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PAPER

LCP1 up-regulated by partial pancreatectomy supports cell proliferation and differentiation†

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The pancreas is the critical organ controlling blood glucose levels and has been shown to rapidly regenerate after injury. In this study, 60% partial pancreatectomy (PPX) was performed on rats and the protein expression profile was acquired using 2-dimensional gel electrophoresis (2-DE)/ MALDI-TOF analysis. In total, 34 proteins were shown to be up-regulated and 27 proteins were down-regulated after PPX. The up-regulated proteins were found to be involved in inflammation and the down-regulated proteins were involved in energy metabolism. Then, we compared the results from previous 4 different omics studies along with our present data and listed several genes which were found to be reproducibly regulated by PPX. The quantification of differentially regulated genes at transcriptional level by real-time PCR analysis showed that the three genes (*Apoa1*, *Lcp1* and *Lipa*) were up-regulated and three genes (*Gatm*, *Ivd* and *Pck2*) were down-regulated. Of these, lymphocyte cytosolic protein 1 (LCP1) was highly (folds = 8.40 ± 2.57) up-regulated by PPX and found to augment cell proliferation in PANC-1 and INS-1 cells. Finally, the validation of islet markers on exogenously expressed LCP1 cells showed up-regulation of genes which are responsible for pancreatic regeneration. These data indicate that the LCP1 may play a critical role in the pancreas regeneration.

Introduction

Identification of transcriptional targets during pancreatic regeneration is one of the approaches for targeting diabetes treatment. There are several experimental conditions applied for studying the mechanism of β-cell regeneration such as pancreatectomy, administration of streptozotocin, wrapping the pancreas in cellophase.[1] Partial pancreatectomy (PPX) has been used as a model of pancreatic regeneration since the 1960s.[2,3] In the PPX animal model, a radical change in cell mass was observed within a week of operation while glucose metabolism recovered to normal levels.[4] There also has been indirect evidence that the islets in adult tissue regenerated in patients with long standing type 1 diabetes.[5]

Pancreatic regeneration involves a balance between proliferation and differentiation of damaged pancreatic tissue. Regeneration is regulated by various metabolites such as β-cellulin[6] and heparin sulfate,[7] growth factors such as clusterin (CLU),[8] fibronectin,[9] glucagons like peptide 1 (GLP1)[10] and regenerating islet-derived proteins (REGs)[11] and transcription factors such as neurogenin 3 (NGN3),[12] paired box gene 4 (PAX4),[13] and pancreatic and duodenal homeobox 1 (PDX1).[14] Although, several new regenerating factors are continuously being reported, genes that have not yet been validated might affect proper understanding on the mechanism of pancreatic regeneration.

A number of proteomic or genomic approaches have been conducted to understand the pathology[15] and isolate cancer markers[16] from the pancreas. The results of these different approaches are dependent on the sample preparation and analytic sensitivity. Previous studies that have utilized the PPX animal model used different sampling conditions such as the percentage of dissection, the time point of sampling and the animal species. As a result, the gene profiles of these studies were not always identical; nevertheless, some proteins (*e.g.* CLU and REGs) were shown to be commonly up-regulated. To the best of our knowledge, there have been four different omics studies on PPX[17–20] and some genes were found to be commonly regulated in these studies.

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In this study, we performed an additional 2-DE analysis and compared the identified genes with the previously reported four PPX studies. We observed that the expression level of LCP1 was significantly increased in PPX. Our present observation may be justified in the light of the previous omics study.[17] Up-regulation of LCP1 appears to be the common response to cancer and inflammation in several organs including the pancreas.[15,16,21,22] Therefore, we investigated the effect of overexpression and knockdown of LCP1 on the cell growth and cell cycle in PANC-1 and INS-1 cells. Furthermore, we validated the expression level of islet marker genes induced by exogenous LCP1 in PANC-1 and INS-1 cells.

Experimental

Partial pancreatectomy and 2-DE/MALDI-TOF analyses

Eight-week-old male Sprague-Dawley rats were anesthetized by i.m. injection of ketamine hydrochloride (40 mg kg^{-1}; Huons, Seoul, Korea) and xylazine hydrochloride (5 mg kg^{-1}; Rompun; Bayer Korea, Seoul, Korea). In the 60% PPX, splenic portion of the pancreas was dissected out. In the sham operation, the abdomen was opened and the pancreas was left intact. The incision was closed using a 4-0 silk thread. After two days, the remaining pancreas was isolated and homogenized in liquid nitrogen. Tissue fragments were suspended in protein lysis buffer and centrifuged to obtain a clear soup. All animal procedures were approved by the Institutional Animal Care and Use Committee at CHA University.

For 2-DE analysis, 1 mg of protein from each pancreas extract was first separated on an immobilized 18 cm pH 3–10 nonlinear gradient strip (Amersham Bioscience, Uppsala, Sweden) and then separated on 9–16% linear gradient polyacrylamide gels. After protein fixation, the gel was stained with Coomassie brilliant blue G-250 for 12 h, scanned with a GS-710 imaging densitometer (Bio-Rad, Hercules, CA, USA) and analyzed with Image Master™ 2-D Platinum software (GE Healthcare, Waukesha, WI). Peptide mass fingerprints of selected spots were analyzed using a 4800 MALDI-TOF/TOF™ Analyzer (Applied Biosystems, Foster City, CA, USA). Mass spectrum was obtained in the reflectron/delayed extraction mode at an accelerating voltage of 20 kV and sum data from either 500 laser pulses. The spectrum was calibrated using trypsin auto-digested peaks (m/z 842.5090 and 2211.1046). Monoisotopic peptide masses were obtained using the Data Explorer 4.4 (PerSeptive Biosystems, Framingham, MA, USA).

Each mass spectrum (MS) was compared with the NCBI non-redundant protein sequence database using the Spectrum Mill MS Proteomics Workbench (Rev A.03.00.015, Agilent, Wilmington, DE, USA). The score is calculated from the points assigned to peaks; matched (Bonus) and unmatched (Penalty). A protein was considered a hit if the protein score was >13, peptide score >10 and SPI >70.

Cell culture, DNA constructs and antibodies

INS-1 (insulinoma cell line) cells were cultured in RPMI 1640 medium (Gibco-BRL, Grand Island, NY, USA) with 10% FBS, 11.1 mM glucose and penicillin/streptomycin (Gibco-BRL, Grand Island, NY, USA). 293T and PANC-1 (human pancreatic carcinoma, epithelial-like cell line) (ATCC CRL-1469) cells were cultured in DMEM medium (Gibco-BRL, Grand Island, NY, USA) with 10% FBS and antibiotics.

The cDNA of *Lcp1* (NM_001012044) from rat pancreas was cloned into the modified pMSCVhyg (antigenic-myc sequence is inserted at multi-cloning site; Clonetech, Mountain View, CA, USA) expression vector. shRNA of *LCP1* was generated using pSilencer Vectors (Applied Biosystems, Austin, TX, USA). The top (shLCP1#1: 5′-GAT CCG ATT GGG TTG TTT GCT GAC TTC AAG AGA GTC AGC AAA CAA CCC AAT CTT TTT TGG AAA-3′, shLCP1#2: 5′-GAT CCG ACC TGG TTG AAG TGA ACC TTC AAG AGA GGT TCA CTT CAA CCA GGT CTT TTT TGG AAA-3′) and bottom (shLCP1#1: 5′-AGC TTT TCC AAA AAA GAT TGG GTT GTT TGC TGA CTC TCT TGA AGT CAG CAA ACA ACC CAA TCG-3′, shLCP1#2: 5′-AGC TTT TCC AAA AAA GAC CTG GTT GAA GTG AAC CTC TCT TGA AGG TTC ACT TCA ACC AGG TCG-3′). The product was integrated into modified pMSCVhyg (H1 promoter was inserted to multi-cloning site) between *BamH* I and *Hind* III (New England BioLabs, Beverly, MA, USA) restriction sites. The following antibodies were used for analysis; mouse anti-c-myc (9E10), and anti-mouse immunoglobulin (KPL, Gaithersburg, MD, USA).

Viral production and transduction

3 μg of the pMSCVhyg expression vector, 3 μg of pVPack-VSV-G (Stratagene, La Jolla, CA, USA) and 3 μg of pVPack-GP (Stratagene, La Jolla, CA, USA) were mixed with 600 μl of 150 mM NaCl and 150 μl of 10 mM PEI (Polyscience, Warrington, PA, USA). 2 days after transfection, supernatant from the packaging cells was collected and filtrated. Viral particles were concentrated at 50 000 *g* for 4 h. The pellet was suspended in the culture media of each target cell containing 8 μg ml^{-1} polybrene (Invitrogen, Carlsbad, CA, USA). Target cells were incubated in viral media for one day.

Reverse transcriptase-polymerase chain reaction (RT-PCR) and real-time polymerase chain reaction (real-time PCR)

RNA was extracted using the TRIzol™ reagent (Invitrogen, Carlsbad, CA, USA). 2 μg of total RNA for each sample was reverse-transcribed using Oligo dT primer by Superscript™ II (Invitrogen, Carlsbad, CA, USA). RT-PCR was performed with AccuPower™ PCR PreMix (Bioneer, Daejeon, Korea). The concentration of each cDNA was normalized with the RT-PCR bands of *Gapdh*. Real-time PCR was performed using the Greenstar™ PCR MasterMix (Bioneer, Daejeon, Korea) and Opticon™ 2 (MJ research, Capital Court, NV, USA). The cycle threshold of each gene was normalized with the *Gapdh* value.

Western blotting

Cell extracts were separated by 12% SDS-PAGE and the proteins were transferred to a PVDF membrane (Millipore, Bedford, MA, USA). The membranes were incubated with the

primary antibody overnight and then with horseradish peroxidase-conjugated secondary antibody for 1 h at room temperature. Immunoreactive bands were visualized using a chemiluminescence detection system (Young In Frontier, Seoul, Korea).

Wounding assay and cell proliferation assay

Wounding assay—using a sterile 1000 μl pipette tip, separate wounds were scratched through the cells. Cells were rinsed gently with phosphate buffered saline (PBS). Pictures were taken at 0 and 48 h. Cell counting assay—5000 cells were set on 12-well plates. The number of cells was counted manually using a hemocytometer three times on the 1st, 3rd, 5th, and 7th day after onset. Cell counting kit-8 assay (CCK-8)—100 μl of INS-1 cell suspension from 50 000–100 000 cells per ml was added to 96-well plate. The protocol was followed according to the instructions provided by the company (Dojindo Molecular Technologies, Rockville, MD, USA). O.D. was measured at 450 nm to determine the cell viability in each well.

Double thymidine block and FACS analysis

50 000 cells were plated on the 60Φ dishes and grown in normal media for one day. Cells were arrested with 2 mM thymidine (Sigma, St Louis, MO, USA) for 28 h, released in normal media, and arrested again with 2 mM thymidine for 24 h. After the media were changed with normal media, the cells were harvested at 0, 4, 8, 10, 12, 14, 16, 20, 24, 28, 32, 36, 40 h, washed with PBS two times and fixed with 200 μl of PBS and 500 μl of ethanol at −20 °C for one day. Cells were washed with PBS one time and then centrifuged at 10 000 rpm for 5 min to remove the ethanol. Staining was performed using 10 μg of propidium iodide (PI, Sigma, St Louis, MO, USA) and 8 μg of RNase A (Qiagen Inc, Valencia, CA, USA) in 200 μl of PBS. The samples were incubated at 37 °C for 10 min. Analysis was performed with a BD FACSCalibur™ (BD Bioscience, Franklin Lakes, NJ, USA). Populations of G0/G1, G2/M and S phase cells were analyzed with ModFit LT software (Verity Software House Inc., Topsham, ME, USA).

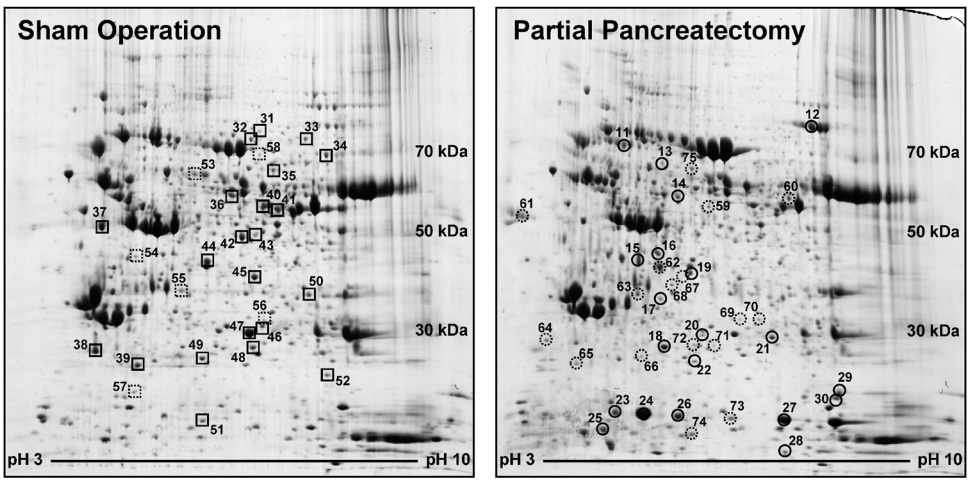
Data processing and statistical analysis

Numerical data were presented as means ± standard error (SE). Two groups of data were compared using the Student's *t* test. p value <0.05 was considered statistically significant. All data were processed using SAS software (SAS institute Inc., Cary, NC, USA).

Results

Identification of up- or down-regulated proteins by PPX using 2-DE analysis

The Image Master™ 2-D Platinum software detected 65 spots, out of which 20 were enlarged spots (at least 2-fold), 22 shrunken spots (at least 2-fold) and 17 newly introduced spots and 6 spots that were disappeared (Fig. 1). Each spot was identified with MALDI-TOF analysis and these spots are shown in Table S1 (ESI†). PPX modulated the expression of



	Number
Increased Spots	20
Non-paired Spots, newly expressed	17
Decreased Spots	22
Non-paired Spots, disappeared	6
Sequenced Spots	65
Total Identified proteins	61

Fig. 1 Comparison of the proteins expressed in pancreas between two 2-DE gels stained with CBB G-250. The size and density of the spots were measured with Image Master™ 2-D Platinum software. The increased spots and decreased spots were defined by the change of at least 2-fold.

proteins involved in cell growth (translationally-controlled tumor protein, member of RAS oncogene family *etc.*), energy metabolism (mitochondrial aldehyde dehydrogenase, phosphoenolpyruvate carboxykinase, propionyl-CoA carboxylase *etc.*), protein expression (eukaryotic translation initiation factor, ribosomal protein *etc.*) and stress (fibrinogen, glutathione *S*-transferase *etc.*). The Clu protein was reported to be a pancreas regenerating factor in a previous report.[8] Sterol esterase and amylase are pancreas-specific proteins.[23,24] Overall, proteins that were up-regulated in the PPX model were involved in stress/inflammation (10 up *vs.* 3 down) and the proteins that were down-regulated were involved in energy metabolism (5 up *vs.* 12 down).

Significantly up- or down-regulated proteins by PPX using 2-DE analysis

Among several protein spots that were regulated by PPX using 2-DE analysis, we have selected protein spots that were significantly up- or down-regulated for further analyses. Our study revealed that 6 up-regulated proteins ALB, APOA1, FGG, LCP1, LIPA, and TCTP showed at least four fold increase (Fig. 2a). Further, we validated and quantified the transcriptional level of these genes by RT-PCR and real-time PCR analyses. The results showed that ApoA1, Lcp1 and Lipa were up-regulated, while Alb, Fgg and Tpt1 were down-regulated at the transcriptional level (Fig. 2b and c). Of these, the mRNA transcript of Lcp1 showed dramatic increase by PPX (folds = 8.40 ± 2.57). Thus, we decided to conduct further functional study on LCP-1 in PANC-1 and INS-1 cell lines.

Similarly, 3 down-regulated protein spots GATM, IVD and PCK2 (Fig. 3a) were further analyzed. The validation of *Gatm*, *Ivd*, and *Pck2* genes by RT-PCR (Fig. 3b) and quantification

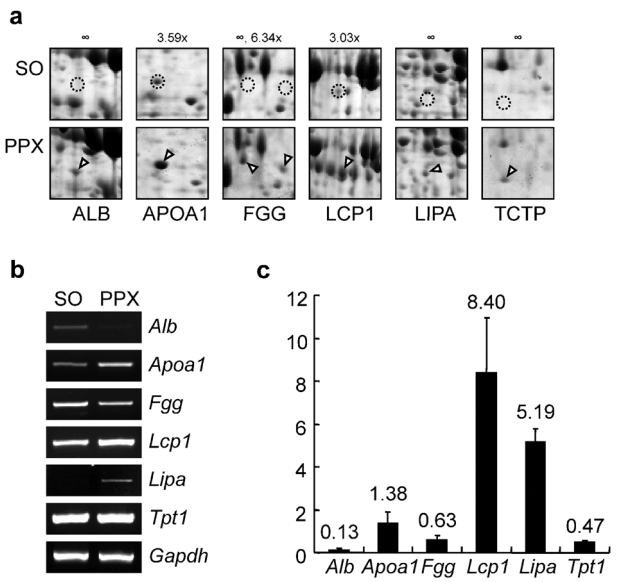


Fig. 2 Up-regulated genes by partial pancreatectomy. (a) Change in spot size on the 2-DE gel. Number above the image is the fold change and '∞' indicates a newly introduced spot. (b) RT-PCR analysis. (c) Real-time PCR. SO, sham operation; PPX, partial pancreatectomy; *Alb, albumin*; *Apoa1*, *apolipoprotein A1*; *Lcp1*, *lymphocyte cytosolic protein 1*; *Lipa*, *lipase A*, *cholesterol esterase*; *Fgg*, *fibrinogen gamma*; and *Tpt1, tumor protein, translationally-controlled 1*.

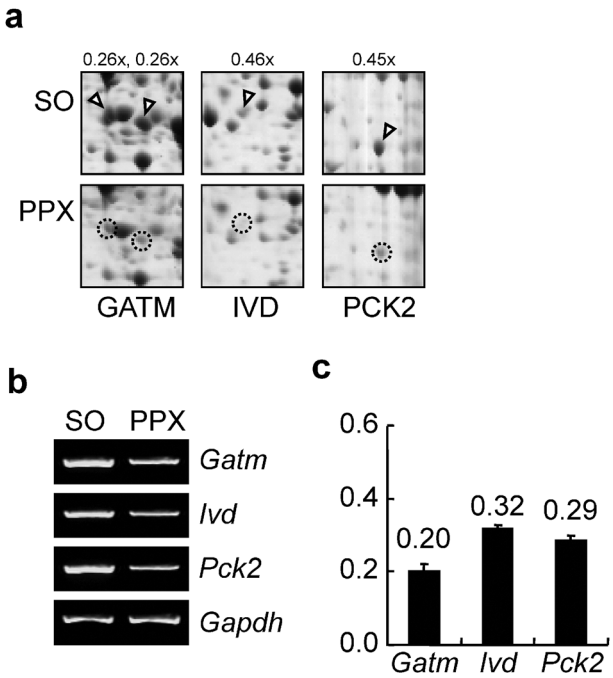


Fig. 3 Down-regulated genes by partial pancreatectomy. (a) Change in spot size on the 2-DE gel. Number above the image is the fold change. (b) RT-PCR analysis. (c) Real-time PCR. SO, sham operation; PPX, partial pancreatectomy; *Gatm*, *glycine amidinotransferase*; *Ivd*, *isovaleryl coenzyme A dehydrogenase*; and *Pck2*, *phosphoenolpyruvate carboxykinase 2*.

by real-time PCR analysis (Fig. 3c) found that all the three genes were down-regulated at the transcriptional level.

Comparative analysis of 5 different omics studies by PPX

We next compared our proteomic data with other four omics studies done by several groups under different experimental conditions[17–19] with the aim of identifying genes that were consistently up- or down-regulated in the PPX model. Our comparison study showed that 17 genes were reproducibly up- or down-regulated in the PPX model (ESI†, Table S2): actin related protein (ARP) homolog, ADP-ribosylation factor (ARF), cytokeratin 8, LCP1, mitogen-activated protein kinase 1 (MAPK1), REG3, serine proteinase inhibitor, sterol esterase (LIPA), translationally-controlled tumor protein (TCTP), X-box binding protein 1 (XBP1), amyloid β precursor like protein (APLP), albumin (ALB), apolipoprotein A-I (APOA1), fibrinogen γ (FGG), isovaleryl coenzyme A dehydrogenase (IVD), glycine amidinotransferase (GATM) and phosphoenolpyruvate carboxykinase 2 (PCK2). The above listed genes were obtained from different experimental approaches performed during PPX. The 6 up-regulated genes and 3 down-regulated genes which were obtained by our proteomic study were compared along with other omics studies in Table S2 (ESI†). We have summarized the results, mode of PPX, sampling time and analytical methods of all the five omics studies carried out on PPX (ESI†, Table S3). Furthermore, we have listed several novel genes and proteins identified from omics studies on PPX (ESI†, Table S4). This information would provide an opportunity to perform further

functional studies on novel genes and proteins that have been identified from five omics studies to expand the knowledge on pancreas regeneration.

LCP1 regulates cell proliferation

LCP1 encoding 627 amino acids sequence was highly conserved between human (NP_002289) and rat (NP_001012044). Peptide homology between human and rat was shown to be 96% (604 aa/627 aa). The proteins sequence of LCP1 consists of two calcium binding motifs and four actin binding domains (Fig. 4a). In order to investigate the overexpression and knockdown effect of LCP-1, we subcloned Lcp1 cDNA into MYC-tagged expression vector. Myc-LCP-1 and Myc-empty vector (Mock) were transfected into PANC-1 and INS-1 cells and validated its expression by Western blot analysis (Fig. 4b). The knockdown efficiency of shLCP1#1 and shLCP1#2 in PANC-1 and INS-1 was confirmed by RT-PCR. The knockdown efficiency of shLCP1#1 was shown to be higher than the shLCP1#2 (Fig. 4c).

In order to evaluate the overexpression and knockdown effect of LCP1 on cell proliferation in PANC-1 and INS-1 cell lines, wounding assay was performed (Fig. 4d). The scratched gap recovered by $34.4 \pm 2.3\%$ in wild type and $36.6 \pm 1.5\%$ in mock-vector transfected group after two days. LCP1-ovexpressing cells showed a $47.3 \pm 2.4\%$ ($p = 0.0004$, $n = 19$) recovery and LCP1-knockdown cells (*shLCP1#1*) showed a $29.0 \pm 1.7\%$ ($p = 0.0021$, $n = 20$) recovery. Collectively, LCP1 appears to effectively induce cell proliferation and significantly recovered the wound in PANC-1 (Fig. 4e). Furthermore, we performed cell counting and cell proliferation assays in INS-1 cells to check the effect of LCP1 on cell growth. The results showed that the LCP1-overexpressing cells grew 1.54 ± 0.09 ($p = 0.0032$, $n = 4$) times faster, while LCP1-knockdown cells (*shLCP1#1*) cells grew 0.80 ± 0.05 ($p = 0.044$, $n = 4$) times slower than the mock group 7 days after onset (Fig. 4f and g). Taken together, our results suggest that LCP-1 increases the rate of cell proliferation in PANC-1 and INS-1 cells.

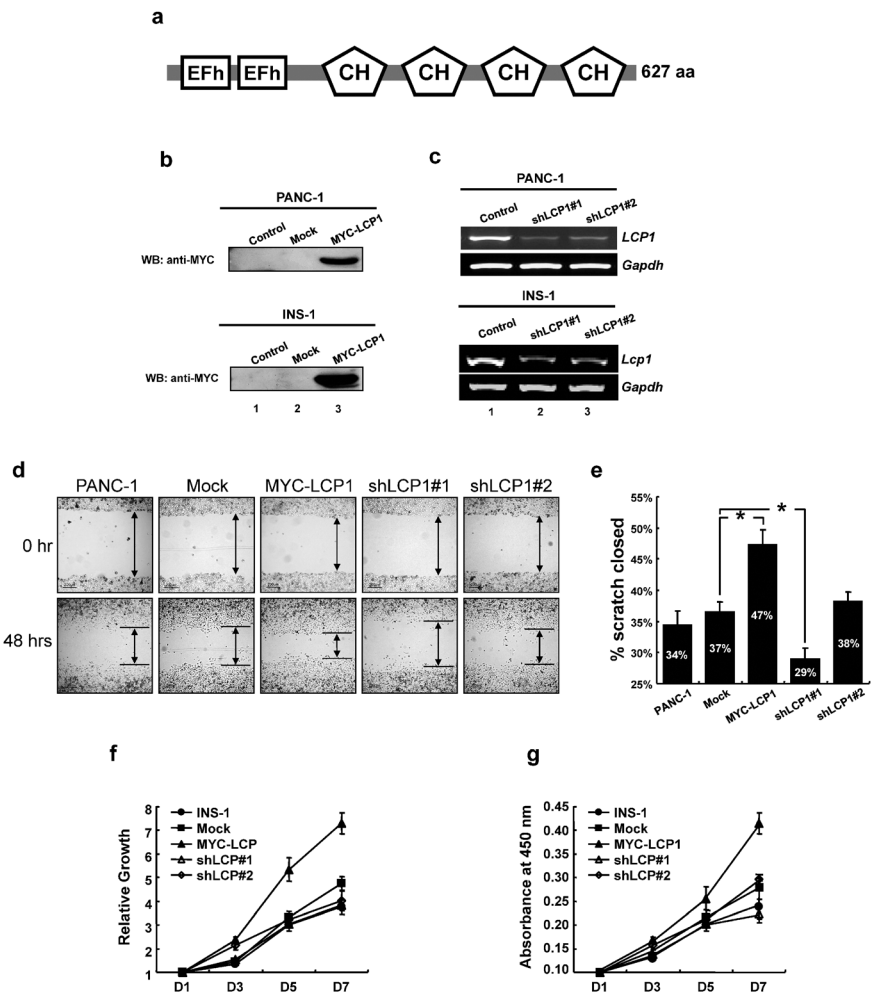


Fig. 4 LCP1 increases cell proliferation. (a) Human LCP1; EF-hands, calcium binding motif (position-13 to 41/53 to 81). CH: calponin homology domain, actin binding domain (position-122 to 234/266 to 373/396 to 501/517 to 622). (b) Overexpression of LCP1 was analyzed by Western blot analysis. (c) Knockdown of LCP1 by RT-PCR analysis. (d) Wounding assay in PANC-1 cells. (e) Graphical representation of wounding healing assay results in PANC-1 cells. Mean of triplicate measurements; bars, standard deviation, $n = 3$. *, $p < 0.05$. (f) LCP1 induced cell growth in INS-1 cells was performed by Cell counting assay. (g) Cell proliferation was analyzed using CCK-8 kit.

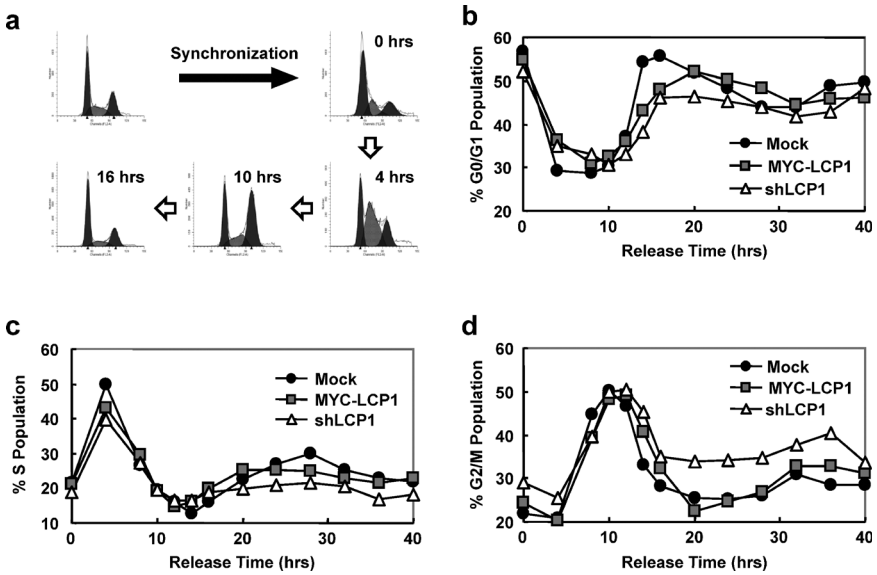


Fig. 5 Cell cycle duration of synchronized cell. (a) Thymidine double blocking and release. (b) Periodic change of G0/G1-population in synchronized cells. (c) Periodic change of S-population in synchronized cells. (d) Periodic change of G2/M-population in synchronized cells.

LCP-1 does not affect the cell cycle process in PANC-1 cells

Thymidine double blocking was performed to synchronize the cells to the G1 phase (Fig. 5a). 78% of mock control cells, 76% of LCP1-overexpressing cells and 71% of LCP1-knockdown cells were synchronized to the G1 and early S phase with 2 mM thymidine (Fig. 5b and c). The synchronized cells periodically changed through thymidine release and the cell cycle duration (gap between nearest local maximum points or local minimum points in Fig. 5b–d) was about 22 h. The result did not show any significant changes in the cell cycle duration between the LCP1 overexpressed and knockdown cells when compared with mock control.

Transcriptional change induced by exogenous LCP1

Overexpression or knockdown of LCP1 was applied to two cell lines and the expression of islet marker genes was investigated by RT-PCR. In PANC-1, *glucokinase*, *ISL1*, *NEUROD*, *NKX2.2*, *NKX6.1*, *PAX6*, *PDX1*, *POU3F4* and *proglucagon* were up-regulated, while *somatostatin* was down-regulated by LCP1-overexpression (Fig. 6a and b). In INS-1, *Isl1*, *Nkx2.2*, *Pax6*, *Pdx1* and *proglucagon* was up-regulated by LCP1-overexpression (Fig. 6c and d). Thus, our results suggest that the overexpression of LCP1 up-regulates islet markers both in PANC-1 and INS-1 cells indicating that LCP1 might play a critical role in pancreas regeneration.

Discussion

In this study, we investigated the protein expression profile in the PPX animal model. In addition, we compared the results of this study with previously reported data with the goal of identifying proteins that were reproducibly altered by PPX under similar conditions. Furthermore, the expression of selected proteins were confirmed by RT-PCR and real-time PCR. Finally, LCP1 was investigated in detail due to its high expression both at transcriptional and translational levels by PPX. Our investigation on the exogenous expression of LCP-1 in PANC-1 and INS-1 cells indicated its significant effect on the cell proliferation and up-regulation of islet marker suggesting its critical role in pancreas regeneration.

PPX is a type of tissue damage that introduces inflammation and metabolic deficiencies. As a well-known model of regeneration, PPX stimulates several regenerating factors that are related to inflammation and energy metabolism. Based on our 2-DE/MALDI-TOF data, PPX stimulates several proteins such as fibrinogen, orosomucoid (α1-acid glycoprotein), platelet-activating factor acetylhydrolase 2 (PAFAH2) and Ba1-647 (haptoglobin) which were involved in acute phase inflammation[25–27] and have been shown to participate in hemostasis and tissue remodeling.[28,29] It is known that the inflammatory response stimulates β cell regeneration.[30]

The results of this study were compared with other four previously reported omics studies that used the PPX model. Yang *et al.* used 90%-PPX rats, collected samples on the 3rd day after surgery and analyzed the samples by 2-DE.[17] De Leon *et al.* examined gene expression variations in total pancreas during 50%-PPX and in response to exendin-4 treatment, collected samples 24 and 48 h after surgery and analyzed the samples by cDNA microarray.[19] Exendin-4 a glucagon-like peptide-1 receptor agonist ameliorates hyperglycemia by stimulating β-cell mass recovery.[31] Shin *et al.* used 60%-PPX rat, collected samples 2 days after surgery and analyzed the samples by 2-DE.[18] Choi *et al.* used 60%-PPX rat, collected samples 2 days after surgery and analyzed the samples by cDNA subtractive hybridization.[20] In this study, 60%-PPX rats were used, samples were collected 2 days after surgery and samples were analyzed by 2-DE and MALDI-TOF analyses. Although the method used in this study was different from the previous studies, several common genes which were found in the expression profiles and its functions are discussed. ARPs contain an actin domain and participate in actin polymerization.[32] ARFs are GTP-binding proteins well-known for their role in membrane trafficking.[33] APLP is

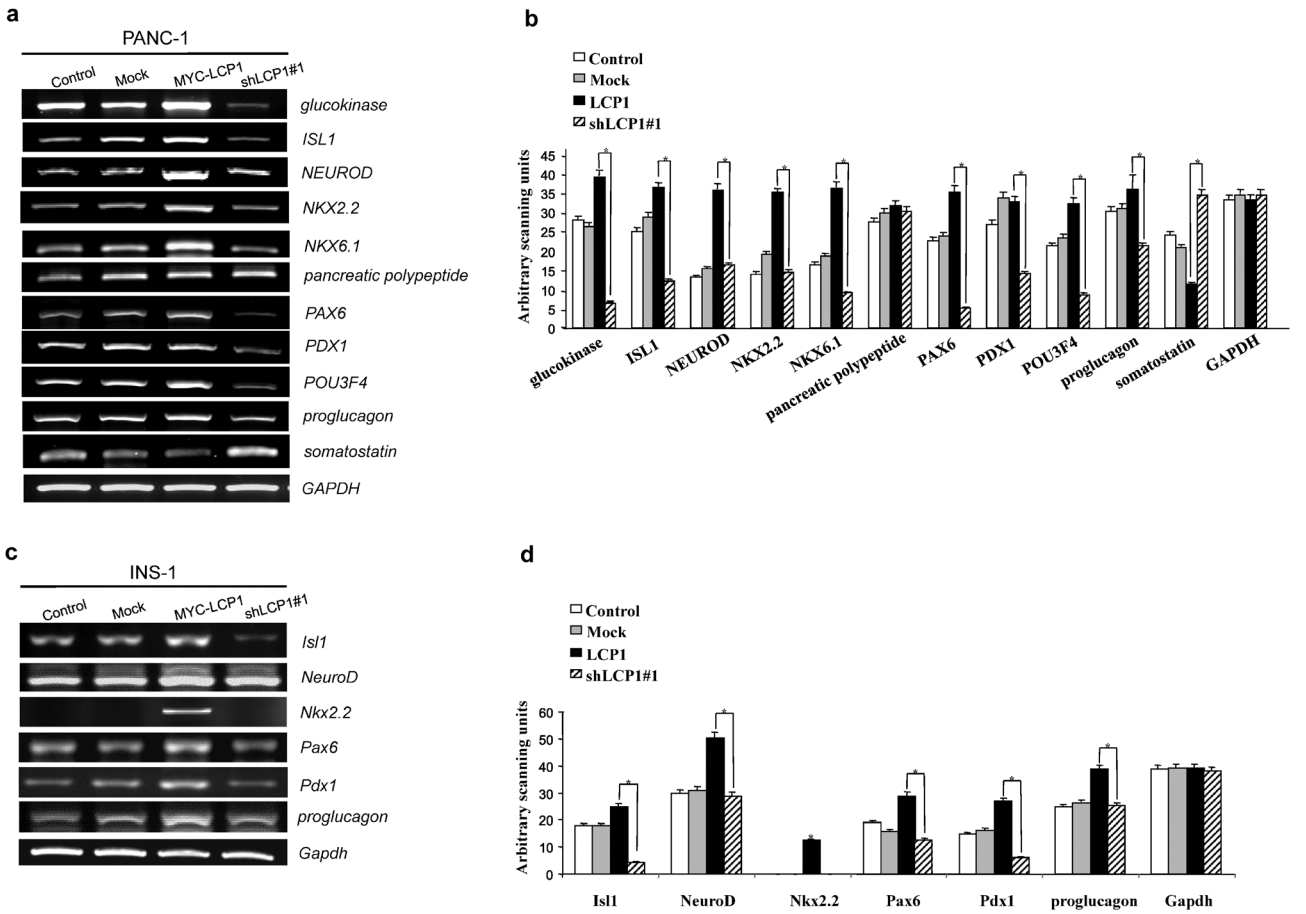


Fig. 6 Regulation of islet differentiation markers by LCP1. Differentiation assay based on RT-PCR analysis. (a) Islet markers in LCP1-overexpressed or LCP1-knockdown PANC-1 cells. (b) Quantification of islet markers in PANC-1 cells was represented from three separate experiments. Mean of triplicate measurements; bars, standard deviation, $n = 3$. *, $p < 0.05$. (c) Islet markers in LCP1-overexpressed or LCP1-knockdown INS-1 cells. (d) Quantification of islet markers in INS-1 cells was represented from three separate experiments. Mean of triplicate measurements; bars, standard deviation, $n = 3$. *, $p < 0.05$.

an isoform of the amyloid β precursor peptide. It splits into several peptides that form amyloid plaques.[34] APLP induces actin stress fiber through p38 MAPK.[35] APOA1 is the major protein component of high density lipoproteins, has an anti-clotting effect[36] and stimulates actin rearrangement.[37] TCTP is highly up-regulated by extracellular stimuli[38] and is localized in actin filaments.[39] XBP1 is a transcription factor that regulates unfolded protein responses in the ER.[40] GATM regulates the rate-limiting step of creatine biosynthesis.[41] Of these, we selected nine genes from our 2-DE analysis for further analysis by RT-PCR. Interestingly, RNA levels of few genes such as *Gatm*, *Ivd* and *Pck2* did not correlate with the 2-DE results, which could be explained by the time gap between transcription and translation. Genes such as *Alb*, *ApoaI*, *Fgg*, *Lcp1*, *Lipa* and *Tpt1* showed variation in its transcriptional level which was correlated with the 2-DE results.

LCP1 was shown to be highly up-regulated by PPX. LCP1 is a member of the actin-bundling proteins, which include filamin.[42] LCP1 is highly up-regulated in TNF-resistant cells and protects the cells from TNF-mediated apoptosis.[43] LCP1 is also related to the progression and metastasis of colorectal cancer.[44] We found that LCP1-overexpressing cells were more proliferative than the mock controls. The cells were synchronized with thymidine and the synchronization efficiency was 70–80%, which was comparable to a previous study that reported a 90% synchronization for PANC-1 cells.[45] The cycle duration of PANC-1 cell was about 22 h, which is shorter than the reported doubling time of PANC-1 (40–60 h).[46,47] This result implies that there is a gap between optimal cell growth and actual cell growth. In our analysis, LCP1 did not change the cell cycle duration and the optimal cell growth. The reason could be the environmental conditions such as the time-dependent state of the medium and intercellular interactions that can affect individual cells. Here, we demonstrated the effect of LCP1 on cell proliferation and differentiation in pancreatic cells. LCP1 affected the transcription of several islet marker genes including *proglucagon*, *NEUROD*, *glucokinase*, *NKX2.2*, *NKX6.1*, *PAX6*, *PDX1* and *POU3F4* in PANC-1 cells. Proglucagon is translated to glucagon in the pancreas and translated to GLP1 and GLP2 in the gastrointestinal tract.[48] Islet cells are induced by PDX1, NGN3, neurogenic differentiation (NEUROD), sequentially.[49] Islet β cells secreting insulin comprise 70–80% of the islet mass[50] and the differentiation of endocrine progenitors into β cells is regulated through a combination of the NK2 homeobox 2 (NKX2.2), PAX4, NKX6.1, musculoaponeurotic fibrosarcoma oncogene homolog A (MAFA), ISL LIM homeobox 1 (ISL1) and

PDX1.[51–53] Islet α cells secreting glucagon constitute 15–20% of islet mass[50] and α cells are induced by POU class 3 homeobox 4 (POU3F4/BRN4), aristaless related homeobox 1, MAFB and PAX6.[54,55] Most of the transcription factors that are listed above can regulate differentiation into pancreatic islet cells. Taken together, our results showing up-regulation of islet markers by exogenous expression of LCP1 suggest that LCP1 has a critical role in pancreas regeneration. Till to date, several omics studies have been conducted on PPX, but there is always a great chance of identifying novel pancreatic regulating genes from each individual study apart from already reported genes. Thus, identifying novel genes and elucidating its functional mechanism during pancreatic regeneration would be a great contribution for the treatment of diabetes disorders.

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