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Proteomic analysis of differential protein expression in early process of pancreatic regeneration in pancreatectomized rats[1]

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Key words

partial pancreatectomy; regeneration; metabolism; stem cell; pancreas proteomics

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Abstract

Aim: A broad-range proteomic approach was applied to investigate the complexity of the mechanisms involved in pancreatic regeneration for identification of new targets of diabetes treatment and potential markers of pancreatic stem cells. **Methods:** A regeneration pancreatic model was induced by 90% partial pancreatectomy (Px) in rats. Changes in the protein expression in regenerating rat pancreas on the third day after Px, as compared with rats that received sham surgery, were analyzed by using 2-D gel electrophoresis (2-DE), mass spectrometry (MS), and mass fingerprinting. **Results:** 2-DE revealed 91 spots with at least 1.5-fold increases in expression at 3 d after pancreatectomy and 53 differentially expressed proteins that were identified by peptide mass fingerprinting (PMF). These included cell growth-related, lipid and energy metabolism-related, protein and amino acid metabolism-related proteins, and signal transduction proteins. Vimentin, CK8, *L*-plastin, hnRNP A2/B1, and AGAT are associated with embryogenesis and cell differentiation, and may be new potential pancreatic stem cells markers. **Conclusion:** The proteome profiling technique provided a broad-based and effective approach for the rapid assimilation and identification of adaptive protein changes during pancreas regeneration induced by pancreatectomy. Our data clarify the global proteome during the pancreatic proliferation and differentiation processes, which is important for better understanding of pancreatic regeneration and for discovering of protein biomarkers for pancreatic stem cells.

Introduction

Recovery of a functional β-cell mass by stimulating pancreatic regeneration is an approach for the treatment of diabetes that is characterized by absolute or relative deficiency of functional pancreatic β-cells. However, the capacity of pancreatic β-cells to regenerate in adults is very limited because of terminal differentiation. This notwithstanding, regeneration of β-cells has indeed been induced in adult animal pancreata by using particular experimental conditions, such as pancreatectomy (Px), administration of streptozotocin (STZ), wrapping the pancreas in cellophane, or using transgenic mice with overexpression of γ-interferon in the β-cells[1]. An understanding of how new β-cells are generated in these conditions is important with respect to the possibility of stimulating the regeneration of β-cells in humans to provide a cure for diabetes.

Many studies have investigated specific markers for the identification of resources such as pancreatic stem or progenitor cells during β-cell regeneration. A number of putative markers that are transiently expressed in embryonic ducts have been suggested as indicators of islet stem/progenitor cells, including cytokeratins, β-galactosidase, PDX-1, tyrosine hydroxylase (TH), and the glucose transporter GLUT 2[1]. However, Dor *et al* reported that neogenesis β-cells were formed by duplication of the pre-existing β-cells rather than differentiation from stem cells in Px rats[2].

Knowledge about molecular events taking place during

pancreatic regeneration would help to identify those molecular factors regulating cell replication and differentiation during the neogenic renewal of pancreatic tissue. Recently, Rafaeloff and colleagues reported the expression of islet neogenesis-associated protein (INGAP) in regenerating hamster pancreas induced by cellophane wrapping, and its expression appeared to stimulate duct cell proliferation, which is a crucial process in pancreatic neogenesis[3]. Min *et al* have reported that clusterin may play essential roles in the neogenic regeneration of pancreatic tissue by stimulating the proliferation and differentiation of duct cells[4,5].

These studies on single pathways have thus far been insufficient to fully delineate the complex molecular mechanisms of pancreatic β-cells regeneration. Although some studies have reported that composite pancreatic extracts from regenerating pancreas can differentiate rat mesenchymal cells into insulin-producing cells[6] or cure diabetes induced by streptozotocin in BALB/c mice and enhance HIT-T15 cell proliferation and insulin secretion[7], it is still unclear which key factors play a role in these processes of differentiation and proliferation. Furthermore, they have focused on cell growth and proliferation, but have ignored other pathological changes such as stress and metabolism in the regenerating pancreas. The proteomic approach offers a high-throughput technology to study a group of proteins simultaneously, which makes it feasible to study the differential protein expression profiles relating to particular pathophysiological conditions. In the current study, a regeneration pancreatic model was induced by 90% Px in rats; the total proteins extracted from the regenerating and non-regenerating pancreas tissue were used in 2-D gel electrophoresis (2-DE), and the proteins that were differentially expressed were identified by matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-ToF-MS). As a result, not only several cell growth and proliferation-related proteins, but also energy metabolism, amino acid metabolism and lipid metabolism-related proteins were found.

Materials and methods

Animal experiments Male specific pathogen-free (SPF) Wistar rats weighing 150–160 g were housed at the SPF animal facility at the Animal Center of Sun Yat-sen University, and allowed access to standard rat chow and water. Rats were randomly assigned to two experimental groups (n=6 per group): Px and sham surgery (Sx). The rats in the Px group were anesthetized with pentobarbital (50 mg/kg bodyweight, ip) and approximately 90% Px was performed as described by Bonner-Weir *et al*[8] with some modifications. Briefly, a midline upper abdominal incision was carried out, and the complete tail portion of the pancreas, together with the spleen and most of the head of the pancreas, were removed by gentle abrasion with a small nipper. The major blood vessels were left intact so as not to compromise other organs. The remnant (residual pancreas) was anatomically well-defined compassion tissue within the common pancreatic duct and the first part of the duodenum. The rats in the Sx group received Sx that consisted of spleen dissection and separation of the duodenum colon ligament. On the 3rd day after surgery, rats were anesthetized, and the remnant pancreatic tissue of the Px rats and the corresponding portion of pancreas of the Sx rats were quickly dissected, cleared on ice and then rapidly stored at -80 °C.

Sample preparation Each sample of tissue (approximately 0.1 g wet weight) was cut into fragments and suspended in 300 μL lysis buffer consisting of 7 mol/L urea, 2 mol/L thiourea, 2% 3-[(3-cholamidopropyl) dimethylammonio]-propanesulfonate (CHAPS), 20 mmol/L Tris, 5 mmol/L tributyl phosphine (TBP), 0.5% immobilized pH gradient (IPG) buffer [pH 3-10 non linear (NL)] and 0.6 mmol/L phenylmethanesulfonyl fluoride (PMSF). The suspension was sequentially homogenized by a rotating blade homogenizer for 25 s and by sonication 15 times, and then centrifuged at 20 000×g at 4 °C for 30 min. Approximately 280 μL of supernatant was obtained and incubated at room temperature for 1.5 h. After addition of 20 mmol/L iodoacetamide (IAA), the supernatant was placed away from light and incubated at room temperature for 1.5 h. A fourfold volume of cold acetone was added slowly and then the supernatant was stored at -20 °C overnight. After centrifugation at 12 000×g at 4 °C for 15 min, the precipitate was obtained and suspended in 200 μL rehydration buffer [7 mol/L urea, 2 mol/L thiourea, 2% CHAPS, 0.5% IPG buffer (pH 3–10 NL) and 0.002% bromophenol blue] and stored at -80 °C for use. The concentrations of the extractions were determined by using the 2D Quant kit (Amersham Biosciences, Uppsala, Sweden) according to the manufacturer's instructions.

Two-dimensional gel electrophoresis For one-dimensional isoelectric focusing (IEF), 600 μg protein extracted from each rat was respectively applied to 24 cm immobilized pH 3–10 non linear gradient strips (Amersham Biosciences, Uppsala, Sweden). IEF was conducted using an IPGPhor II system (Amersham Biosciences, Uppsala, Sweden) according to the following procedure: 1 h at 200 V, 1 h at 500 V, 1 h at 1000 V then 8–12 h at 8000 V until 80 000 V h. After IEF, the strips were equilibrated for 25 min with gentle shaking in 10 mL of a solution containing 50 mmol/L Tris-HCl, 6 mol/L urea, 30% glycerol, 2% w/v sodium dodecylsulfate (SDS),

and a trace of bromophenol blue. For the second dimensional separation, the IPG strips were placed on the top of 12% SDS-polyacrylamide gel electrophoresis (PAGE) gels and sealed with 0.5% *w/v* agarose in SDS electrophoresis buffer (25 mmol/L Tris, 192 mmol/L glycine, 0.1% *w/v* SDS). The molecular weight markers used were the LMW Calibration Kit for SDS Electrophoresis (Amersham Biosciences, Uppsala, Sweden). Electrophoresis was performed at 10 °C in the Ettan DALTsix system (Amersham Biosciences, Uppsala, Sweden), according to the following program: 2.5 W per gel for 30 min, then 17 W per gel for 5 h until the bromophenol blue front had migrated to the end of the gel. Gels were stained with Coomassie blue for 15 min and destained with 10% glacial acetic acid for 12 h. The gels were scanned in Imagescanner II (Amersham Biosciences, Uppsala, Sweden), and the 2-D images were analyzed with ImageMaster 2D Platinum software 5.0 (Amersham Biosciences, Uppsala, Sweden) according to the protocols provided by the manufacturer. To account for experimental variation, we ran and analyzed 6 gels for each experimental group.

Spot handling Selected protein spots were subjected to fully automated spot handling in the Ettan Spot Handling Workstation (Amersham Biosciences, Uppsala, Sweden). The methods included spot picking, digestion, extraction of tryptic peptides, and spotting on Ettan MALDI target slides which were automatically run overnight.

In the automated procedure, gel plugs were cut by a 1.4-μm picking head, and washed twice in 50% methanol/50 mmol/L ammonium bicarbonate and once in 75% acetonitrile before drying. For digestion, 10 μL trypsin solution (0.02 μg/mL; sequencing grade, Promega) was added before incubation at 37 °C for 2 h. Extraction was performed in 2 steps by the addition of 50% acetonitrile and 0.1% trifluoroacetic acid. The pooled extract was dried and dissolved in 3 μL matrix (5 mg/mL recrystallized α-cyano-4-hydroxy-cinnamic acid). In the final step before MALDI-ToF (time-of-flight) analysis, 0.3 μL dissolved sample was spotted on the target slides.

Protein identification Peptide mass fingerprinting (PMF) was performed by using an Ettan MALDI-ToF Pro (Amersham Biosciences, Uppsala, Sweden). For each sample, spectra were acquired in the delayed extraction and reflector mode, and an average of 200 spectra that passed the accepted criterion of peak intensity were automatically selected and accumulated. Using ProFound data acquisition[9], spectrum processing and database searches were performed in automatic mode with internal calibration using trypsin autolysis peaks (*m/z* 842.509 and *m/z* 2211.104).

Results

2-D gel separation of proteins To analyze the proteome in relation to β-cell proliferation and differentiation in rats, a rat Px-induced pancreatic regeneration model was established. The proliferating pancreatic tissue derived from rats 3 d after undergoing Px was processed for 2-D electrophoresis to isolate the candidate proteins that were differentially regulated when compared with control tissue from Sx rats. In order to measure the reproducibility of the technique, 2-DE for the regenerating and normal pancreas from the Px and Sx rats was repeated 6 times. For the regenerating pancreatic tissues, a total of 1315±28 spots were detected, and 1098±19 spots were matched with an average matching rate of 83.5%. For the control pancreatic tissues of the Sx rats, 1369±28 spots were detected in total, and 1110±21 spots were matched, with an average matching rate of 81.1%. The regeneration 2-DE maps were compared with the control 2-DE maps, and a total of 997±16 spots were matched. The patterns of protein expression by the pancreas after Sx and Px are shown in Figure 1. The average volume of each spot was calculated using 3 gels (selected from the 6 gels in each group), and the ratio of the average volume of each spot in the Px pancreas relative to the Sx pancreas were determined by using ImageMaster 2D Platinum 5.0 software (Figure 2). Among 91 spots in the gels that had a significant and at least 1.5-fold change in abundance after Px, 41 protein spots were upregulated and 50 spots were downregulated (selection criteria: $P<0.05$ according to Student's *t*-test). Figure 1A and 1B show that differentially expressed proteins (indicated with a circle) were found to have a 1.5-fold or greater difference in intensity between the regeneration and control groups. Figure 2 shows the images from 2-D-PAGE focusing on some areas containing differentially expressed proteins. All the 91 differentially expressed proteins were selected for subsequent analysis by mass spectrometry.

Protein identification All 91 spots of interest were specifically digested by trypsin and 89 spots had nearly perfect peptide masses for PMF analysis. The PMF maps were obtained by MALDI-ToF-MS and calibrated against trypsin auto-degraded peaks (*m/z* 842.509 and *m/z* 2211.104). PMF of the selected spots and a subsequent database search revealed the identity of these proteins as summarized in Table 1. The NCBInr database (comprehensive, non-identical protein database) and a database of homology predicting protein sequences from the rat genome were searched for theoretical protein digest patterns matching the experimentally determined masses. Figure 3 shows the PMF map of spot #8170, which database searching revealed was vimentin.

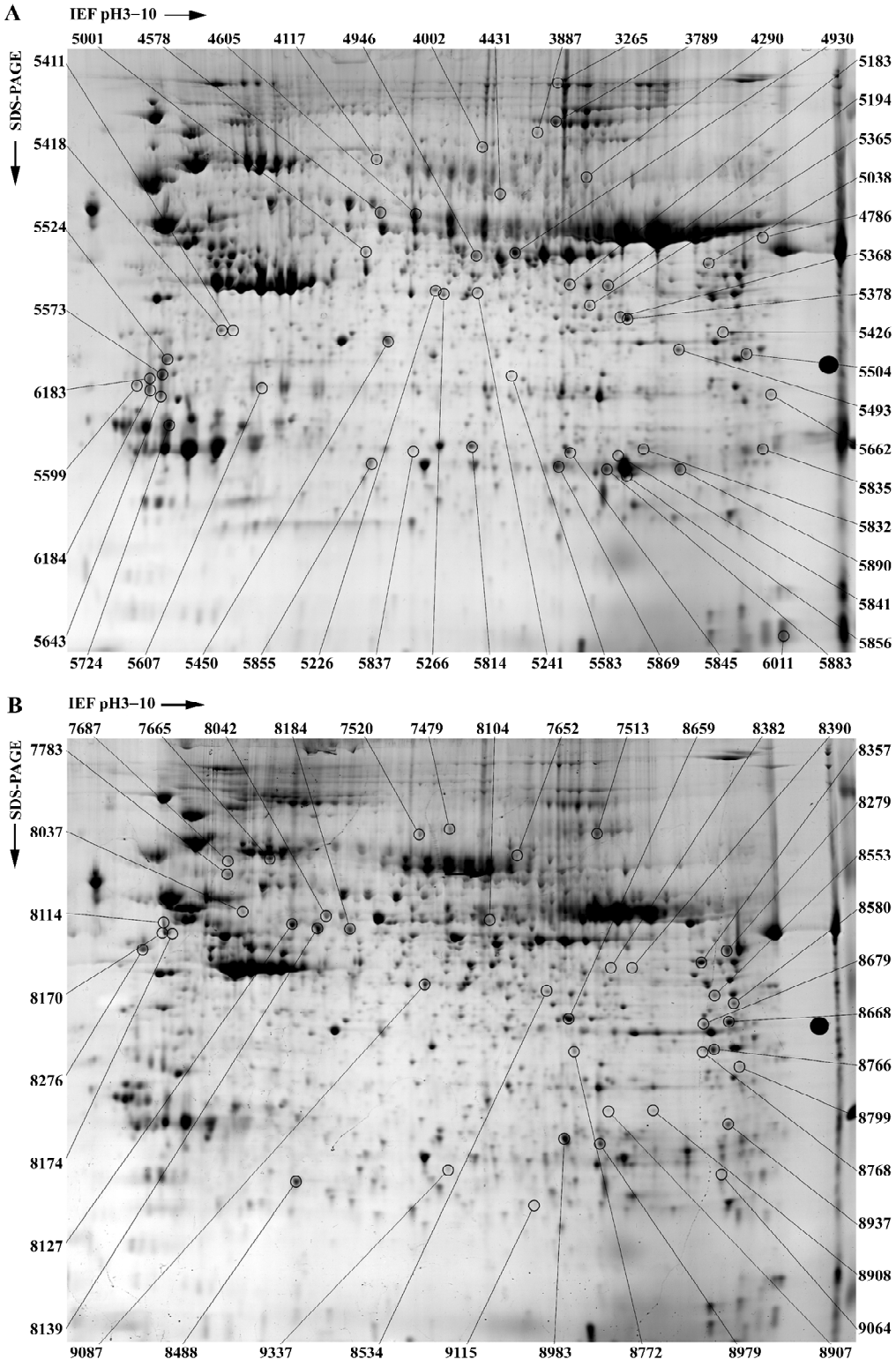


Figure 1. Representative Coomassie blue-stained 2-D gels loaded with extracts from the pancreas of Sx (A) or Px (B) rats. Spots showing downregulation (A) or upregulation (B) of expression level are indicated (circles). The identification numbers of the spots correspond to the numbers in Table 1. Some spots were identified as being the same proteins (eg #8114, #8170, #8174, and #8276 were all identified as being vimentin; #5411 and #5418 were both identified as being eukaryotic translation initiation factor 2, subunit 1).

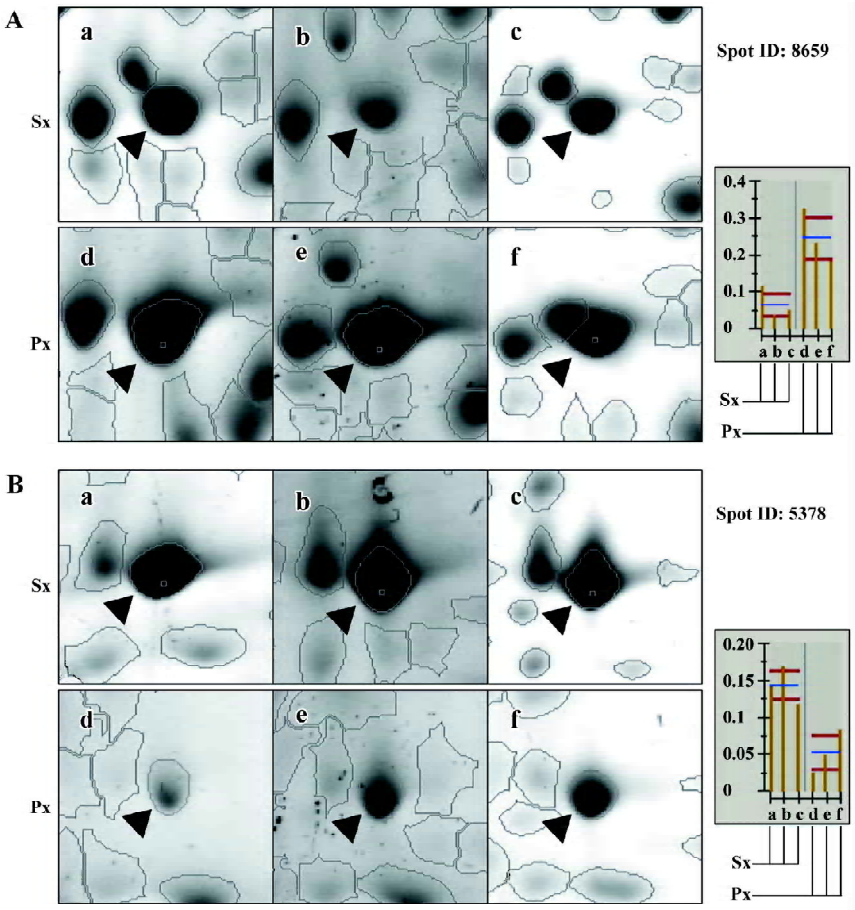
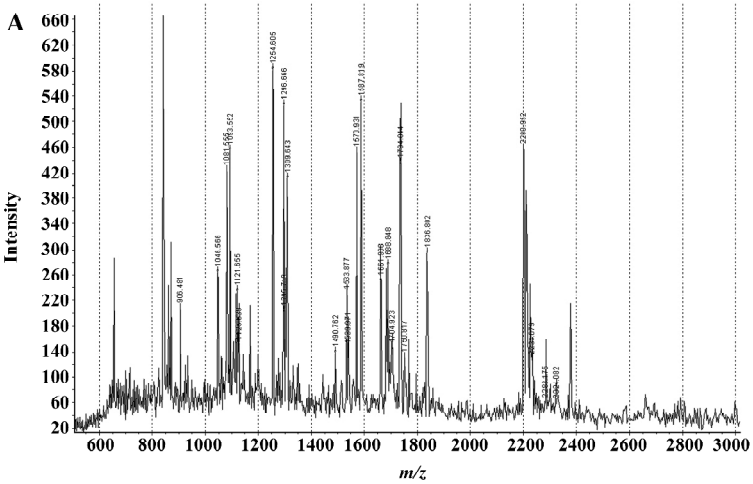


Figure 2. Images from 2D-PAGE focusing on the corresponding areas in all analyzed gels. The differentially expressed spots were detected with ImageMaster 2D Platinum 5.0 software. (A) spot #8659 was upregulated; (B) Spot #5378 was downregulated.



B

1 MSTRSVSSSS YRRMFGGSGT SSRPSSNRSY VTTSTRTYSL GSALRPSTSR
51 SLYSSSPGGA YVTRSSAVRL RSSMPGVRLL QDSVDFSLAD AINTEFKNTR
101 TNEKVELQEL NDRFANYIDK VRFLEQQNKI LLAELEQLKG QGKSRLGDLY
151 EEEMRELRRQ VDQLTNDKAR VEVERDNLAE DIMRLREKLQ EEMLQREEAE
201 STLQSFRQDV DNASLARLDL ERKVESLQEE IAFLKKLHDE EIQELQAQIQ
251 EQHVQIDVDV SKPDLTAALR DVRQQYESVA AKNLQEAEEW YKSKFADLSE
301 AANRNNDALR QAKQESNEYR RQVQSLTCEV DALKGTNESL ERQMREMEEN
351 FALEAANYQD TIGRLQDEIQ NMKEEMARHL REYQDLLNVK MALDIEIATY
401 RKLLEGEESR ISLPLPNFSS LNLRETNLES LPLVDTHSKR TLLIKTVETR
451 DGQVINETSQ HHDDLE

Figure 3. Identification of rat vimentin protein corresponding to spot #8170 of the 2-DE gel by PMF and database searches. (A) MALDI-PMF of spot #8170. (B) Sequence of vimentin with indications of the detected peptides (underline). The sequence coverage of vimentin reached 45%.

Table 1. List of protein spots identified by peptide mass fingerprinting.

Spot ID[a]	pVal[b]	Name[c]	NCBI ID[d]	Coverage[e] (%)	pI[f]	M_W[g] (kDa)	Ratio[h]
Cell growth and proliferating							
	Up-regulation						
7513	0	Liver regeneration-related protein LRRG03	gi|33187764	29.8	7.3	76.38	2.98
7687	0.002	Similar to lymphocyte cytosolic protein 1 (L-plastin)	gi|34875362	13.9	5.9	80.27	3.03
7783	0.002	Hnrpk protein (Hnrpk)	gi|38197650	26.7	5.2	51.01	2.71
8037	0.138	Regulator of G-protein signalling 3 (RGS)	gi|18644718	11	5.5	106.36	∞
8042	0.001	Cytokeratin 8 polypeptide (CK8)	gi|203734	21.2	5.5	52.69	3.54
8114	0	Vimentin	gi|14389299	38.6	5.1	53.71	4.27
8170	0	Vimentin	gi|14389299	45	5.1	53.71	6.75
8174	0	Vimentin	gi|14389299	45.1	5.1	53.71	2.88
8276	0	Vimentin	gi|14389299	36.3	5.1	53.71	3.32
8148	0.042	Actin-related protein 3 homolog (Arp 3)	gi|34879484	22.2	9.2	55.27	5.21
8534	0.001	Coordinates of Rat Map Kinase Erk2 with an Arginine Mutation at Position 52 (ERK2)	gi|1942172	24.2	6.7	42.11	2.54
7479	0.001	Albumin	gi|19705431	29.8	6.1	68.7	15.76
7652	0.001	Albumin	gi|19705431	33.2	6.1	68.7	7.93
8659	0.017	Annexin 1 (ANXA1)	gi|6978501	28.9	7	38.81	3.83
8668	0.011	Heterogeneous nuclear ribonucleoprotein A3 variant b (hnRNP A3)	gi|34327779	38.4	8.7	37.07	5.25
8679	0		gi|34327779	40.1	8.7	37.07	2.55
8766	0	Similar to heterogeneous nuclear ribonucleoprotein A2/B1 (hnRNP A2/B1)	gi|34855868	49.6	8.7	35.99	2.10
8937	0	Galectin-3	gi|1170759	25.6	8.6	27.18	3.35
9337	0.041	Proteasome (prosome, macropain) subunit, beta type 3	gi|8394082	26.8	6.1	22.95	2.85
	Down-regulation						
4946	0	*L*-arginine: glycine amidinotransferase (AGAT)	gi|13591949	38.3	7.2	48.22	0.58
5724	0.047	Similar to cisplatin resistance-associated overexpressed protein	gi|34873078	11.4	9.2	42.88	0.27
Glucose metabolism							
	Up-regulation						
7665	0.001	Similar to ATPase, H+ transporting, V1 subunit A, isoform 1	gi|34869154	19.4	5.4	68.25	7.43
8553	0	Aldolase A	gi|6978487	40.1	8.8	39.33	2.87
8768	0	Lactate dehydrogenase A (LDH)	gi|8393706	43.4	8.7	36.43	2.24
8908	0.028	Similar to glyceraldehyde-3-phosphate dehydrogenase	gi|34932247	12.5	6.8	43.94	∞
8983	0	Phosphoglycerate mutase 1	gi|8248819	52	7.1	28.83	2.19
	Down-regulation						
4290	0.027	Similar to phosphoenolpyruvate carboxykinase 2	gi|34874156	20.1	6	35.78	0.40
5001	0	3-methyl-2-oxobutanoate dehydrogenase (lipoamide) alpha chain precursor	gi|66044	47.2	7.8	50.15	0.40
5038	0	Fumarase	gi|227665	31	9.3	54.45	0.64
5832	0.042	Electron-transfer-flavoprotein, beta polypeptide	gi|51259425	35.3	7.8	27.67	0.34
5883	0.003	Ubiquinol-cytochrome-c reductase Rieske iron-sulfur protein precursor	gi|111883	30.9	9.2	27.67	0.22
Lipid metabolism							
	Up-regulation						
9087	0	Preproapolipoprotein A-I	gi|55747	43.6	5.5	30.07	6.50
	Down-regulation						
4578	0	Pancreatic lipase related protein 1 (PLRP1)	gi|14091772	30	5.8	52.36	0
4605	0	Pancreatic lipase related protein 1 (PLRP1)	gi|14091772	37.2	5.8	52.36	0.15
4930	0	Pancreatic lipase	gi|6981376	33.1	6.3	51.42	0.52
5368	0.011	Similar to acyl-Coenzyme A dehydrogenase family, member 8	gi|34866733	39.5	6.6	28.45	0.33
5662	0.006	2,4-dienoyl-CoA reductase (NADPH2)	gi|111287	26.6	9.3	36.11	0
5841	0.002	Chain F, Enoyl-Coa Hydratase Complexed with Octanoyl-Coa	gi|3212683	24.9	6.4	28.27	0.30

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Spot ID[a]	pVal[b]	Name[c]	NCBI ID[d]	Coverage[e] (%)	pI[f]	M_W[g] (kDa)	Ratio[h]
5845	0.001	Enoyl Coenzyme A hydratase, short chain, 1, mitochondrial	gi|17530977	36.6	8.8	31.5	0.61
Amino acids metabolism							
	Down-regulation						
5183	0.001	Similar to 2-amino-3-ketobutyrate-coenzyme A ligase	gi|34867034	27.4	7.9	45.18	0
5194	0.001	Similar to 2-amino-3-ketobutyrate-coenzyme A ligase	gi|34867034	21.9	7.9	45.18	0.60
5226	0.015	Isovaleryl Coenzyme A dehydrogenase	gi|6981112	21.5	8.5	46.42	0.32
5241	0.033	Isovaleryl Coenzyme A dehydrogenase	gi|6981112	17.5	8.5	46.42	0
5365	0	Branched chain aminotransferase 2, mitochondrial (BCAT)	gi|11693174	38.2	9.1	44.26	0.45
5378	0	Branched chain aminotransferase 2, mitochondrial (BCAT)	gi|11693174	33.3	9.1	44.26	0.37
Regulation of gene expression (Level on DNA, RNA, Protein)							
	Down-regulation						
3789	0.009	Eef2 protein	gi|38511951	12.2	6.4	93.54	0.52
3887	0.037	Similar to Impdh1 protein	gi|34855047	12.7	6.1	60.72	0
4431	0.034	Stress-induced-phosphoprotein (Hsp70/Hsp90-organizing protein)	gi|20302113	15.3	6.4	62.55	0
4786	0	Similar to eukaryotic translation initiation factor 2, (eIF2) subunit 3, structural gene X-linked	gi|34880581	29.7	9.1	51.06	0.47
5411	0.001	Eukaryotic translation initiation factor 2, subunit 1 (eIF2)	gi|9506571	34.3	5	36.09	0.05
5418	0	Eukaryotic translation initiation factor 2, subunit 1 (eIF2)	gi|9506571	38.7	5	36.09	0.36
5493	0	Rhodanese	gi|57069	39.7	8	33.16	0.53
5450	0	Acidic ribosomal protein P0	gi|11693176	44.2	5.9	34.2	0.25
5814	0	High mobility group (HMG) box 1	gi|52789475	36.7	5.6	24.88	0.06
Stress and imflammation							
	Up-regulation						
8139	0	Fibrinogen gamma chain precursor	gi|1346007	25.4	5.4	50.61	5.19
8279	0.001	Serpinh1 protein (HSP47)	gi|55824765	27.1	9	46.54	2.8
8357	0.039	Similar to serine (or cysteine) proteinase inhibitor, clade A (alpha-1 antiproteinase, antitrypsin), member 9	gi|34935459	20.1	9.9	46.82	5.67
8488	0	Similar to serine (or cysteine) proteinase inhibitor, clade B, member 1b	gi|34875374	35.9	5.9	42.71	∞
8799	0	Fibrinogen alpha subunit	gi|790486	19.3	7.8	60.47	4.58
	Down-regulation						
5607	0.001	Annexin A4	gi|55742832	30.1	5.4	35.88	0.08
5835	0.043	beta-globin	gi|56252	36.7	9	15.97	0.60

[a] Spot ID: defined according to spot positions in 2-D gel indication as in Figure 1.
[b] pVal: the possibility that the observed match is a random event.
[c] Name: name of each matched protein in NCBInr database.
[d] NCBI: NCBInr database accession number.
[e] Coverage: percent of identified sequence to the complete sequence of the known protein.
[f] pI: theoretical isoelectric point of the matching protein.
[g] M_W: theoretical molecular weight of the matching protein in kDa.
[h] Ratio: the ratio of average volume of each spot in Px pancreas to Sx pancreas.

A total of 75.82% (69/91) of selected spots could be identified by MALDI-ToF-MS analysis, whereas some corresponded to the same protein. For example, spots #8114, #8170, #8174, and #8276 were identified as the same protein, vimentin (Table 1, Figure 1B). In total, 53 differentially expressed proteins were identified by PMF, including cell proliferation-related proteins, lipid and energy metabolism-related proteins, protein and amino acid metabolism-related proteins, and signal transduction and acute-phase response proteins (Table 1).

Discussion

The rat partial pancreatectomy model has been widely

used to study diabetes and pancreatic stem cells because it provides a setting in which the remnant pancreas undergoes regeneration to compensate for the inefficiency of β-cells. In the present study we attempted to address the molecular basis of β-cell neogenesis at the tissue level *in vivo*, regardless of whether new β-cells are differentiated from stem cells or duplicated from old β-cells. The strategy used has advantages, and should more accurately reflect the molecular regulation mechanism than cell-level studies *in vitro* could, and it should also reveal other important pathological changes accompanying β-cell neogenesis, which have remained undetected in previous studies. Our study has identified several proteins whose expression was significantly altered in pancreatectomized rats. In the following sections, the possible functions of these proteins will be discussed.

Cell growth and proliferation Several proteins that appear to be involved in cell growth, proliferation and related processes were found to be upregulated in pancreatectomized rats (Table 1). ANXA1 has a significant role in several physiological and pathological processes, including anti-inflammation[10], cell growth, differentiation, apoptosis, membrane fusion, endocytosis and exocytosis[11–14], and regulation of endocrine function[15]. ERK2 (one member of the ERK family) appears to be the major transducer of proliferative signals to the nucleus[16]. A series of studies provide evidence for Hnrpk protein involvement in cellular processes such as proliferation and apoptosis[17–19]. In breast cancer cells, Hnrpk significantly enhances cell proliferation[18].

Other proteins associated with embryogenesis and cell differentiation have been found to be upregulated in pancreatectomized rats. *L*-plastin has been detected in the early stages of intestinal epithelial cell differentiation until day 14.5, and was localized to the basal surface of the epithelium, but by day 16.5 no *L*-plastin was detected in the epithelium[20]. This result indicates that *L*-plastin plays a role during intestinal epithelial cell differentiation. Cytokeratin 8 is the early and fundamental keratin expressed during development in many vertebrates[21,22], and is the main keratin present in hyperproliferative human cells[23,24]. Vimentin is another intermediate filament (IF) protein, which has already been reported to be rapidly induced during the process of epithelial-mesenchymal transition and in rapidly proliferating porcine and human pancreatic duct cells[25,26]. However, mature pancreatic epithelial cells no longer express vimentin protein[27]. A study of the hnRNP A2/B1 expression revealed a regulated expression pattern during fetal development, and down-regulation in normal adult tissues[28], but re-overexpression occurred during lung cancer progression[29]. *L*-arginine: glycine amidinotransferase (AGAT), which is downregulated in pancreatectomized rats, catalyzes the committed step in creatine biosynthesis. A series of studies found that AGAT fulfiled a function in energy metabolism while also playing an important role during early embryonic development; during embryogenesis AGAT is preferentially expressed around the blastopore and later in the notochord of the neurula and tailbud stages[30].

The potential identification, expansion, and differentiation of adult pancreatic stem cell(s) raises the possibility of there being enough islets for widespread β-cell replacement therapy. Recent immunohistochemical observations suggest that the expression of PDX-1 in pancreatic duct epithelium is upregulated under conditions of pancreatic regeneration in Px rats, whereas it is transiently expressed in the embryonic stem cells in foregut endoderm and functionally directs them toward a pancreas-specific cell fate in pancreogenesis[1,31–33]. The authors of these studies considered that the duct cells expressing the PDX-1 protein transiently regained their multipotency as progenitor cells in the adult pancreas, and that PDX-1 protein would be one of the markers of adult pancreatic stem cells. According to this hypothesis, these differentially expressed proteins related to embryogenesis and cell differentiation may be potential markers of pancreatic stem cells. It is exciting that vimentin protein has been considered as another useful marker for a low-level differentiation stage of pancreatic ductal cells, and one that correlates with the precursor/progenitor stage during the process of β-cell neogenesis[34]. Using a proteomic approach, our own work revealed that other new proteins (*L*-plastin, hnRNP A2/B1 and AGAT) shared similar characters with PDX-1, besides CK8, and vimentin. We postulate that these proteins are associated with β-cell neogenesis and may be new potential stem cell markers.

Glucose metabolism Four proteins involved in the glycolytic cycle were induced 3 d after Px. Aldolase A catalyzes a reversible aldol condensation, which cleaves fructose 1,6-bisphosphate to yield two different triose phosphates, glyceraldehyde-3-phosphate and dihydroxyacetone phosphate. Glyceraldehyde 3-phosphate dehydrogenase catalyzes the phosphate-independent irreversible oxidation of *D*-glyceraldehyde 3-phosphate to 3-phosphoglycerate. The enzyme phosphoglycerate mutase catalyzes a reversible conversion of 3-phosphoglycerate to 2-phosphoglycerate. *L*-lactate dehydrogenase (LDH) is the enzyme involved in the final step of anaerobic glycolysis, which catalyzes the interconversion of L-lactate and pyruvate with nicotinamide adenine dinucleotide (NAD+) as a coenzyme.

Several proteins were found to be functionally related to aerobic oxidation, the citric acid cycle and gluconeogenesis:

lipoamide, fumarase and phosphoenolpyruvate carboxykinase were downregulated after pancreatectomy. Lipoamide is a cofactor in the pyruvate dehydrogenase complex, which catalyzes the oxidative decarboxylation of pyruvate with concomitant formation of CO_2, acetyl-CoA and NADH. Fumarase catalyzes a reversible hydration of fumarate to *L*-malate. Phosphoenolpyruvate carboxykinase plays a central role in glucose homeostasis as one of the rate-limiting enzymes in gluconeogenesis, which catalyzes the decarboxylation and mononucleoside triphosphate (NTP)-dependent phosphorylation of oxaloacetate (OAA) to form phosphoenolpyruvate (PEP) and nucleoside diphosphate (NDP).

Taken together, these data suggest that the pancreas displays substantial anaerobic glycolysis in pancreatectomized rats; the flux of metabolites from glucose into lactate is enhanced in pancreatic neogenesis. However, the modified expression of these enzymes may fulfill other biological functions. For example, aldolase A also has an effect on the promotion of cell growth when overexpressed[35], and GAPDH is a key transcriptional coactivator necessary for entry into S phase[36].

Lipid metabolism Pancreatic lipase secreted by the exocrine pancreas into the duodenum of the intestine, cleaving triglycerides into monoglycerides and free fatty acids, was downregulated in Px rats. In addition to pancreatic lipase, the lipase gene family also encodes other two homologous proteins, pancreatic lipase related proteins 1 and 2 (PLRP1 and PLRP2). PLRP1 has displayed no significant activity with respect to any of the substrates tested, and its physiological role is still unknown. Studies on the expression pattern of PLRP1 in rats showed that the mRNA encoding PLRP1 was mainly expressed shortly after birth and then decreased to a low level as compared with pancreatic lipase[37]. In the present study, pancreatic lipase and PLRP1 were both downregulated on d 3 after Px, the determination of which ultimately required a study of sequence expression patterns during pancreatic regeneration.

Three enzymes involved in fatty acid oxidation had decreased expression levels after pancreatectomy. Acyl-CoA dehydrogenase catalyzes the dehydrogenation of fatty acyl-CoA to produce a double bond between the α and β carbon atoms (C-2 and C-3), yielding a trans-Δ^2-enoyl-CoA. Enoyl-CoA hydratase plays a key role in fatty acid metabolism by catalyzing the reversible addition of water to trans-Δ^2-unsaturated enoyl-CoA thioesters. 2,4-Dienoyl-CoA reductase an auxiliary enzyme was needed for β oxidation of the common unsaturated fatty acids.

It has been shown that the rate of lipid peroxidation is reduced in regenerating pancreas following Px, which accords with the general hypothesis that increased cell proliferation is associated with a decreased rate of lipid peroxidation[38]. This suggest that the decrease in lipid peroxidation is another important event during pancreatic regeneration. However, defective triglyceride digestion (pancreatic lipase) and insufficient fatty acid catabolism would ineffectively generate high-energy metabolites and phospholipids required for cytoplasmic membrane formation[39]. In this context, modulation of defective lipid metabolism in pancreatectomized rats might be of therapeutic value.

Amino acid metabolism and protein synthesis The expression levels of several enzymes related to amino acid metabolism were downregulated. 2-Amino-3-ketobutyrate-coenzyme A ligase is an enzyme associated with the conversion of *L*-threonine to glycine through a 2-step biochemical pathway[40]. Isovaleryl-CoA dehydrogenase catalyzes the conversion of acyl-CoA thioesters to the corresponding trans-2-enoyl-CoA, which is involved in leucine degradation[41]. Branched chain aminotransferases (BCAT) catalyze the transamination of the branched chain amino acids leucine, isoleucine, and valine to their respective α-keto acids, α-ketoisocaproate, α-keto-h-methylvalerate, and α-ketoisovalerate[42].

Protein synthesis also seems to be inhibited in the remnants of the pancreas after Px. Several components of the translational machinery that regulates protein synthesis were observed to be downregulated in our pancreatic regeneration model. Eukaryotic initiation factor eIF2, which is composed of 3 subunits (a, b, and c), mediates the binding of the initiator methionyl-tRNA (Met-tRNAi) to the ribosome during the initiation of translation of all cytoplasmic mRNAs in eukaryotic cells[43]. Eukaryotic elongation factor 2 (eEF2) promotes ribosomal translocation and is involved in eukaryotic polypeptide chain elongation[44] and some types of post-translational modification[45–47]. We also found another factor: high-mobility-group (HMG) protein, a non-histone DNA-binding protein, was downregulated in pancreatectomized rats. HMG box proteins are generally considered to participate in maintaining the structure of chromatin and to mediate gene expression, replication, recombination and repair[48].

The effects of amino acid metabolism and protein synthesis on growth and development of the pancreas are largely unknown. Ip *et al* reported that pancreatic protein synthesis was transiently low in animals at birth[49]. Our data suggest that the decrease in protein synthesis of regenerating pancreas was similar with that in pancreatic postnatal development.

The proteome profiling technique used in the present study provided a broad-based and effective approach for

the rapid assimilation and identification of adaptive protein changes during pancreatic regeneration induced by pancreatectomy. Changes in the expression of proteins that we documented after Px reflect the involvement of various regulation mechanisms: transcription, translation, post-translation, signal conduction, cell cycle, apoptosis, cellular energy and metabolic pathways, which would be valuable targets for further investigation. Additionally, information about the dynamic expression pattern of the regenerating pancreas at different time points after Px would also be very valuable. Recently, Shin *et al* investigated differential expression on the 2nd day after 60% Px[50], which can be added to our data from the 3rd day after 90% Px to illuminate the molecular mechanism of pancreatic regeneration. In summary, our data elucidate the global proteome during pancreatic proliferation and differentiation, which is very important and will lead to a better understanding of the regulation mechanism of pancreatic regeneration, and ultimately assist in reaching the target of discovering protein biomarkers for pancreatic stem cells.

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