Phosphorylation of carboxypeptidase B1 protein regulates β-cell proliferation

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Abstract. A reduction in pancreatic islet β-cells leads to the onset of diabetes. Hence, the identification of the mechanisms inducing β-cell proliferation is important for developing a treatment course against the disease. It has been well established that post-translational modifications (PTMs) of proteins affect their functionality. In addition, PTMs have been suggested to play important roles in organ regeneration. Therefore, in this study, we investigated PTMs associated with pancreatic regeneration using two-dimensional electrophoresis. Four carboxypeptidase B1 (CPB1) proteins were identified at different isoelectric points, with the same molecular weight. The motif of CPB1 PTMs was identified by mass spectrophotometry, and the downregulation of CPB1 phosphorylation in pancreatectomy was confirmed. The dephosphorylation of CPB1 induced β-cell proliferation. We thus surmise that the altered PTM of CPB1 is associated with pancreatic regeneration.

Introduction

The loss of β-cell mass in pancreatic islets is closely associated with type 1 and 2 diabetes mellitus. The regeneration of β-cells for curative treatment of the disease is an important topic of research. New β-cells are derived from either exogenous sources, such as stem cells or endogenous sources, such as facultative pancreatic progenitors (1-3). Pancreatic injury models (4-7) have suggested several endogenous sources for β-cell regeneration. Surviving β-cells may generate new β-cells by self-duplication (8), and new β-cells may arise through transdifferentiation from other pancreatic cells, such as α- and δ-cells (9,10). Moreover, β-cell neogenesis may develop from multipotent pancreatic progenitor cells (11,12). These studies indicate the plasticity of the pancreas. However, the molecular mechanisms of β-cell neogenesis are not well known.

A number of studies have identified genes differentially expressed in pancreatic neogenesis by using 'omics' methods, such as cDNA microarray analysis and two-dimensional electrophoresis (2-DE) (13-18). De León *et al* suggested endoderm transcription factor Foxa2 (also known as HNF3β) as a potential candidate for the improvement of pancreatic growth and function (15). Moreover, lymphocyte cytosolic protein 1 (LCP1), which is upregulated by partial pancreatectomy, has been suggested as an important protein for pancreatic regeneration owing to an increase in pancreatic cell proliferation and regulation of islet markers (18). In addition, various transcription factors, such as neurogenin 3 (19), paired related homeobox 1 (Prrx1) (20) and pancreatic and duodenal homeobox 1 (Pdx1) (21) regulate pancreatic regeneration. However, protein activity or function is regulated by the expression level, as well as post-translational modifications (PTMs). Mutations of PTM sites cause various diseases (22); in particular, differences in PTMs between chronic pancreatitis and non-pancreatitis have been reported (23). Pdx1 is known to be an important regulator of β-cell maturation, and its activity is a prerequisite for the regulation of blood glucose homeostasis. Various studies have suggested that Pdx1 activity is regulated by phosphorylation as one of the types of PTMs (21,24-28). Frogne *et al* recently identified a single phosphorylation site in Pdx1 by isoelectric focusing (IEF) (29). In a partial hepatectomy model, Yes-associated protein (YAP) activity was found to regulate liver regeneration by phosphorylation (30). Moreover, the phosphorylation of RelA (p65), a subunit of nuclear factor-κB (NF-κB), has been reported as an important mechanism in liver regeneration and cancer (31). Therefore, the identification of PTM alterations during injury and regeneration may provide novel mechanistic insight into organ regeneration. In this study, to analyze the potential association between PTMs and β-cell replication, we investigated PTMs associated

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with pancreatic regeneration using 2-DE analysis and further examined the tyrosine phosphorylation level of CPB1.

Materials and methods

Partial pancreatectomy. To investigate proteins associated with pancreatic regeneration following pancreatectomy, 8-week-old male Sprague-Dawley rats (Daehan Experimental Animals, Seoul, Korea) were anesthetized with isoflurane (Santa Cruz Animal Health®, Paso Robles, CA, USA) and divided into 3 treatment groups as follows: partial pancreatectomy (n=2; approximately 90% pancreatectomy), sham surgery (n=2) and no surgery (n=2). The animals were allowed free access to standard diet and water before and after the operation. Partial pancreatectomy was executed according to Bonner-Weir *et al* (32). In brief, 8-week-old male Sprague-Dawley rats (Daehan Experimental Animals) were anesthetized with 3% isoflurane in oxygen (O_2)/nitrous oxide (N_2O) mixtures, and 90% of the pancreatic tissue was then removed by gentle abrasion with cotton swabs, leaving the tissue between the common bile duct and the loop of the duodenum intact. For sham operation, the same surgical procedure was carried out without the removal of pancreatic tissue. On the 3rd day after surgery, the remnant pancreatic tissues were isolated under inhaled anesthesia (3% isoflurane in O_2/N_2O mixture) and the rats were then euthanized. The tissues were fixed with neutral buffered formalin (NBF) for histological examination, and immediately stored at -80˚C. The study protocol was approved by the Institutional Animal Care and Use Committee (IACUC) at Konyang University in accordance with the National Institutes of Health (NIH) Guidelines for the Care and Use of Laboratory Animals.

2-DE. Isolated pancreatic tissues were solubilized using a previously described method (33). Solubilized proteins were quantified with a Bradford protein assay kit (Bio-Rad, Hercules, CA, USA) and then stored at -80˚C until use. For resolution across the pH 4-7 range, 1 mg of protein extracted from the sham-operated and pancreatectomized pancreases was mixed with modified rehydration buffer [6 M urea, 2 M thiourea, 4% CHAPS, 2.5% DTT, 10% isopropanol, 5% glycerol and 2% immobilized pH gradient (IPG) buffer pH 4-7], loaded into a cup on the anodic side of a pH 4-7 IPG strip (GE Healthcare Bio-Sciences, Pittsburgh, PA, USA), and incubated for 12 h. Following rehydration, first-dimension IEF was performed on a Multiphor II IEF system (GE Healthcare Bio-Sciences). To separate the second dimension, an equilibrated IPG gel strip was placed on an 8-16% linear gradient SDS polyacrylamide gel and the gels were subjected to electrophoresis using the Ettan-DALT system (GE Healthcare Bio-Sciences). After 2-DE, the gels were stained using colloidal Coomassie brilliant blue (CBB) G-250 (Sigma-Aldrich, St. Louis, MO, USA). The stained gel was scanned with a GS-710 calibrated imaging densitometer (Bio-Rad), and proteins differentially expressed between pancreatectomized and sham operation samples were identified using the Melanie III image analysis software (Swiss Institute for Bioinformatics, Geneva, Switzerland). Two-DE analysis was performed three times independently. Differences among protein spots were analyzed using a two-way Student's t-test.

Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF/MS) and protein identification. For the identification of differentially expressed proteins, MALDI-TOF/MS analysis of selected spots was performed as previously described (33). Selected spots sliced from the gel were stained with CBB and then digested with trypsin. Extracted peptides were subjected to MALDI-TOF/MS analysis on a Voyager DE-STR MALDI-TOF mass spectrometer (Applied Biosystems, Foster City, CA, USA). All acquired spectra of samples were processed using Voyager™ 5.1 software (Applied Biosystems) in the default mode. Averages of 500 spectra were obtained for each sample, and scans were performed twice. Spectra were calibrated automatically upon acquisition using an external 3-point calibration. Peaks were manually assigned using the DATA Explorer™ software package (Applied Biosystems), and spectra were used to search against non-redundant protein sequence databases available online (SWISS-PROT and/or NCBInr Data Bank). The peptide mass fingerprinting data were applied to ProFound and MASCOT search engines (http://prowl.rockefeller.edu/; http://www.matrixscience.com/search_form_ select.html) for protein identification based on ProFound and MASCOT scores. The Z score of ProFound is the distance to the population mean in units of the standard deviation.

Western blot analysis. Pancreatic tissues and cell lines were lysed using RIPA buffer containing 50 mM Tris-HCl (pH 7.4), 1% NP-40, 0.25% sodium deoxycholate, 150 mM NaCl, 1 mM EDTA, and protease inhibitor cocktail (Roche, Basel, Switzerland). To investigate differences in CPB1 phosphorylation, we performed immunoprecipitation. Briefly, 800 μg of lysate was precleared with protein A-agarose for 2 h at 4˚C. The supernatant was incubated with anti-CPB1 (GenWay Biotech, San Diego, CA, USA) by shaking overnight at 4˚C, followed by incubation with protein A-agarose for 2 h. The beads were resuspended in 2X sample buffer and boiled for 10 min. The proteins were resolved on 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) gels and transferred onto polyvinylidene difluoride membranes (Millipore, Billerica, MA, USA). Immunoblots were incubated in 5% skim milk (Difco, Franklin Lakes, NJ, USA) for 1 h and probed with anti-tyrosine (1:1000; sc-7020; Santa Cruz Biotechnology, Inc., Dallas, TX, USA), anti-CPB1 (1:1000; GWB-31DED0; GenWay Biotech, San Diego, CA, USA) and anti-β-actin (1:3000; sc-47778; Santa Cruz Biotechnology, Inc.) primary antibodies overnight, followed by incubation with a horseradish peroxidase-conjugated anti-mouse or anti-rabbit secondary antibody for 2 h. Immunoreactive bands were detected using an enhanced chemiluminescence kit (ECL; Thermo Scientific, Waltham, MA, USA).

Immunohistochemical analysis. To identify alterations in CPB1 protein phosphorylation levels in the pancreas following pancreatectomy, the sham-operated and partially pancreatectomized pancreases were removed and fixed with NBF overnight at room temperature. Fixed tissues were embedded in paraffin and the paraffin blocks were sliced at 5 μm thicknesses using a tissue microtome (Leica, Nussloch, Germany). The slides were blocked with 10% goat serum (Vector Laboratories, Inc., Burlingame, CA, USA) and incubated with phospho-Tyr antibody (sc-7020; Santa

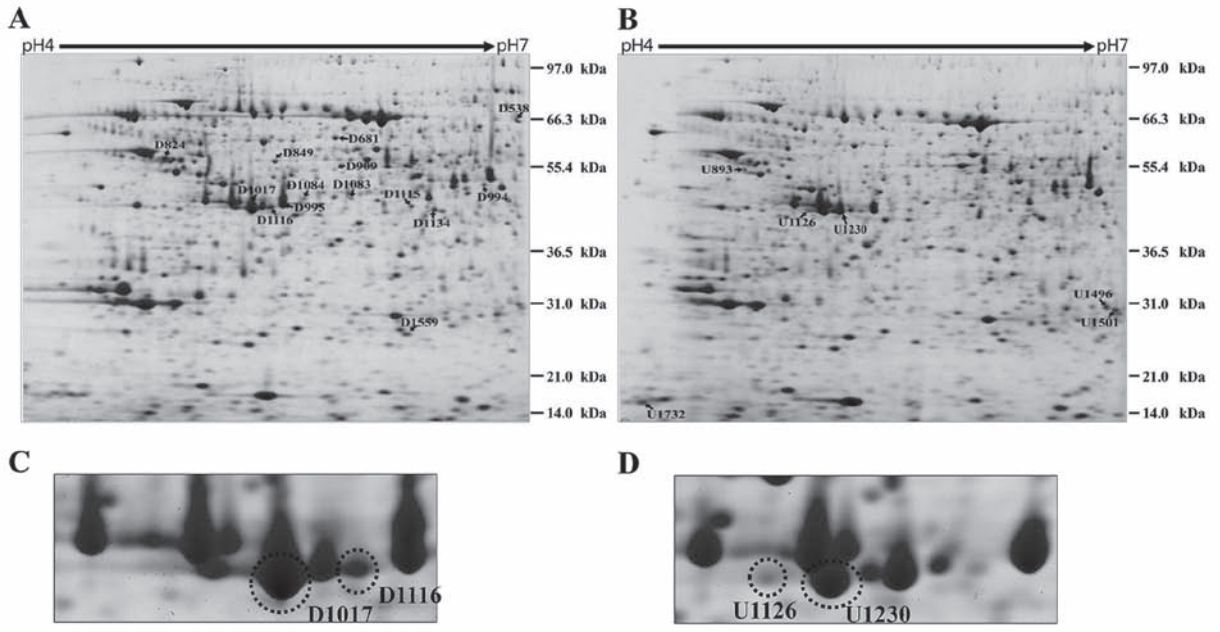

Figure 1. Two-dimensional electrophoresis (2-DE) profiling of pancreatic proteins in (A and C) sham-operated and (B and D) partially pancreatectomized rats. The arrows in (A) indicate downregulated proteins in rats subjected to partial pancreatectomy vs. the sham-operated rats, and the arrows in (B) indicate upregulated proteins in rats subjected to partial pancreatectomy vs. the sham-operated rats. (C and D) Carboxypeptidase B1 (CPB1) proteins of different isoelectric points on the 2-DE profile.

Cruz Biotechnology, Inc.), and CPB1 antibody (GWB-31DED0; GenWay Biotech) as primary antibodies at 4˚C overnight and then with Cy2-conjugated goat anti-rabbit IgG (111-225-144) and Cy3-conjugated goat anti-mouse IgG (115-165-146) secondary antibodies (both from Jackson Immuno Research, West Grove, PA, USA) as secondary antibodies at room temperature for 1 h. Nuclei were counterstained with 4',6'-diamidino-2-phenylindole dihydrochloride (Molecular Probes, Waltham, MA, USA). The stained cells were examined under a confocal microscope (Carl Zeiss, Oberkochen, Germany).

DNA constructs and mutagenesis. Total RNA was isolated from the rat pancreases using TRI Reagent® (Ambion, Waltham, MA, USA) according to the manufacturer's instructions. Approximately 1.2 kb cDNA of CPB1 (NM_012533.1) was obtained from rat pancreatic RNA by RT-PCR using the following primers: 5'-GCC GCC ACC ATG TTG CTG CTA CTG GCC CT-3' (forward) and 5'-GGT CCA ATT GGT CAA CAC ACC CA-3' (reverse), and the cDNA was cloned (CPB1OE) into the E*co*RI site of pcDNA3-EGFP (Addgene, Cambridge, MA, USA). Mutations of 6 tyrosine sites of the CPB1 protein (Y298F, Y304F, Y310F, Y312F, Y314F and Y316F) were introduced (CPB1OE-mutY) using the QuikChange® Site-Directed Mutagenesis kit (Stratagene, La Jolla, CA, USA) according to the manufacturer's instructions. Mutated sites were confirmed by sequencing.

Cell culture and transfection. RIN-m insulinoma cells obtained from the American Type Culture Collection (ATCC, Manassas, VA, USA) were cultured in RPMI-1640 (ATCC) supplemented with 10% fetal bovine serum (Gibco, Carlsbad, CA, USA) and 1% penicillin/streptomycin (Lonza, Basel, Switzerland). The cells were maintained at 37˚C in a humidified atmosphere containing 5% CO_2. The RIN-m cells were transfected with control pcDNA3-EGFP (Addgene), a rat *CPB1* full-length cDNA vector (CPB1OE) and mutant rat *CPB1* full-length cDNA vector (CPB1OE-mutY) using Lipofectamine 2000 (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions. To establish stable cell lines, transfected clones were cultured in selective media containing 150 μg/ml G418 (Sigma-Aldrich).

Cell viability. To identify the association between the phosphorylation of CPB1 protein and cell viability, stable cell lines (control, CPB1OE and CPB1OE-mutY) were seeded in 12-well plates at a density of $5x10^5$ cells/well. Cell viability was measured at 6, 24 and 48 h. Cells were collected and mixed with 0.4% trypan blue stain solution (Gibco), after which unstained cells were counted under a microscope (Olympus, Tokyo, Japan).

Statistical analyses. All graphed data are presented as the means ± standard deviation. The results were analyzed using analysis of variance (ANOVA) or a Student's t-test. P-values of <0.05 and <0.01 were considered to indicate statistically significant and highly statistically significant differences, respectively.

Results

Identification of proteins up- or downregulated by partial pancreatectomy. To identify proteins associated with pancreatic regeneration, we compared proteins in pancreatic tissues obtained from rats at 3 days after partial pancreatectomy with those of sham-operated rats by 2-DE analysis with IEF gel electrophoresis on pH 4-7 linear IPG strips. Independent experiments were each carried out in duplicate. Melanie III detected differential intensity in 20 spots; 14 proteins were downregulated and 6 were upregulated in the rats subjected to compared with the sham-operated rats (Fig. 1A and B).

Table I. Identification of proteins differentially expressed in partial pancreatectomy in the pH 4-7 range.

Spot ID	Est'd Z[a]	Accession no.	Protein information	Coverage (%)	pI	kDa
Downregulated proteins						
D538	2.39	NP_001101847.2	Phosphoenolpyruvate carboxykinase 2 (mitochondrial)	33	8.4	71.79
D681	2.4	NP_599153.2	Serum albumin precursor	43	6.1	71.23
D824	1.55	AAA40788.1	α-1-antitrypsin precursor	50	5.7	46.03
D849	2.39	AAA41082.1	Vitamin D-binding protein	46	5.8	55.49
D909	1.85	EDL88549.1	*Rattus norvegicus* (Norway rat) albumin, isoform CRA_a	27	6.8	53.46
D994	2.22	NP_001004206.1	Proliferation-associated protein 2G4	47	6.4	44.07
D995	2.4	P00731.2	Carboxypeptidase A1	40	5.5	47.19
D1017	2.18	NP_036665.1	Carboxypeptidase B precursor	26	5.4	48
D1083	1.47	NP_001101857.2	Succinyl-CoA ligase [ADP-forming] subunit β, mitochondrial	33	6.1	47.8
D1084	1.82	NP_599153.2	Serum albumin precursor	24	6.1	71.23
D1115	2.3	EDL77312.1	*Rattus norvegicus* (Norway rat) rCG25777, isoform CRA_a	41	5.9	42.36
D1116	2.3	NP_036665.1	Carboxypeptidase B precursor	49	5.4	48
D1134	2.4	NP_036724.1	Isovaleryl-CoA dehydrogenase, mitochondrial precursor	34	8.5	46.44
D1559	2.2	NP_476484.1	Protein DJ-1	71	6.3	20.24
Upregulated proteins						
U893	2.3	NP_112402.1	Vimentin	46	5.1	53.75
U1126	2.04	NP_036665.1	Carboxypeptidase B precursor	39	5.4	48
U1230	55[b]	NP_036665.1	Carboxypeptidase B precursor	39	5.4	48
U1496	2.36	XP_345279.4	Amylase 2a5, pancreatic	27	9.3	50.58
U1501	2.36	NP_445742.1	Phosphoglycerate mutase 1	56	7.1	28.97
U1732	2.16	XP_573831.1	Ribosomal protein P2-like	84	4.4	11.69

[a]Z score is the distance to the population mean in units of standard deviation. It also corresponds to the percentile of the search in the random match population. Conceptually, this 95th percentile is different from 95% confidence that the search is a correct identification. The following is a list for Z score and its corresponding percentile in an estimated (Est'd) random match population: [(Z: percentile) 1.282:90, 1.645:95.0, 2.326:99.0, 3.090:99.9)]. [b]Mowse score is -10xlog(P), where P is the probability that the observed match is a random event. Protein scores >51 are significant ($p<0.05$).

The differentially expressed proteins were identified by MALDI-TOF/MS (Table I). Among the identified proteins, phosphoenolpyruvate carboxykinase 2 (PCK2) has been reported to be differentially expressed in pancreatectomy (18). Amylase is also specifically expressed in the pancreas (34). Succinate-CoA ligase ADP-forming β subunit (Sucla2) and isovaleryl-CoA dehydrogenase (Ivd), which are involved in the mitochondrial Krebs cycle, were downregulated upon pancreatectomy. Mitochondrial function and plasticity are associated with glucose circulation (35). Interestingly, carboxypeptidase B (CPB1) precursor protein was identified from 4 spots at different PIs among the 20 spots (Fig. 1C and D). Therefore, we selected CPB1 for further study.

Post-translational modification of CPB1. PTMs of proteins such as phosphorylation or dephosphorylation induce shifts in the PI and regulate the functional activity of enzymes (36). Therefore, we investigated alterations in the phosphorylation level of CPB1 in normal, sham-operated and partially pancreatectomized pancreases using the immunoprecipitation method. Phosphorylation of tyrosine residues of the CPB1 protein was significantly reduced by partial pancreatectomy (Fig. 2A). Immunohistochemistry confirmed that the phosphorylation of the tyrosine residue of CPB1 in partial pancreatectomy decreased (Fig. 2B). These results suggested that PTM of the CPB1 protein may be associated with pancreatic regeneration.

Reduced phosphorylation of CPB1 induces pancreatic cell proliferation. An increase in β-cell mass is derived from β-cell neogenesis, proliferation and hypertrophy, and β-cell proliferation gradually declines with age (37). However, partial pancreatectomy models in mice and rats have been reported to enhance β-cell replication (38). Therefore, we investigated

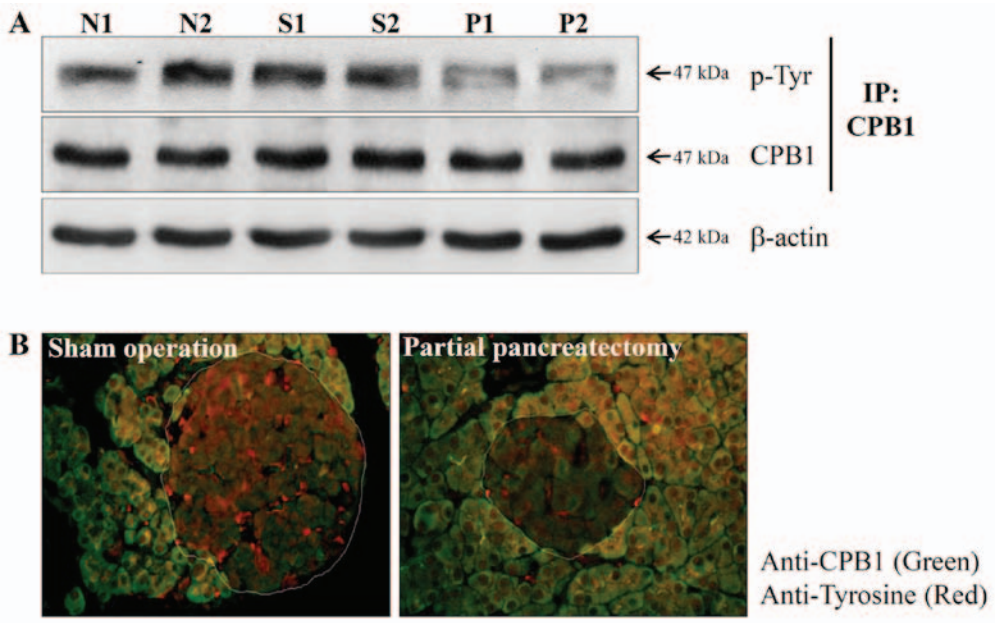

Figure 2. Phosphorylation of carboxypeptidase B1 (CPB1) protein is decreased by partial pancreatectomy. (A) The phosphorylation levels of CPB1 protein among normal (N), sham-operated (S), and partial pancreatectomized (P) pancreases were analyzed by western blotting after immunoprecipitation with anti-CPB1. (B) The phosphorylated tyrosine of CPB1 protein between sham operation and partial pancreatectomy was observed by immunohistochemistry. Images were captured at x200. Green, CPB1 protein; red, phosphorylated tyrosine.

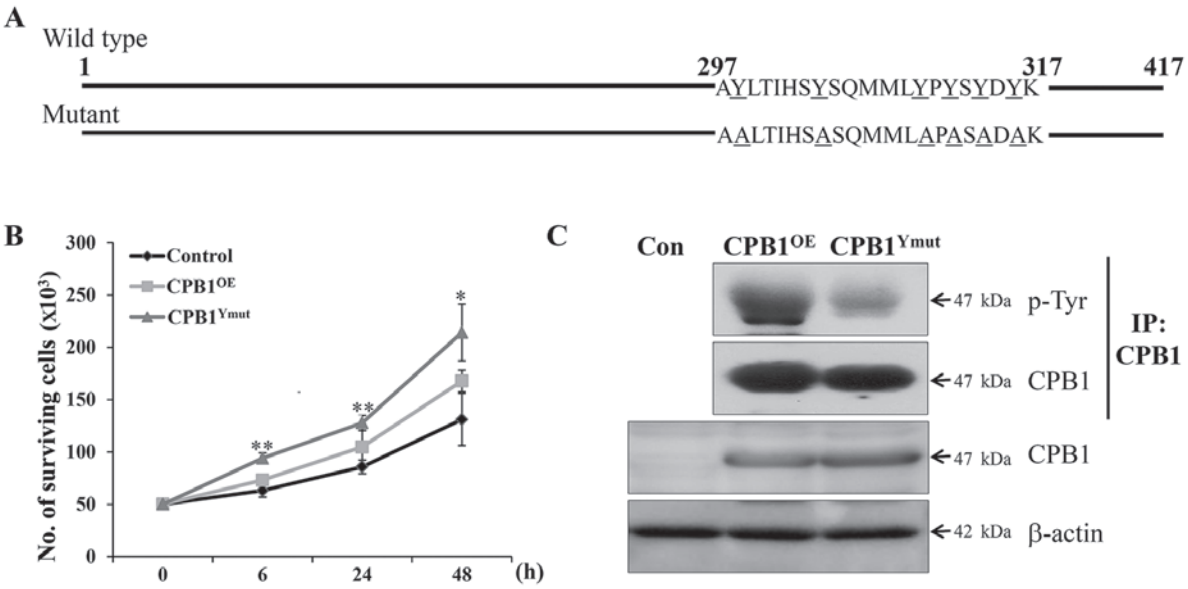

Figure 3. Reduced phosphorylation of carboxypeptidase B1 (CPB1) protein promotes pancreatic cell survival. (A) Schematic diagram representing the part of wild-type CPB1 modified by partial pancreatectomy as identified by mass spectrophotometry. In mutant CPB1, phenylalanine is substituted by tyrosine in the modification site. (B) Overexpression of CPB1 increases pancreatic cell survival, and decreased phosphorylation of CPB1 further increases cell survival. Data represent means ± SDs of three independent experiments. $^{**}P<0.01$; $^{*}P<0.05$. (C) CPB1-overexpressing stable cell lines were identified by western blot analysis, and the phosphorylation levels of wild-type and mutant proteins were investigated by immunoprecipitation.

whether the PTM of CPB1 was involved in β-cell replication. First, the PTM region of the CPB1 protein was mutated by phenylalanine-to-tyrosine substitution to reduce the phosphorylation level (Fig. 3A). Mutations at 6 sites (Y298F, Y304F, Y310F, Y312F, Y314F and Y316F) were confirmed by sequencing.

Rat β-cell lines overexpressing wild-type or mutant CPB1 were established. CPB1-overexpressing (CPB1OE) cell lines showed higher proliferation than the control (Fig. 3B). In addition, reduced tyrosine phosphorylation in the CPB1OE-mutY cell line resulted in increased proliferation compared to that in CPB1OE cells. To confirm the reduced tyrosine phosphorylation of CPB1, we performed immunoprecipitation of the CPB1 antibody and detected phosphorylated CPB1 by western blotting. Tyrosine phosphorylation of CPB1 in CPB1OE-mutY cell lines decreased (Fig. 3C). Therefore, we clearly demonstrated that the dephosphorylation of CPB1 was associated with pancreatic regeneration.

Discussion

In the present study, we applied 2-DE analysis to investigate PTMs of proteins related to pancreatic regeneration. However, the present study has certain limitations, one of these being the small sample size. Although the experimental group was not sufficiently large enough for a comprehensive protein hunt, we used it only to screen for proteins differentially expressed in pancreatectomy, and we do not claim complete coverage. We observed 20 spots, including 4 CPB1 spots having different PIs, and we confirmed the modification of CPB1 by MALDI-TOF/MS. Carboxypeptidases have been examined for their functions in digestion, and several studies on the synthesis of mature proteins or their regulation of biological processes are underway. On the basis of their active site mechanism, carboxypeptidases are divided into three classes: metallocarboxypeptidases, serine carboxypeptidases, and cysteine carboxypeptidases. CPB is a metallocarboxypeptidase, and it has two isoforms: CPB1 and CPB2 (39). CPB was the first pancreas-specific protein identified from proteins differentially expressed between the normal pancreas and pancreatic carcinoma (40). Moreover, CPB has been suggested as a serum marker for acute pancreatitis and dysfunction of pancreatic transplants (41-43).

CPB1 is secreted as a procarboxypeptidase form, together with other pancreatic proenzymes, and is converted to the active form through the action of trypsin (44-46). Only few PTMs of CPB1 have been reported thus far. 3-Nitrotyrosine has been reported to be a type of oxidative PTM of CPB1, and nitration of tyrosine plays an important role in regulating enzyme activity. Sites of tyrosine nitration are located at Try-198 and Tyr-248 in the catalytic site of CPB1 (47,48). However, there is no report on the association between PTMs of CPB1 and pancreatic regeneration. We identified and confirmed PTMs of CPB1 by 2-DE and mass spectrometry that may be associated with partial pancreatectomy. In islet cells and β-cells, AMP-activated protein kinase (AMPK) regulates enzyme activity by phosphorylation, and active AMPK inhibits insulin secretion and proinsulin gene expression (49). Hussain *et al* suggested that the phosphorylation of CREB-binding protein involved in the cyclic AMP signaling pathway affects β-cell proliferation (50). Moreover, Rab-GTPase-activating protein, having a critical role in glucose and lipid metabolism, regulates phosphorylation by glucose in rat β-cells (51). Khoury *et al* reported a statistical analysis of PTMs in the SWISS-PROT database; phosphorylation is the most widely experimentally observed PTM (52). Moreover, protein phosphorylation plays important roles in activation, inactivation, or modification of protein function. Therefore, we compared CPB1 phosphorylation levels between pancreases from sham-operated and partially pancreatectomized mice using immunoprecipitation and immunohistochemistry. We confirmed a reduction in CPB1 phosphorylation by partial pancreatectomy. To our knowledge, this study is the first to identify the association between CPB1 dephosphorylation and pancreatic regeneration. As exact phosphorylation sites of CPB1 have not been reported to date, we substituted 6 anticipated tyrosines for phenylalanine and focused on the PTMs of CPB1 and pancreatic regeneration. We found that CPB1 dephosphorylation affected β-cell proliferation. However, the identification of the exact phosphorylation sites and mechanisms warrants further studies. Glucagon-like peptide-1, cyclin D1, parathyroid hormone-related protein (PTHrP), hepatocyte growth factor/c-Met signaling, and double-stranded RNA-dependent protein kinase have been identified as candidate proteins functioning in pancreatic β-cell proliferation (53-57). 5'-*N*-ethylcarboxyamidoadenosine, an adenosine kinase inhibitor, and domperidone, a dopamine D2 receptor antagonist, were recently identified as small-molecule enhancers of β-cell proliferation for the treatment of diabetes (58,59). In addition, injection of amino-terminal peptides of PTHrP enhanced β-cell proliferation in partially pancreatectomized mice (60). Therefore, as a next step, it needs to be determined if peptides containing the dephosphorylation site of CPB1 indicated in Fig. 3A can enhance β-cell proliferation *in vivo*. Diabetes induced by a reduction in pancreatic β-cells may be treatable by replenishing the β-cell mass. However, pancreas or islet transplantation has limited applicability owing to difficulties such as shortage of organ donors and immunorejection of the transplanted tissue. Therefore, the identification of β-cell proliferation mechanisms forms the basis for developing efficient diabetes treatment approaches. In this study, we provided evidence supporting the relevance of CPB1 phosphorylation in β-cell proliferation.

Acknowledgements

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