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Proteomics as a Systems Approach to Pancreatitis

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Abstract

Proteomics is an approach to looking at the identity, amount, proteolysis, compartmentalization and post-translational modification of a large number of proteins simultaneously in a cell or tissue. Recently, proteomics has begun to be applied to the study of pancreatitis to ascertain mechanisms of disease and search for biomarkers of disease. Most mechanistic work has been carried out in animal models of acute pancreatitis. In eight studies, 97 proteins have been reported to increase, 55 to decrease and 23 to undergo proteolysis. Proteins showing increases are most often related to stress, inflammation or the cytoskeleton while decreases are seen in digestive enzymes and proteins related to metabolism. Many protein changes however, are not consistent between studies and only the most recent studies are rigorous and quantitative. By contrast, biomarker studies have focused on pancreatic juice and plasma of humans with disease and often are directed at distinguishing chronic pancreatitis from cancer. Chronic pancreatitis has also been investigated in tissue sections of histological samples. In this review the results of studies to date are described as well as coverage of the methods used and special issues that must be considered. Areas are pointed out that are worthy of future study.

Keywords

Proteomics; mass spectrometry; biomarkers; acute pancreatitis; pancreatic juice

Proteomics involves the characterization of the complete set of proteins in a tissue, cell, organelle or body fluid compartment. While traditional biochemical techniques focus on one or a few individual proteins, proteomics can identify and quantitate hundreds or even thousands of proteins. Often this is hypothesis generating rather than hypothesis testing. Because it allows evaluation of the entire system of proteins, it is also considered one type of systems biology. Due to alternative splicing and post translational modification, the proteome is more complex and dynamic than the genome and transcriptome. The fact that protein translation, post-translational modification, and degradation are also highly regulated contributes to the dynamic nature of the proteome.

While earlier work utilized large format 2D-gels to separate proteins in a complex mixture followed by N-terminal sequencing of individual proteins, modern research relies heavily on mass spectrometry (MS)-based techniques. MS allows identification of peptide sequences

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and utilizing information from the genome, the identification of proteins. For this to work efficiently for any complex biological sample either the proteins or peptides or both need to undergo preliminary separation prior to mass spectrometry. This usually involves gel electrophoresis or liquid chromatography resulting in a larger number of samples with a reduced protein content. For technical issues of sample fractionation and mass spectrometry techniques see (1).

Proteomics has been used to study the exocrine pancreas and its diseases in various manners including understanding physiology, identification of biomarkers of disease, and understanding the mechanism of disease. This review summarizes research related to normal function and pancreatitis. In addition to summarizing the current state of knowledge we summarize issues and difficulties in interpreting the data and present future avenues for research. For comparable research on pancreatic cancer the reader is referred to several recent reviews (2,3).

Tissue Proteomics in Acute Pancreatitis

A number of studies have evaluated changes in the amount of individual proteins in the pancreas or isolated acinar derived cells as a way to gain insight into experimental models of pancreatitis and to identify potential therapeutic targets (Table 1). Most of these studies have been based on the premise that proteins could increase either through synthesis or migration into the pancreas and decrease by degradation or possibly secretion. A few studies have tried to decrease complexity by first isolating a subcellular fraction which also allows evaluation of protein change by translocation within the cells. The number and functional class of proteins reported to show changes is given in Table 2. As shown in the table a much smaller number of proteins have been consistently observed between studies. Only one study, however, has been specifically targeted at proteolysis.

Protein separation by two dimensional gel electrophoresis (2DE) followed by mass spectrometry (MS) to identify proteins

The earliest proteomic studies utilized pancreatic AR42J cells as a model for rat acinar cells and simulated pancreatitis by hyperstimulation with caerulein. Yu et al (4) separated AR42J cell proteins by 2D gel electrophoresis and utilized silver staining and image analysis to quantitate protein “spots” which were then identified by matrix-assisted laser desorption/ionization (MALDI)-time of flight (TOF) mass spectrometry of tryptic peptides. Five proteins which increased three fold in response to 24 h caerulein treatment were identified as HSP90, mitochondrial ATP synthase beta chain, tubulin beta chain, 3-mercapto-pyruvate sulfurtransferase, and mitochondrial ATP synthase subunit D. In a subsequent report the same group of investigators used a shorter (12 h) caerulein treatment and a two fold change cutoff identified 10 upregulated and four downregulated proteins (5). Both up and down regulated proteins included stress proteins. In a more recent study, Li et al (6) treated AR42J cells for 20 min with taurocholic acid to model biliary pancreatitis and identified 23 proteins by 2DE that were upregulated 1.5 fold and 16 proteins that were down regulated. KEGG (Kyoto Encyclopedia of Genes and Genomics) analysis was used to identify pathways; the changed proteins were predominately involved in metabolic pathways, stress response, intracellular calcium regulation and cytoskeleton. These results show that the number of proteins identified depends on the threshold change selected as a cutoff and that additional information can be gained by looking at pathways involving proteins rather than just the amount of individual proteins.

The 2DE approach combined with MALDI-TOF/TOF (tandem mass spectrometry) has also been applied to the pancreas of rats with acute pancreatitis. A TOF/TOF instrument allows fragmentation of selected peptides and determination of their amino acid sequence. Fetaud et

al (7) studied acute caerulein pancreatitis 6 hours after the first of two hourly injections of caerulein. Pancreatitis was confirmed by an increase in serum amylase. The 2DE system used allowed identification of 125 proteins from AP and control samples. Comparative image analysis of silver stained protein spots showed 22 increased, 14 decreased and several newly appearing spots interpreted as the activated form of carboxypeptidase species. Proteins increased were related to the inflammatory response, protease activation, proteolysis, and response to cellular stress as listed in the Gene Ontology (GO) annotation database. Several increased proteins (albumin, apolipoprotein A1, α -1-macroglobulin) were likely entering the tissue from plasma. In this study as with most subsequent ones, attention was paid to the peptide sequence established by MS/MS and matched against the UniProt Knowledgebase (UniProtKB) rat database using the program MASCOT. Criteria were established for the validity of the identification. The authors also confirmed changes by immunoblotting of several proteins identified by mass spectrometry. In another study, the 2DE approach combined with tandem MS/MS was also used to evaluate rat pancreas from hyperlipidemic rats (8) and after administering taurocholate retrograde into the pancreatic duct to induce pancreatitis (9). This study used 2D DIGE (Difference Gel Electrophoresis) by labeling proteins from different pancreases with different fluorophores and running the combined mixture on a single gel to facilitate comparison. They identified 39 proteins (23 enhanced; 16 decreased) with fold changes of at least 1.5. Multiple stress proteins were increased and digestive enzymes were decreased. A problem with all of the 2D GE studies is that an excised gel spot may contain more than one protein making quantitation by spot density not always an accurate measure of individual protein change.

Peptide separation by IEF followed by LC-MS/MS

For global proteomic analysis, improved computational analysis has resulted in studies shifting away from 2DE of proteins to multidimensional separation of peptides prepared by tryptic digestion of pancreatic extracts. Two studies have used isoelectric focusing (IEF) of peptides after which fractions were eluted and subjected to reversed phase HPLC (rpHPLC) with fractions spotted on MALDI plates for tandem MS/MS. In the first study the effects of heat shock protection on caerulein induced pancreatitis was studied (10). Proteins from the four groups were quantitated by isobaric tagging of peptides with iTRAQ reagent to allow relative quantitation. Only proteins with at least two nonredundant peptides identified with Phenix software and the International Protein Index (IPI) Rat database were used for quantitation. The effect of heat stress was confirmed by Western blotting of HSP70 and HSP27. A total of 573 proteins were identified and differential expression was defined as an experimental ratio of >1.50 or <0.66 . Using these criteria, 83 proteins were differentially expressed in AP (46 increased; 37 decreased), 66 proteins were differentially expressed with heat treatment and 102 proteins were differentially expressed between AP and AP following heat stress. Individual proteins are listed in the publication; the proteins increased in AP were primarily associated with inflammatory and stress responses while the proteins decreased were mainly digestive enzymes and proteins related to metabolic processes. Note that this type of analysis evaluated a much greater number of identified proteins than the 2DE studies.

Another study by the same research group (11) evaluated the low molecular weight proteome, or peptidome, in the same model of caerulein pancreatitis in the rat. Their workflow involved strong homogenization followed by centrifugation after which peptides in the soluble fraction were enriched by centrifugal ultrafiltration through a 10 kDa cutoff filter prior to fractionation and mass spectrometry as described above but using an LTQ Orbitrap instrument. Because they were looking for released peptides the samples were not trypsinized and database searching was not limited to specific proteases or cleavage sites. Protein and peptides were selected based on scores designed to keep the false discovery rate

below 1%. Between one and four thousand peptides were identified in each run (2 control and 2 AP) and are given in their supplementary data. Gene ontology (GO) database was used to classify the proteins. In control samples the most common categories were cytoskeleton, translation, metabolic process and unknown. Peptides that increased in AP were most concentrated in cytoskeleton and inflammatory response. Immunoblots were used to confirm a decrease of tubulin α and β chain native protein in pancreatitis.

2D LC-MS/MS separation of Peptides

This approach, now one of the most frequent, uses two dimensional liquid separation. Most commonly, following trypsinization, strong cation exchange is used to generate 10-15 fractions followed by rpHPLC of each fraction prior to mass spectrometry. Such a technique can identify up to several thousand proteins in a complex mixture. However, to simplify and obtain more functionally relevant information the cell or tissue can be fractionated using cell biological techniques. Because of the relevance of the rough endoplasmic reticulum to protein synthesis and ER stress, Chen et al (12) carried out a quantitative proteomic analysis of pancreas RER from normal and AP rats. Following induction of arginine and caerulein pancreatitis, RER was purified by sucrose gradient fractionation, the ER pellets digested with trypsin, and the resulting peptides labeled with iTRAQ and separated by 2D LC-MALDI-MS/MS. A total of 469 unique RER proteins were determined with 95% confidence by use of Protein Pilot software searching the IPI rat database. Functional categorization used DAVID (The Database for Annotation, Visualization and Integrated Discovery). RER proteins belonged to functional categories including ribosomal proteins, translocon subunits, chaperones, secretory proteins and glyco- and lipid-processing enzymes (12). The function of these proteins and their relation to RER of other tissues has been reviewed by the same group (13). A total of 37 proteins were changed in AP, 25 in arginine induced, 6 in caerulein induced and 6 common to both models (12). Digestive enzymes decreased in both models of AP although more so in arginine-induced pancreatitis. The cluster of proteins showing the greatest increase was fibrinogen alpha, beta and gamma chains. Four tubulin isoforms increased more than two fold in arginine-induced AP as did several translation factors. Changes in several proteins were confirmed by immunoblotting and fibrinogen was localized to acinar cells by immunohistochemistry.

A second organelle that has attracted attention and is relevant to pancreatitis is the zymogen granule. Several groups have purified rat zymogen granules and characterized the proteome of the granule membrane and contents (14-17). Over two hundred proteins have been identified including many not previously known to exist on the granule including a number of small G proteins. A challenge to determining the complete organelle proteome is to eliminate contaminating proteins. As advances are made in the sensitivity of mass spectrometers ever more proteins have been identified even though the ZGs are 98% pure. In addition some proteins known from other types of studies have not been found in these studies consistent with a low copy number per granule. It would be interesting to compare ZG from control and pancreatitis animals to look for changes that might contribute to the inhibition of secretion seen in experimental AP. Also it would be useful to analyze the content of human ZG.

Proteomics in Chronic Pancreatitis

Proteomics has been applied to chronic pancreatitis (CP) in several ways. One has been the characterization of pancreatic stellate cells (PSCs) because of the important role of this cell type in secreting extracellular matrix proteins (18). PSCs can be isolated in tissue culture under conditions where acinar and other cell types fail to survive. Depending on conditions they can be kept quiescent or activated to secrete extracellular matrix as occurs in vivo in CP and pancreatic cancer. In two studies, the complement of proteins in immortalized rat and

mouse PSC cell lines were characterized in the serum starved quiescent state or the serum driven activated state (19,20). In both studies the approach was to carry out 1D gel electrophoresis and then divide the gel into 10 pieces each of which was trypsinized and subjected to rPHPLC and mass spectrometry. A maximum of 1500-2000 proteins were identified and the relative abundance analyzed by spectral counting in which the number of times a peptide is observed is divided by the total spectral count of all peptides. Activated (proliferating) rat PSCs had 176 proteins exclusive to or of statistically higher abundance and 375 proteins exclusive to or higher in quiescent cells (19). These proteins were analyzed by GO and KEGG pathway analysis. The ribosome pathway was the highest represented in active PSCs while for quiescent cells, the highest number of associated categories were: Regulation of actin cytoskeleton, Focal adhesions, Insulin signaling and Lysosomes. Essentially similar results although with higher numbers of proteins, were reported in the study of mouse PSCs (20). A third study evaluated proteins secreted by an immortalized human PSC line, the RLT-PSC (20). A total of 641 proteins were identified in the secretome from activated cells compared to only 46 from quiescent cells. The proteins were classified by bioinformatic tools and eleven were confirmed by immunoblotting (21).

Another approach used to study CP is the analysis of tissue sections cut from formalin-fixed, paraffin-embedded human tissue for global proteomic analysis (22,23). Unstained sections were deparaffinized, rehydrated and homogenized followed by heating to hydrolyze crosslinks. Lysates were then digested with trypsin and separated by LC-MS/MS. In the study by Pan et al, mild CP, severe CP, pancreatic adenocarcinoma(PAC) and normal pancreas was evaluated with 5 specimens per group and each analyzed in duplicate (22). In total, 3330 unique peptides were observed in at least two runs and 700-800 proteins quantitated. The different groups were compared by a label free analysis. Their results show that proteins can be identified from archival specimens and that the spectrum of proteins is different for different diseases. Not surprisingly, proteins related to extracellular matrix and fibrosis as well as inflammatory proteins were enhanced in CP. In the study by Paulo et al (23), CP and pancreatic cancer were compared to normal pancreas using 3 specimens per group. Their study identified 525 nonredundant proteins. When proteins were limited to those appearing in 2 of the 3 specimens per group, there were 162 normal proteins, 62 for CP and 101 for PAC. Selected proteins of interest expressed exclusively in CP include collagen $\alpha 1$, collagen $\alpha 3$, and filamin A while agrin, laminin and vitronectin were exclusive to normal pancreas. Some of this exclusiveness is most likely due to only the most abundant proteins being identified. Part of the reason for fewer proteins being identified in the second study was that less material (fewer and thinner sections) was analyzed. However, the proteins observed in the two studies illustrated the same points. Whether this approach will be useful diagnostically is uncertain because of the labor involved but it could identify proteins that could then be visualized by immunohistochemistry. It might also be useful to analyze fresh tissue samples from surgical specimens of CP and possibly couple this to laser capture microscopy.

Proteomics of Pancreatic Juice

Proteomic analysis of pancreatic juice has been carried out at this time only in humans and has focused on a search for biomarkers of CP or pancreatic adenocarcinoma (PAC). A priori, pancreatic ductal fluid should contain proteins from the zymogen granule content, plasma proteins leaking or transcytosed into ductal fluid, proteins shed from the apical membrane of acinar and ductal cells, and intracellular proteins released as a result of damage either by underlying pathology or by the collection process. Published studies to date support these as sources with the most frequently identified proteins being digestive enzymes and plasma proteins. Because pancreatic juice is primarily collected during surgery or by ERCP, a technique that carries some risk, there is very little data on control subjects.

In studies of PAC, the controls are sometimes patients with CP (24). The best controls are probably from patients undergoing study for abdominal pain but where no pancreatic pathology is observed (25,26). To avoid these problems, Paulo, Conwell and colleagues have developed a procedure to endoscopically collect what they term “pancreatic fluid” from the duodenum near the entry of the common duct after aspirating the gastric content (26, 27). This limits the risk to subjects and allows timed collections before and after secretin stimulation but has the disadvantage of contamination by bile, gastric juice and duodenal fluids and proteins. This is not an issue for quantitation of unique pancreatic proteins or proteins increased with pancreatic disease but prevents classifying all the normal pancreatic juice proteins.

As with pancreatic tissue, the methods of analysis for pancreatic juice have evolved. Early work used 2D electrophoresis to separate proteins as spots which were identified by mass spectrometry (24, 28, 29). The proteins were either run on separate gels and compared by image analysis or fluorescently labeled with different fluorophores and run together on a gel. These studies generally yielded only a few identified proteins. In one study 22 proteins were identified with 7 consistently changed in PAC. Gronborg et al (30) used 1D SDS gel electrophoresis followed by LC-MS/MS in three patients with PAC where pancreatic juice was collected at surgery. 170 proteins were identified in at least one patient but only 23 were common to all three patients. In addition to expected pancreatic proteins they found a number of “cancer-associated” proteins including annexin5, CEACAM5, CEA and several S100 proteins. Chen et al (31) compared pancreatic juice from a chronic pancreatitis patient to a pooled sample from 10 “normal” subjects who had benign pancreatic disease, mostly cystic neoplasms. Quantitation was carried out by use of ICAT labeling followed by 2D LC-MS/MS. Seventy two proteins were identified and 27 were differentially expressed. Proteins upregulated in CP included fibrinogen β chain, plasminogen/plasmin, NCAM1, serum albumin, α 1b-glycoprotein, and α 2-macroglobulin. Some of these, however, have also been reported to be upregulated in pancreatic cancer. The proteome of normal pancreatic juice was studied by Doyle et al (25) who collected fluid by ERCP from 3 female subjects with clinical abdominal symptoms but no apparent pancreatic pathology after investigation. Proteins were denatured in urea, trypsinized and peptides separated by rpHPLC. Peptides were identified from 285 proteins but only 172 were deemed of high quality and for only 90 was identification of at least two distinct peptides found which is currently considered necessary for high quality identification. In this study secretin stimulation changed the relative quantity but not the identity of the proteins observed. While the biggest groups of proteins were digestive enzymes and plasma proteins only 42 proteins overlapped with the prior group of patients with PAC. This study was limited by the resolving power of the 1D HPLC separation.

The approach of collecting pancreatic juice from the duodenum endoscopically and analyzing by 1D gel electrophoresis followed by LC-MS/MS, a procedure the authors term GeLC-MS/MS has been used to compare pancreatic juice from 9 individuals with CP to 9 controls with chronic abdominal pain (26). A total of 1391 proteins were identified and quantitated by spectral counting using the IPI human database. There were 413 proteins identified in CP but not in the non-pancreatic disease controls. Of the 721 proteins found in both groups, 77 proteins were upregulated in CP and 38 downregulated. Proteins exclusive to or up-regulated in CP included proteins associated with fibrosis, inflammation, and pain while the predominant down regulated group was pancreatic digestive enzymes. This study benefited from both the inclusion of a larger number of subjects and a more powerful analytic approach. It should be noted that the IPI database has recently been closed and replaced by UniProtKB which has complete data sets for many species including human, rat and mouse.

Several conclusions can be drawn from this data. First, the normal human pancreatic juice proteome is not yet fully understood. Animal studies, most likely using rats, could be useful to understand the various routes that proteins enter pancreatic juice. Animal studies could also allow the study of acute pancreatitis which is not possible in humans. The limited amount of sample in mice, however, would be difficult to overcome. Second, to resolve the full spectrum of proteins it is necessary to use two dimensions of separation prior to mass spectrometry analysis. Using SDS gel electrophoresis as the first dimension may have the added advantage of denaturing proteases. Whether trypsin activation of other proteases can occur during tryptic digestion of pancreatic juice needs evaluation. One approach to this issue is to evaluate peptides without the constraint of assuming tryptic peptides. Third, there appears to be an overlapping spectrum of additional juice proteins in CP and PAC. Finally, it seems unlikely that MS analysis of pancreatic juice will become a diagnostic tool but the use of targeted approaches such as protein chips based on the proteins identified by MS could be practical for pancreatic fluid collected in the duodenum or by ERCP.

Serum or plasma proteomics

Evaluation of serum or plasma has primarily been used to search for predictive biomarkers of disease, particularly pancreatic carcinoma (32). A few studies have evaluated pancreatitis. Papachristou et al (33) carried out surface-enhanced laser desorption/ionization (SELDI)-TOF MS on serum from patients with mild AP and severe AP. This technique which evaluates peak clusters without identifying specific proteins found several clusters which had good ability to discriminate severe pancreatitis. A prospective study with larger sample size would be necessary to determine if this approach has better predictive value than simple plasma parameters. Studies of plasma from patients with chronic pancreatitis have primarily been evaluated as a control for cancer. In a recent study, Pan et al, (34) evaluated plasma pools from pancreatic cancer, chronic pancreatitis and nonpancreatic disease control. They depleted the seven most abundant plasma proteins and then isotopically labeled the proteins followed by 2D-LC separation. Over 900 proteins were identified in studies comparing pancreatic cancer to controls and chronic pancreatitis to pancreatic cancer. Because chronic pancreatitis was not compared to controls the information obtained was more related to pancreatic cancer and not chronic pancreatitis. It would be interesting to compare information from AP and CP to plasma from normal subjects.

In a related study, Mittal et al (35) evaluated the proteome of mesenteric lymph during acute pancreatitis in rats induced by taurocholate injection. Using iTRAQ labeling and LC-MS/MS after depleting high abundance proteins, 285 proteins were identified with 8 being increased in pancreatitis. Of these 7 were digestive enzymes and the other was a glutathione S-transferase.

Conclusions and Recommendations for Future Proteomic Studies of Pancreatitis

Proteomics as a field is now relatively mature and can be applied to specific diseases such as pancreatitis. However, to be effective certain principles need to be followed. First, expertise is needed in both the pathobiology of the pancreas and in mass spectrometry. It is rare, some would say impossible, to have this in one investigator so the most common approach is a collaboration between two individuals. They must have access to high quality instrumentation which is available for extensive time periods and the bioinformatics capability to analyze the resulting data and its interpretation. Second, studies need to be quantitative and collect data in a manner that objective, sometimes statistical, criteria can be applied. Current standards include the requirement for finding of at least two peptides to identify a protein and the establishment of criteria to keep the false discovery rate low.

Finally, just measuring protein abundance in the pancreas will provide limited insight. Studies to define organellar localization, proteolysis, translocation and post-translational modification will provide more in depth knowledge

One issue that is clear from this review is the necessity for complimentary human and experimental animal studies. Human studies have to focus on blood and pancreatic juice but need animal studies for comparison and mechanistic understanding. Tissue studies of pancreatitis, especially acute pancreatitis can only be carried out on experimental models but the findings need to then be used for targeted evaluation of human pathological specimens.

The methods of sample fractionation used to date show that two orthogonal methods of protein or peptide separation are necessary to resolve a complete proteome or subproteome. Most success has come from 2D-LC using cation exchange followed by rpHPLC or 1D GE followed by rpHPLC. IEF can also be used for the first dimension. All these approaches generate large numbers of samples and need to be coupled to a mass spectrometer with appropriate analytic capabilities. This latter point becomes critical in order to evaluate the phosphoproteome as has been done in other tissues (36). Both the method of sample preparation and the sensitivity of the mass spectrometer influence the number of proteins identified. In a recent study evaluating pancreatic cancer biomarkers by crude cell fractionation, 1D gel electrophoresis and HPLC of 20 gel slices, over 2000 proteins were identified (37). However, since most cell types are estimated to contain around 5000 proteins and the pancreas contains multiple cell types, it is clear that not all low abundance proteins are yet being identified.

The pancreas and pancreatic juice presents one challenge not seen in other tissues that needs to be considered. This is the presence of a large number of proteases activated by trypsin. If trypsin is added to pancreatic juice the other zymogens will be activated and cleave proteins at different sites making identification difficult. Thus either a different protease in the presence of trypsin inhibitor needs to be used (38) or the proteins separated first on a denaturing gel to separate and inactivate the various proteases as carried out in the studies of Paulo et al (26, 27)

A number of additional proteomic studies that could be carried out to further understand pancreatitis include: 1) Analysis of pancreatic juice in animal models to understand the normal protein content and the effect of pancreatitis. 2) A more complete analysis of the proteome of human pancreatic juice. 3) Analysis of the proteome of pancreatic nuclei, zymogen granules, mitochondria and cytosol in experimental pancreatitis and normal tissue to enhance protein coverage and consider translocation. 4) Characterization of the proteome of isolated pancreatic acini and purified duct fragments. 5) Evaluation of the phosphoproteome of the pancreas in acute pancreatitis. Finally it is important that the results of proteomic analysis be interpreted in a systems approach to look at pathways or ensembles of proteins and how this data interrelates to transcriptional and metabolic data.

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Table 1

Summary of proteomic studies of acute pancreatitis

Species	Inducing Agent	Protein/peptide separation	MS	Proteins detected	Proteins Changed	Quantitation	Ref
AR42J Cells	Caerulein	2DE	Micromass MALDI-TOF	NA	5 Increased	Gel Spot Density	4
AR42J Cells	Caerulein	2DE	Micromass MALDI-TOF	NA	10 Increased 4 Decreased	Gel Spot Density	5
AR42J Cells	Taurolithocholic Acid	2DE	4800 MALDI-TOF/TOF	NA	23 Increased 16 Decreased	Gel Spot Density	6
Rat	Caerulein	2DE	4800 MALDI-TOF/TOF	125	22 Increased 14 Decreased	Gel Spot Density	7
Rat	Heat Shock Caerulein	IEF, rpHPLC	4800 MALDI-TOF/TOF	573	46 Increased 37 Decreased	iTRAQ	10
Rat	Caerulein	Ultrafiltration, IEC, rpHPLC	LTQ Orbitrap XL	489	17 Increased (Peptidome)	Number of peptides	11
Rat	Hyperlipidemia Taurocholic Acid	2D-DIGE	4700 MALDI-TOF/TOF	NA	23 Increased 16 Decreased	DIGE	9
Rat	Arginine	Sucrose Gradient	4800 MALDI-TOF/TOF	469	16 Increased	iTRAQ	12

Table 2
Proteins reported changed in experimental acute pancreatitis

INCREASED – 97 proteins	Reference
Inflammatory Response – 36 proteins	
Apolipoprotein A1	7,9,10
Complement C3	7,10
Fibrinogen (α , β , and γ chains)	9,12
Hemopexin	9,10
Murinoglobulin	7,10
Serum albumin	7,9,10
Response to Stress – 13 proteins	
Clusterin	9,10,11
Hypoxia Upregulated Protein 1 (ORP-150)	5,6
Pap2 (Reg 3A)	7,10
Protein Disulfide Isomerase	5,9
78 KDa Glucose Regulated Protein (Bip)	6,9
Metabolism – 13 proteins	
Mitochondrial ATP synthase beta chain	4,9
Cytoskeleton – 7 proteins	
Various Tubulins	4,12
Other – 27 proteins	
DECREASED – 55 proteins	
Digestive Enzymes and ZG membrane proteins – 21 proteins	
Amylase	9,10,12
Bile salt activated lipase	9,10,12
Carboxypeptidase B	9,12
Chymotrypsin B	10,12
Ribonuclease	10,12
Triglyceride Lipase, PLRP-1, and PLRP-2	9,10,12
Metabolism – 13 proteins	
Isocitrate Dehydrogenase	6,9
Response to Stress – 4 proteins	
78 KDa Glucose Regulated Protein (Bip)	5,12
Other -17 Proteins	
PROTEOLYSIS – 23 proteins	
Inflammatory Response – 9 proteins	
Complement C3	11,12

INCREASED – 97 proteins	Reference
Response to Stress – 3 proteins	
Cytoskeleton – 3 proteins	
Translational Response -2 proteins	
Other – 6 proteins	

The table includes all proteins uniquely identified with a change in amount of greater than 1.5 fold. Individual proteins reported more than once are listed with references. Note that 78 KDa Glucose Regulated Protein is listed in both increased and decreased categories as reported by different studies.