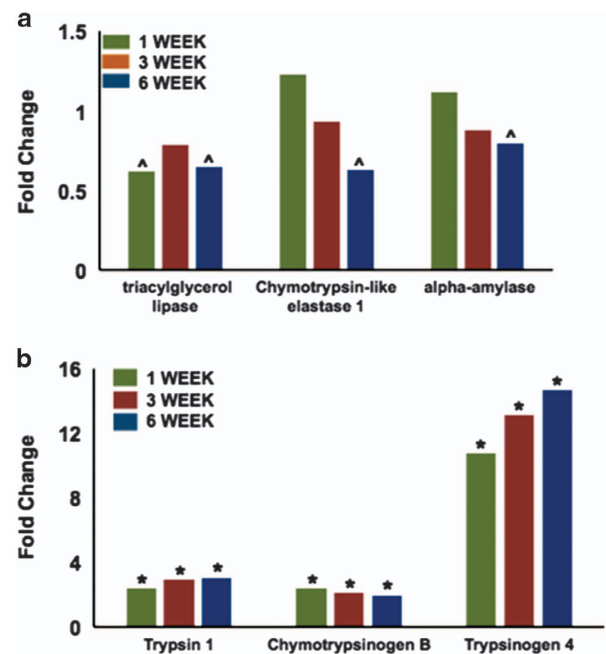
Figure 4 (**a**) Fold change of three pancreatic enzymes following 1, 3, and 6 weeks of caerulein injection vs. phosphate-buffered saline (PBS) control provided by quantitative analysis of spectral counts. (^$P<0.05$). (**b**) Fold change of three trypsin proteins following 1, 3, and 6 weeks of caerulein injection vs. PBS control provided by quantitative analysis of spectral counts (*$P<0.001$).

significantly higher levels after caerulein treatment ($P<0.001$; Figure 4b). Of these proteins, trypsinogen 4, which is a homolog to human mesotrypsingen, showed marked upregulation following a single week of caerulein treatment (~11-fold increase) and was further increased to 15-fold at 6 weeks.

Relationship between proteomic signatures in caerulein-induced pancreatitis with human disease. To confirm the clinical relevance of the peptide signatures identified in the caerulein model, our data were compared with a previously published study characterizing the proteome changes that occur in human CP. Importantly, although the tissue in this study was not subjected to LCM, it was processed in a method similar to that reported here for protein extraction. A comparison of these two LC-MS/MS identified proteome data sets revealed significant correlation in the fold change detected in the 252 common proteins (Pearson's $R = 0.725$; $P < 0.0001$; Figure 5).

DISCUSSION

We describe a robust method that allows for reproducible quantitative mass spectrometry analysis of proteins isolated via LCM of FFPE biopsies. This method was highly efficient requiring only 10,000 cells. In this proof-of-principle study, LCM was used to isolate acinar cells from the pancreas of mice with caerulein-induced pancreatitis. This well-characterized animal model provided a surrogate to demonstrate that the LCM-MS/MS method was robust and capable of identifying canonical proteomic alterations relevant to the pathogenesis of acute and CP. Thus, the LCM-MS/MS methodology represents a viable platform that could be applied to better understand disease pathogenesis and biomarker discovery.

The ability to characterize the proteome from archived FFPE tissue represents an important technical advance. In theory, this approach could allow for an abundance of high-quality, retrospective analyses of proteomic data across various disease states for which archival tissue is available. To date, the ability to characterize gene expression using FFPE biopsies has significantly advanced owing to innovative platforms and methods such as Nanostring, RNA seq, among others.[28] In contrast, the ability to characterize quantitatively protein expression from these same types of FFPE tissue samples
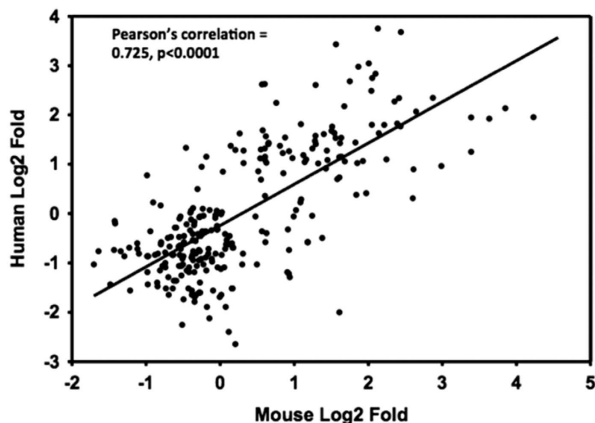
Figure 5 Comparison of 6-week caerulein mouse proteomic data vs. human proteomic data that characterized chronic pancreatitis using liquid chromatography tandem-mass spectrometry (LC-MS/MS) analysis of proteins retrieved from formalin-fixed paraffin-embedded (FFPE) biopsies. The correlation between human and mouse protein expression was assessed by generation of Pearson's correlation coefficient using a comparison (of the log 2) of ratios of pancreatitis vs. normal expression of all common proteins in the two data sets. A simple linear regression line was fitted to the data points.

has been more limited. One particular difficulty associated with mass spectrometry proteome analysis of LCM isolates from archived FFPE biopsies has been suboptimal protein/peptide quality.[29] This factor can lead to a limited ability to derive meaningful biologic data from these analyses. Additionally, inefficient protein recovery becomes more labor intensive, requiring greater instrument usage and ultimately increased cost of analysis. Taken together, these limitations have reduced enthusiasm for improving upon proteomic analysis of FFPE tissue for widespread use in the field. The technology described in this study provides a means of overcoming these limitations and allows for effective FFPE tissue proteomic analysis.

There remains a large, unmet need for improvements in the diagnostic modalities for CP. Since the morphologic changes that are specific for this diagnosis occur late in the disease course, there is especially a need for biomarkers of early CP. The accuracy of available tests to detect early or intermediate CP is poor.[3] Thus, these biomarkers would be clinically relevant for use in patients with a consistent clinical history, risk factors, and/or genetic predisposition for CP. The early diagnosis of CP would permit more aggressive life style modifications, as well as the opportunity to study mechanisms of disease progression and therapeutic interventions to retard disease progression. Ultimately, the goal would be to prevent complication, including pain, malnutrition, and endocrine and exocrine failure due to the effects of pancreatic inflammation and fibrosis. The use of tissue from animal models of early CP as well as tissue specimens from patients at various stages of disease will also permit examination of the changes in protein expression during disease progression. Furthermore, this methodology may help to further understand the pathogenesis in the subset of patients with CP who progress to PDAC.

We propose that the LC-MS/MS analysis platform validated in this study may be of further use in applications of clinical relevance to CP. First, the use of this method could help identify novel biomarkers related to pathogenesis of the disease. Although our report used murine samples, its applicability to FFPE tissue indicates it is a highly versatile method that can also be used effectively on human tissue. For example, it is plausible that analysis of acinar regions might uncover secreted proteomic signatures that can be tested as potential biomarkers in urine or pancreatic secretions from patients. Similarly, this method could also be applied to other regions of the pancreas including islets, stroma or ducts, and to other conditions given the widespread availability of archived FFPE biopsies. Finally, proteomic biomarkers identified from FFPE tissue could be validated as a potential diagnostic marker by comparison with proteins in pancreatic fluid secretions, which can be obtained via endoscopic procedures.[30]

Use of the well-characterized caerulein model of pancreatitis allowed for validation of proteomic signatures that might be expected in an inflammatory state that has relevance to human disease. Indeed, it was reassuring that the LC-MS/MS approach confirmed many of the well-known changes that occur during the progression of pancreatitis. This includes detection of the enhanced expression of proteases, the diminished expression of digestive enzymes such as amylase and lipase (reflecting the poorly functioning organ), and the presence of proteins indicative of the fibrotic process that is known to accompany the progression of the disease.[31] This

reliable identification of these expected changes in protein expression detected by the mass spectrometer supports the ability of the described method to characterize the proteome in an FFPE biopsy from LCM isolated tissue.

Animal models are often used to better understand the underlying physiology in correlating human disease. Unfortunately, the reliability of these models to characterize accurately the human disease often leads to conclusions determined to be non-relevant in humans.[32,33] The comparison of the caerulein mouse to human CP proteomic data described here and the ensuing correlation (comparing 252 common proteins) substantiates the caerulein mouse as one model that retains some relevance to understanding the pathophysiological mechanisms of CP.[6] Additionally, this comparison further demonstrates the capability of the methods described herein to generate information relevant to disease physiology.

Since this represents a proof-of-principle study, it is not without limitations related to the approach. First, this report used a single model of pancreatic disease. Although this model is the most widely used and well-characterized murine model of CP, it requires continued delivery of exogenous supramaximal cholecystokinin analog (3 days per week) to initiate and maintain pancreatitis.[34] Therefore, although the phenotype appears histologically similar to human CP, the mechanism of disease is distinct. Indeed, supramaximal cholecystokinin itself may alter expression of some acinar proteins.[35] The caerulein model also has the inherent disadvantage of being reversible upon cessation of caerulein. However, the relatively short-term nature of treatment in this study should negate this concern. Second, if eventually this technique was applied to archival clinical samples, there may be variability in the methods by which tissues will have been preserved. Similarly, although this method heavily enriches for acinar cells, it is not by definition completely "pure" in its isolation capabilities. This could affect results, and thus any study will require extensive numbers to ensure training and validation cohorts for potential biomarkers are feasible. Third, from a technical perspective, the abundance of enzymatic activity present within pancreatic tissue may result in data that is challenging to interpret. Although this is possible, we were able to recover meaningful proteomic signatures in the present study that reflected many known pathogenic mechanisms of disease. These data suggest that quality data on peptide signatures can still be obtained, in spite of the presence of a cellular environment in the pancreas that includes abundant protein degrading proteases.

Overall, this study represents an advance that leverages proteomics to improve our capability to analyze archival tissue specimens. Future application of this technology to clinical samples will inform our understanding of pathogenesis, and aid in identifying new biomarkers related to diagnosis and treatment of pancreatic diseases.

CONFLICT OF INTEREST

Study Highlights

WHAT IS CURRENT KNOWLEDGE

✓ There are no curative therapies for chronic pancreatitis (CP).

✓ Pathogenesis and biomarkers of disease in CP are not well characterized. As such, diagnosis remains difficult.

✓ Previous proteomic analysis from animal and human tissue suggests significant changes in protein expression during CP.

WHAT IS NEW HERE

✓ Laser capture microdissection followed by mass spectrometry facilitates characterization of proteomic changes in specific cellular compartments of the pancreas, such as acinar cells.

✓ Three hundred and seventy proteins demonstrated significant changes in expression that correlated with previous data obtained from human CP samples.

TRANSLATIONAL IMPACT

✓ This method may be used to identify novel biomarkers of disease in human and animal models of CP.

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