GASTROINTESTINAL, HEPATOBILIARY, AND PANCREATIC PATHOLOGY

Pancreatic Protein Tyrosine Phosphatase 1B Deficiency Exacerbates Acute Pancreatitis in Mice

Ahmed Bettaieb,^{*} Shinichiro Koike,^{*} Samah Chahed,^{*} Santana Bachaalany,^{*} Stephen Griffey,[†] Juan Sastre,[‡] and Fawaz G. Haj^{*§¶}

From the Department of Nutrition^{*} and the Comparative Pathology Laboratory,[†] University of California Davis, Davis, California; the Department of Physiology,[‡] University of Valencia, Burjassot, Spain; the Division of Endocrinology, Diabetes and Metabolism,[§] Department of Internal Medicine, and the Comprehensive Cancer Center,[¶] University of California Davis, Sacramento, California

Accepted for publication
April 6, 2016.

Address correspondence to
Fawaz G. Haj, Ph.D., or Ahmed
Bettaieb, Ph.D., Department of
Nutrition, University
of California Davis, 3135
Meyer Hall, Davis,
CA 95618. E-mail: fghaj@ucdavis.edu
or abettaieb@ucdavis.edu.

Acute pancreatitis (AP) is a common and devastating gastrointestinal disorder that causes significant morbidity. The disease starts as local inflammation in the pancreas that may progress to systemic inflammation and complications. Protein tyrosine phosphatase 1B (PTP1B) is implicated in inflammatory signaling, but its significance in AP remains unclear. To investigate whether PTP1B may have a role in AP, we used pancreas PTP1B knockout (panc-PTP1B KO) mice and determined the effects of pancreatic PTP1B deficiency on cerulein- and arginine-induced acute pancreatitis. We report that PTP1B protein expression was increased in the early phase of AP in mice and rats. In addition, histological analyses of pancreas samples revealed enhanced features of AP in cerulein-treated panc-PTP1B KO mice compared with controls. Moreover, cerulein- and arginine-induced serum amylase and lipase were significantly higher in panc-PTP1B KO mice compared with controls. Similarly, pancreatic mRNA and serum concentrations of the inflammatory cytokines IL-1B, IL-6, and tumor necrosis factor- α were increased in panc-PTP1B KO mice compared with controls. Furthermore, panc-PTP1B KO mice exhibited enhanced cerulein- and arginine-induced NF- κ B inflammatory response accompanied with increased mitogen-activated protein kinases activation and elevated endoplasmic reticulum stress. Notably, these effects were recapitulated in acinar cells treated with a pharmacological inhibitor of PTP1B. These findings reveal a novel role for pancreatic PTP1B in cerulein- and arginine-induced acute pancreatitis. (*Am J Pathol* 2016, 186: 2043–2054; <http://dx.doi.org/10.1016/j.ajpath.2016.04.012>)

Acute pancreatitis (AP) is the most common gastrointestinal cause for hospitalization in the United States and is associated with an overall mortality of up to 5%.^{1,2} The incidence of AP increases in parallel with its triggering risk factors, such as alcohol abuse, duct obstruction, and obesity.^{3–5} AP arises with local inflammation on activation of proteolytic pancreatic enzymes within the acinar tissue and may lead to systemic inflammatory response and complications.^{6,7} Specific therapy for AP is lacking, and understanding the molecular mechanisms underlying its pathogenesis is fundamental for management and therapy of the disease at early and late stages.

Different animal models have been generated to investigate AP pathogenesis, and one of the most common is cerulein-induced pancreatitis.⁸ Administration of cerulein at high concentrations causes secretion of lipase and amylase,

death of acinar cells, and infiltration of inflammatory cells into the pancreas, which are also observed in human pancreatitis.^{9,10} Cerulein action involves activation of NF- κ B, promotion of oxidative stress, and the release of proinflammatory cytokines.^{11,12} In addition, cerulein treatment alters pancreatic tyrosine phosphorylation and modulates the activities of protein tyrosine kinases and protein tyrosine phosphatases (PTPs).^{13,14}

The roles of PTPs in AP remain largely unexplored, but some studies demonstrate altered PTP expression and

Supported by NIH grants R01DK090492 (F.G.H.), R01DK095359 (F.G.H.) and K99DK100736 (A.B.).

Disclosures: None declared.

Current address of A.B., Department of Nutrition, University of Tennessee Knoxville, Knoxville, TN.

activity in rodent models of AP. Cerulein-induced AP is associated with increased expression of the SH2 domain-containing phosphatases SHP1 and SHP2.^{15,16} In addition, protein tyrosine phosphatase 1B (PTP1B) expression increases during the early stages of cerulein-induced AP and is suppressed by pharmacological inhibitors of mitogen-activated protein kinases (MAPKs).^{16,17} Moreover, T-cell protein tyrosine phosphatase (TCPTP) expression increases in the early stages of the disease, and pancreatic TCPTP deficiency mitigates cerulein-induced AP in mice.¹⁸ These studies implicate PTPs in AP, but additional investigation into the contribution of these enzymes to disease pathogenesis is warranted.

PTP1B (encoded by *PTPNI*) is an abundant, widely expressed nonreceptor phosphatase that is localized to the endoplasmic reticulum (ER).¹⁹ PTP1B is an established regulator of epidermal growth factor receptor,²⁰ integrin,^{21,22} and insulin receptor²³ signaling. Whole-body PTP1B knockout (KO) mice exhibit improved systemic insulin sensitivity, enhanced glucose tolerance, and resistance to high-fat diet-induced obesity.^{24,25} Tissue-specific PTP1B deletion helped define the functions of this phosphatase in many tissues, including muscle,²⁶ liver,²⁷ and brain.^{28,29} In addition, several studies implicate PTP1B in pancreas function. A compound knockout mouse model demonstrates that PTP1B global deficiency mitigates the severe diabetes caused by insulin receptor substrate 2 deletion.³⁰ In this model, PTP1B deficiency increases islet area and delays diabetes.³⁰ Moreover, PTP1B modulates ER stress signaling in the glucose-responsive MIN6 β -cell line.³¹ Furthermore, mice with pancreatic PTP1B deficiency display comparable glucose tolerance to controls, but when subjected to robust and prolonged challenges, exhibit mild glucose intolerance.³² Herein, we investigated the effects of pancreatic PTP1B deficiency on experimental acute pancreatitis using cerulein- and arginine-induced models. Alterations in inflammatory response were determined in cerulein/arginine-treated versus nontreated control and pancreas PTP1B knockout mice, and the underlying molecular mechanisms investigated.

Materials and Methods

Animal Studies

All animal studies were conducted using male mice and rats, according to federal guidelines, and approved by the Institutional Animal Care and Use Committee at University of California Davis and Ethical Committee for Animal Experimentation and Wellbeing of the University of Valencia, respectively. Generation of pancreas PTP1B knockout mice was previously described.³² Mice were maintained on a 12-hour light-dark cycle in a temperature-controlled facility, with free access to food and water. Mice were fed standard laboratory chow (Purina laboratory chow, 5001) at weaning. Acute pancreatitis was induced in 10- to 12-week-old control and pancreas PTP1B knockout mice using cerulein and

arginine. For cerulein-induced pancreatitis, mice were fasted overnight, then received 12 i.p. injections of cerulein (50 μ g/kg body weight) at 1-hour intervals or dimethyl sulfoxide as a vehicle control. Animals were sacrificed at 14, 24, and 48 hours after the initial injection, and blood was collected to determine serum amylase and lipase using commercial kits (Sigma, St. Louis, MO), according to the manufacturer's instructions. In addition, serum concentrations of IL-1B, TNFA, and IL-6 were determined by multiplex electrochemiluminescence (Meso Scale Discovery, Rockville, MD). Another group of mice was used for arginine-induced AP, as previously described, with slight modifications.³³ Briefly, 10- to 12-week-old mice received a single i.p. injection of 5 g/kg body weight L-arginine monohydrochloride in 0.9% sodium chloride (pH 7.0). A control group was injected i.p. with 0.9% sodium chloride as a vehicle control. Mice were sacrificed at 48 and 72 hours after arginine injection. For rat studies, Wistar rats were placed under deep anesthesia with isoflurane before treatment with 3.5% sodium taurocholate in 0.9% sodium chloride. AP was induced by a retrograde infusion of the solution. At 1 and 6 hours after induction of pancreatitis, rats were anesthetized again and pancreata were harvested and snap frozen in liquid nitrogen.

Cell Studies

Murine pancreatic acinar 266-6 cells³⁴ were purchased from ATCC (Manassas, VA) and cultured in Dulbecco's modified Eagle's medium containing 25 mmol/L glucose, 10% fetal bovine serum, 50 U/mL penicillin, and 50 μ g/mL streptomycin. Pharmacological inhibition of PTP1B was achieved by treating cells with 400 μ mol/L PTP1B inhibitor [3-(3,5-dibromo-4-hydroxybenzoyl)-2-ethyl-N-(4-[(2-thiazolylamino)sulfonyl]phenyl)-6-benzofuran] sulfonamide (Cayman Chemical, Ann Arbor, MI),³⁵ for 2 hours before cerulein treatment (2 μ mol/L for 24 hours).³⁶ For phosphatase activity assays, 266-6 cells were lysed in NP40 buffer (10 mmol/L Tris-HCl, pH 7.4, 150 mmol/L NaCl, 1 mmol/L EDTA, 1% NP-40, 5% glycerol, and proteases inhibitors) and PTP1B was immunoprecipitated (10 μ g antibody/mg of lysate) using PureProteome beads (Millipore, Billerica, MA). Phosphatase activity was determined using p-nitrophenyl phosphate (5 mmol/L), as we previously described.³⁷ Phosphatase activity was monitored by measuring the absorbance of p-nitrophenyl phosphate at 405 nm using a plate reader (GloMax-Multi Detection System; Promega Corp., Madison, WI).

Biochemical Studies

Tissues and cells were lysed using radioimmunoprecipitation assay buffer (10 mmol/L Tris-HCl, pH 7.4, 150 mmol/L NaCl, 0.1% sodium dodecyl sulfate, 1% Triton X-100, 1% sodium deoxycholate, 5 mmol/L EDTA, 1 mmol/L NaF, 1 mmol/L sodium orthovanadate, and protease inhibitors). Lysates were clarified by centrifugation, and protein

Table 1 Primer Sequences Used to Quantitate *IL1b*, *IL6*, *TNFA*, and *TBP* Expression

Gene	Forward primer	Reverse primer
<i>Il1b</i>	5'-AGCTTCAGGCAGGCAGTATC-3'	5'-AAGGTCCACGGGAAAGACAC-3'
<i>Il6</i>	5'-ACAACCACGGCCTTCCCTACTT-3'	5'-CACGATTTCCAGAGAACATGTG-3'
<i>Tbp</i>	5'-TTGGCTAGGTTTCTGCGGC-3'	5'-GCCCTGAGCATAAGGTGGAA-3'
<i>Tnfa</i>	5'-GACGTGGAAGTGGCAGAAGAG-3'	5'-TGCCACAAGCAGGAATGAGA-3'

concentrations were determined using bicinchoninic acid protein assay kit (Pierce Chemical, Dallas, TX). Proteins were resolved by SDS-PAGE and transferred to polyvinylidene difluoride membranes. Immunoblotting of lysates was performed with antibodies for PTP1B (Millipore), pERK1/2 (Tyr202/Thr204), pp38 (Thr180/Tyr182), p38, pJNK (Thr183/Tyr185), JNK, pIKK α / β (Ser178/180), IKK α / β , pI κ B α (Ser32), I κ B α , pNF- κ B p65 (Ser536), NF- κ B p65, and NF- κ B p50 (all from Cell Signaling Technology, Beverly, MA) and cleaved caspases 8, 9, and 3, SHP1, ERK1/2, pPERK (Thr980), PERK, pEIF2 α (Ser51), eIF2 α , sXBP1, IRE1 α , and tubulin were from Santa Cruz Biotechnology (Dallas, TX). Antibodies for pIRE1 α (Ser724) were purchased from Abcam (Cambridge, MA). After incubation with the appropriate secondary antibodies, proteins were

visualized using Luminata Forte Western HRP substrate (Millipore). Pixel intensities of immunoreactive bands were quantified using FluorChem Q Imaging software version 9900 (Alpha Innotech, San Jose, CA). For phosphorylated proteins, data were presented as phosphorylation level normalized to protein expression; and for nonphosphorylated proteins, as protein expression normalized to tubulin.

Total RNA was extracted from pancreas using TRIzol reagent (Invitrogen Carlsbad, CA). cDNA was generated using high-capacity cDNA synthesis kit (Applied Biosystems, Foster City, CA). *Il-1 β* , *Il-6*, and tumor necrosis factor (Tnf)- α mRNA were assessed by SYBR Green quantitative real-time PCR (iCycler; BioRad, Hercules, CA) using the Δ CT method with appropriate primers (Table 1) and normalized to TATA-box binding protein.

Figure 1 Increased pancreatic PTP1B protein expression in rodent models of acute pancreatitis (AP). **A:** Schematic depicting time course for cerulein- and arginine-induced experimental AP in mice. Total pancreas lysates of wild-type mice that were administered cerulein (Cer; **B**) or arginine (Arg; **C**) and immunoblotted for PTP1B, SHP1, and tubulin. Representative immunoblots are shown. **D:** Total pancreas lysates of rats that were administered taurocholate (Taur) then immunoblotted for PTP1B, SHP1, and tubulin. Representative immunoblots are shown. Bar graphs represent expression of PTP1B normalized to tubulin. Data are presented as means $\pm$ SEM (**B–D**). $n = 8$ per group (**B**, Cer); $n = 6$ per group (**C**, Arg, and **D**, Taur). ** $P < 0.01$ versus Cer/Arg/Taur-treated animals. A.U., arbitrary units.

Figure 2 Pancreatic PTP1B deficiency exacerbates cerulein (Cer)- and arginine (Arg)-induced pancreatic injury. **A:** Total pancreas lysates from control and panc-PTP1B knockout (KO) mice were immunoblotted for PTP1B and tubulin. Representative immunoblots are shown. **B:** Hematoxylin and eosin–stained sections of pancreas with cerulein-induced pancreatitis. In nontreated groups, control and panc-PTP1B KO mice appear normal with no pathological changes; necrosis is increased in knockout compared with focal area of necrosis in control mice at 24 hours after injection; vacuolization (arrows), inflammation (arrowheads), and necrosis (asterisks) are increased in knockout compared with control mice 48 hours after injection. **C:** Acute pancreatitis was induced by i.p. injections of cerulein and arginine and pancreatic injury determined by measuring serum amylase and lipase. **D:** IL-1 β , IL-6, and TNF- α mRNA levels were determined from at least six mice per group. **E:** Serum levels of IL-1B and IL-6, and TNFA, in control and knockout mice with cerulein-induced AP at the indicated times. Data are presented as means $\pm$ SEM (C–E). $n =$ at least 5 mice per group (C–E). * $P < 0.05$, ** $P < 0.01$ versus Cer/Arg-treated mice; $^{\dagger}P < 0.05$, $^{\dagger\dagger}P < 0.01$ versus panc-PTP1B KO mice. Scale bar = 50 μ m (B). A.U., arbitrary units., KU, kilo units.

Histological Analysis

A portion of the pancreas was fixed in 4% paraformaldehyde overnight, embedded in paraffin, and sections (5 μ m thick) were stained with hematoxylin and eosin to observe morphological changes. Initial histological analysis was performed by an experienced histopathologist (S.G.) in a blinded manner without knowledge of treatment groups. Histological scoring of pancreatic sections was performed to grade the extent of pancreatic parenchyma edema (0, no edema; 1, interlobular edema; 2, interlobular and moderate intralobular edema; 3, interlobular and severe intralobular edema), cell vacuolization (0, none; 1, <20% acini with vacuoles; 2, <50%

acini; 3, >50% acini), inflammation (0, no inflammation; 1, inflammatory cells present at intralobular; 3, inflammatory cells present at interacini), and acinar cell necrosis (0, no necrosis; 1, <10% necrosis; 2, <40% necrosis; 3, >40% necrosis), as previously described.^{38,39}

Statistical Analysis

Data are expressed as means $\pm$ SEM. Single data point comparisons were performed using Tukey's-Kramer honestly significant difference analyses using JMP Pro program version 11 (SAS Institute Inc., Cary, NC). Differences were considered significant at $P < 0.05$.

Results

PTP1B Expression Increases in Rodent Models of Acute Pancreatitis

Pancreatic PTP1B protein expression was determined in two different mouse models of AP. Pancreatitis was induced in wild-type mice by repetitive injections of cerulein or a single injection of arginine, as detailed in *Materials and Methods*. Mice were sacrificed at different times after injection, and PTP1B protein expression was determined at 14, 24, and 48 hours after initial cerulein injection, and 48 and 72 hours after arginine injection (Figure 1A). Immunoblots of pancreas lysates demonstrated significant increase in PTP1B protein expression on cerulein and arginine administration (Figure 1, B and C). In addition, SHP1 protein expression was increased on induction of AP, consistent with published reports.^{15,17} To ensure that these observations were not limited to mouse models, pancreatic PTP1B expression was determined in taurocholate-induced pancreatitis rat model and comparable increases in expression were also observed (Figure 1D). Together, these data demonstrate increased PTP1B protein expression in different rodent models of chemical-induced AP.

Pancreatic PTP1B Deficiency Exacerbates Cerulein- and Arginine-Induced Acute Pancreatitis

Increased pancreatic PTP1B expression in rodent models of AP prompted us to investigate its potential role in disease pathogenesis. To that end, we used mice with pancreas PTP1B deficiency (panc-PTP1B KO).³² Immunoblots of total pancreas lysates demonstrated efficient deletion of PTP1B in knockout mice compared with controls, as

previously reported (Figure 2A).³² In addition, selective deletion of PTP1B in the pancreas of knockout mice was previously demonstrated.³² Accordingly, this mouse model is suitable for determining PTP1B contribution to pancreatitis. We evaluated the severity of cerulein- and arginine-induced pancreatitis in control and panc-PTP1B KO mice. Histological analysis of hematoxylin and eosin–stained pancreatic sections was performed to grade pathological changes, including edema, cell vacuolization, inflammation, and necrosis, as detailed in *Materials and Methods*. In control and panc-PTP1B KO mice, cerulein administration caused an increase in edema, cell vacuolization, inflammation, and necrosis at 24 and 48 hours after the first injection, with highest grades at 24 hours (Figure 2B and Table 2). Scores for edema and necrosis in panc-PTP1B KO were significantly higher than control mice at 24 hours. In addition, 48 hours after the first injection, panc-PTP1B KO exhibited higher scores of vacuolization, inflammation, and necrosis compared with control mice (Table 2). Consistent with histological findings, serum amylase and lipase, which are commonly used markers for AP diagnosis,^{40,41} were significantly different between control and panc-PTP1B KO mice (Figure 2C). Under basal conditions, serum amylase and lipase were comparable between control and knockout mice. Cerulein and arginine administration led to significant increase in amylase and lipase but to a higher level in panc-PTP1B KO compared with control mice. Similar findings were observed in two additional independent cohorts of mice ($n = 6$ to 8 per group, data not shown). During acute pancreatitis, activation of NF- κ B enhances the release of proinflammatory cytokines, such as IL-1 β and IL-6 and TNF α . Pancreatic mRNA of IL-1 β , IL-6, and Tnf- α increased after cerulein and arginine administration, and levels were significantly higher in panc-PTP1B KO than control mice

Table 2 Histological Scoring of Pancreatic Tissues of Control and Panc-PTP1B KO Mice

Variable	Edema	Vacuolation	Inflammation	Necrosis
Control				
Cer (–) ($n = 11$)	0.00 $\pm$ 0.00	0.27 $\pm$ 0.19	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00
Cer (24 hours) ($n = 13$)	1.61 $\pm$ 0.36**	0.69 $\pm$ 0.13**	1.610 $\pm$ 0.36**	0.10 $\pm$ 0.00
Cer (48 hours) ($n = 11$)	1.45 $\pm$ 0.24**	0.54 $\pm$ 0.15	1.00 $\pm$ 0.30**	0.18 $\pm$ 0.00
KO				
Cer (–) ($n = 10$)	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00
Cer (24 hours) ($n = 12$)	2.58 $\pm$ 0.19**†	0.91 $\pm$ 0.08**	2.33 $\pm$ 0.25**	1.25 $\pm$ 0.13**†
Cer (48 hours) ($n = 11$)	1.72 $\pm$ 0.23**	1.00 $\pm$ 0.00**††	2.36 $\pm$ 0.27**††	1.18 $\pm$ 0.22**††

Hematoxylin and eosin–stained pancreas sections were scored to grade the extent of acinar edema, cell vacuolation, inflammation, and acinar cell necrosis. Data are presented as means $\pm$ SEM from two independent cohorts.

** $P < 0.01$ indicates significant difference between mice without and with cerulein administration.

† $P < 0.05$, †† $P < 0.01$ indicate significant difference between control and panc-PTP1B KO mice.

Cer, cerulein; KO, knockout.

Figure 3 Regulation of cerulein- and arginine-induced NF- κ B inflammatory response by pancreatic PTP1B. Total pancreas lysates from control and panc-PTP1B knockout (KO) mice treated with cerulein (Cer; **A**) and arginine (Arg; **B**) were immunoblotted for pIKK α , pI κ B α , pNF- κ B p65, and their respective unphosphorylated proteins, NF- κ B p50, PTP1B, and tubulin. Representative immunoblots are shown. **C**: Bar graphs represent normalized data for pIKK α /IKK α , pI κ B α /I κ B α , pNF- κ B p65/NF- κ B p65, and NF- κ B p50/tubulin. Data are presented as means $\pm$ SEM (**C**). $n = 6$ to 8 animals per treatment (**C**). * $P < 0.05$, ** $P < 0.01$ versus Cer/Arg-treated mice; $^{\dagger}P < 0.05$, $^{\dagger\dagger}P < 0.01$ versus panc-PTP1B KO mice. A.U., arbitrary units.

(Figure 2D). Similarly, serum levels of IL-1B, IL-6, and TNFA were increased in control mice after cerulein administration but were significantly higher in panc-PTP1B KO than control mice (Figure 2E). Together, these data indicate that pancreatic PTP1B deficiency exacerbates cerulein- and arginine-induced AP in mice.

Pancreatic PTP1B Deficiency Increases Cerulein- and Arginine-Induced NF- κ B Inflammation

The mechanisms of cerulein and arginine action involve activation of the nuclear transcription factor NF- κ B, leading to increased cytokine expression in the pancreas and inflammation.⁴² NF- κ B is activated early in AP in leukocytes and pancreatic acinar cells.⁴³ Proinflammatory cytokines, such as TNFA, activate I κ B kinase complex (IKK) to phosphorylate inhibitor of NF- κ B (I κ B).⁴⁴ I κ B phosphorylation triggers its ubiquitination and subsequent degradation, leading to the dissociation of NF- κ B dimers (p65 and p50) and translocation to the nucleus for transcription

activation.⁴⁵ In addition, several reports implicate PTP1B in modulating the inflammatory response.^{46–50} Accordingly, we evaluated NF- κ B signaling in panc-PTP1B KO and control mice in cerulein- and arginine-induced pancreatitis. Cerulein and arginine induced IKK α , I κ B α , and NF- κ B p65 phosphorylation and increased NF- κ B p50 expression (Figure 3). Notably, cerulein- and arginine-induced IKK α , I κ B α , and NF- κ B p65 phosphorylation and NF- κ B p50 expression were increased in panc-PTP1B KO compared with control mice. These findings are in keeping with the increased pancreatic proinflammatory cytokines in cerulein- and arginine-treated knockout mice.

Pancreatic PTP1B Deficiency Regulates Cerulein- and Arginine-Induced MAPK Signaling

To further investigate the molecular basis for AP in panc-PTP1B KO mice, we determined activation of MAPKs. MAPKs, including ERK1/2, p38, and JNK1/2, are rapidly and transiently induced during experimental AP in

Figure 4 Pancreatic PTP1B deficiency regulates cerulein- and arginine-induced mitogen-activated protein kinase signaling. Total pancreas lysates from control and panc-PTP1B knockout (KO) mice treated with cerulein (Cer; **A**) and arginine (Arg; **B**) were immunoblotted for pERK1/2, pp38, pJNK1/2, and their respective unphosphorylated proteins and tubulin. Representative immunoblots are shown. **C**: Bar graphs represent normalized data for pERK/ERK, pp38/p38, and pJNK/JNK. Data are presented as means $\pm$ SEM (**C**). $n = 6$ to 8 animals per treatment (**C**). * $P < 0.05$, ** $P < 0.01$ versus Cer/Arg-treated mice; †† $P < 0.01$ versus panc-PTP1B KO mice. A.U., arbitrary units.

rodents.⁵¹ This is believed to be a component of the cellular stress response in the onset of inflammation in the pancreas. In addition, JNK and ERK have been implicated in the up-regulation of PTP1B expression on cerulein treatment.¹⁶ Under basal conditions, MAPK activation was comparable between control and panc-PTP1B KO mice (Figure 4). However, cerulein and arginine administration led to increased phosphorylation of ERK1/2, p38, and JNK in control mice, and that was significantly higher in panc-PTP1B KO mice. These findings establish increased MAPK phosphorylation in pancreata of cerulein- and arginine-treated panc-PTP1B KO mice and are consistent with the elevated inflammatory response in these mice.

Pancreatic PTP1B Deficiency Regulates Cerulein- and Arginine-Induced ER Stress

The ER is highly responsive to the energy status of the cell and plays an important role in the folding of newly

synthesized proteins. Unfolded protein response is used by cells to counter the deleterious effects of ER stress and is triggered by transmembrane sensors, such as PKR-like ER-regulated kinase (PERK), that detect unfolded proteins in the ER and convey information through their cytosolic domain.⁵² More important, activation of ER stress is associated with AP⁵³ and treatment with ER chaperones mitigates the disease in animal models.⁵⁴ In addition, we previously demonstrated that PTP1B knockdown in MIN6 β -cells enhanced PERK and inositol requiring enzyme 1 α (IRE1 α) signaling.³¹ We evaluated activation of ER transmembrane proteins PERK and IRE1 α and their downstream targets α -subunit of eukaryotic translation initiation factor 2 (eIF2 α) and X-box binding protein 1 (XBP1), respectively. Consistent with previous reports,^{54,55} cerulein and arginine administration induced ER stress in the pancreas (Figure 5). Notably, pancreatic PTP1B deficiency potentiated cerulein- and arginine-induced ER stress signaling, as assessed by increased PERK, eIF2 α , and IRE1 α phosphorylation

Figure 5 Regulation of cerulein- and arginine-induced endoplasmic reticulum (ER) stress by PTP1B. Total pancreas lysates from control and panc-PTP1B knockout (KO) mice treated with cerulein (Cer; **A**) and arginine (Arg; **B**) were immunoblotted for pPERK, peIF2α, pIRE1, and their respective unphosphorylated proteins, cleaved caspases 8, 9, and 3 and tubulin. **C**: Representative immunoblots are shown. Bar graphs represent normalized data for pPERK/PERK, peIF2α/eIF2α, pIRE1/IRE1, caspase 8, 9, 3, and sXBP1/tubulin. Data are means ± SEM (**C**). $n = 6$ to 8 animals per treatment (**C**). * $P < 0.05$, ** $P < 0.01$ indicate significant difference between untreated and Cer/Arg-treated mice; † $P < 0.05$, †† $P < 0.01$ versus panc-PTP1B KO mice. A.U., arbitrary units.

and sXBP1 expression. The unfolded protein response is deployed as a compensatory mechanism to restore homeostasis, but if it fails then apoptosis commences. We assessed cerulein- and arginine-induced expression of initiator and effector caspases in control and knockout mice. Both cerulein and arginine induced procaspase 8, 9, and 3 cleavage in controls but that was significantly enhanced in panc-PTP1B KO mice (Figure 5). Collectively, these findings establish increased ER stress and cleaved caspase

expression on pancreatic PTP1B deficiency during the early phase of cerulein/arginine-induced AP.

PTP1B Pharmacological Inhibition Exacerbates Cerulein-Induced Inflammation in Acinar Cells

As a complementary approach, we investigated the effects of PTP1B pharmacological inhibition in murine pancreatic acinar 266-6 cell line. Cells were treated with PTP1B

Figure 6 Pharmacological inhibition of PTP1B in acinar cells exacerbates cerulein (Cer)-induced inflammation. **A:** Murine pancreatic acinar 266-6 cells were pretreated with PTP1B inhibitor for 2 hours, then exposed to cerulein for 24 hours. Cell lysates were immunoblotted for PTP1B, SHP1, TCPTP, and tubulin. Representative immunoblots are shown. Cell lysates from control and cerulein-treated cells, with and without PTP1B inhibition were immunoblotted for pIKK α , pI κ B α , pNF- κ B p65 (**B**), pp38, pJNK1/2 (**C**), pPERK, pelf2 α , caspase 3 (**D**), and respective unphosphorylated proteins and tubulin.

pharmacological inhibitor (PTP1Bi) without and with cerulein stimulation, as detailed in *Materials and Methods*. Consistent with findings from total pancreas lysates, PTP1B protein expression was increased in acinar cells on cerulein treatment (Figure 6A). Similarly, SHP1 and TCPTP expression was also increased in cerulein-treated acinar cells. In addition, PTP1B pharmacological inhibition did not alter PTP1B protein expression but significantly decreased its activity in cells without cerulein (1.00 ± 0.07 without PTP1Bi versus 0.66 ± 0.01 with PTP1Bi) and with cerulein treatment (3.71 ± 0.25 without PTP1Bi versus 1.93 ± 0.34 with PTP1Bi). Cerulein treatment increased IKK α , I κ B α , and NF- κ B p65 phosphorylation and elevated NF- κ B p50 expression in acinar cells (Figure 6B). In addition, PTP1B inhibitor-treated cells exhibited increased NF- κ B inflammatory response compared with nontreated cells on cerulein stimulation. Moreover, PTP1B pharmacological inhibition exacerbated cerulein-induced increase in MAPK activation and ER stress in acinar cells (Figure 6, C and D). These findings demonstrate that PTP1B pharmacological inhibition in acinar cells recapitulates the effects of its pancreatic deficiency in cerulein-induced AP.

Discussion

The development of acute pancreatitis involves a complex cascade of events that occurs in acinar cells, but the underlying cellular and molecular mechanisms are not fully understood. In this study, we investigated the role of pancreatic PTP1B in experimental AP using rodent models. We established increased pancreatic PTP1B expression

during the early stages of AP in mice and rats. In addition, pancreatic PTP1B deficiency exacerbated cerulein- and arginine-induced pancreatitis. This was associated with increased cerulein- and arginine-induced NF- κ B inflammatory response and elevated MAPK signaling and ER stress in knockout mice. These findings define a previously unknown role for PTP1B in chemical-induced acute pancreatitis.

Alterations in gene and protein expression during the early stages of AP play a significant role in disease progression and severity.⁵⁶ In this regard, pancreatic PTP1B expression increased during experimental AP using cerulein and arginine in mice and taurocholate in rats, establishing that it is not specific to a particular chemical or rodent model. Similarly, PTP1B expression and activity increased in cerulein-treated acinar cells. These findings are consistent with a previous report of elevated PTP1B expression during the early stages of AP.¹⁷ In most cases, increased PTP1B protein expression correlates with increased enzymatic activity.^{57,58} Indeed, in cerulein-treated acinar cells, increased PTP1B expression was mirrored by elevated enzymatic activity. However, several post-translational modifications dynamically regulate PTP1B activity⁵⁹ and alterations of PTP1B activity during AP in rodents and humans remain to be determined. Increased PTP1B expression is often, but not always, associated with states of elevated inflammation, such as obesity and diabetes.^{60,61} PTP1B is induced by inflammation *in vivo*, and TNF- α treatment leads to increase in PTP1B mRNA and protein in insulin- and leptin-responsive tissues in mice, whereas TNF- α deficiency blocks diet-induced increase in expression.⁶² Of note, PTP1B expression during the early stages of AP is

comparable to that of other PTPs, such as SHP1, SHP2,^{15,16} and TCPTP.¹⁸ However, pancreatic TCPTP deficiency mitigates cerulein-induced AP in mice¹⁸ compared with the effects of PTP1B deficiency observed herein. Thus, additional investigations into the regulation of PTPs and their contribution to AP pathogenesis are warranted.

Genetic and pharmacological approaches helped define the role of PTP1B in AP. Pancreatic PTP1B deficiency exacerbated chemical-induced AP, as evidenced by pancreas histology, increased serum amylase and lipase, and enhanced serum and pancreatic IL-1B, TNFA, and IL-6.

A limitation of this study is that panc-PTP1B KO mice exhibit PTP1B deletion in the endocrine and exocrine pancreas,³² so the precise contribution of acinar cells cannot be delineated. However, the effects of PTP1B deficiency *in vivo* were recapitulated in inhibitor-treated acinar cells, suggesting that they were likely cell autonomous, although we cannot rule out effects of nonacinar cells. Moreover, we cannot rule out that PTP1B pharmacological inhibition did not alter activity of other phosphatase(s), although activity of the closely related TCPTP was not significantly altered in inhibitor-treated acinar cells (data not shown). Additional studies are needed to determine the effects of acinar cell-specific PTP1B deficiency and PTP1B pharmacological inhibition *in vivo* on the progression and development of AP.

The molecular mechanisms underlying pancreatic PTP1B actions in AP remain to be determined but may encompass direct and/or indirect modulation of inflammatory, MAPK, and ER stress responses. First, pancreatic PTP1B deficient mice exhibited increased proinflammatory cytokines that play a significant role in the development and severity of the disease.⁶³ TNF- α exacerbates acinar cell injury, IL-1 β regulates the development of AP, and IL-6 is a major mediator of the acute-phase response. Consistent with increased proinflammatory cytokines, knockout mice exhibited enhanced cerulein- and arginine-induced NF- κ B signaling. NF- κ B inflammatory response is activated early in AP and plays an important role in disease pathogenesis.^{64,65} Previous studies implicate PTP1B in the control of inflammatory processes but are somewhat inconsistent. Knockdown of PTP1B increases production of TNFA and IL-6 in TLR-triggered macrophages.⁴⁶ In addition, PTP1B deficient macrophages display increased inflammatory activity *in vitro* and *in vivo*.⁴⁷ Moreover, PTP1B deficiency exacerbates inflammation and accelerates leukocyte trafficking *in vivo*.⁴⁸ Furthermore, PTP1B deficiency accentuates the effects of proinflammatory stimuli in both rodent and human macrophages.⁴⁹ On the other hand, myeloid cell-specific PTP1B deficiency protects against high-fat diet- and lipopolysaccharide-induced inflammation.⁵⁰ The reason(s) for these differences is not apparent and could depend on the experimental systems and/or regulation of inflammatory signaling by PTP1B in a stimulus/tissue-dependent manner. At any rate, given the current findings, it is reasonable to stipulate that the effects of pancreatic PTP1B deficiency during experimental AP could be mediated, at least partly,

through exacerbation of the inflammatory response. Second, pancreatic PTP1B deficiency affected MAPK signaling that is already implicated in pancreatitis. In particular, pancreatic PTP1B deletion was associated with increased cerulein- and arginine-induced MAPK activation, indicative of elevated stress, and is consistent with previous studies implicating MAPKs in AP.⁶⁶ Of note, studies using MAPK pharmacological inhibitors suggest that JNK and ERK regulate cerulein-induced PTP1B expression.¹⁶ The mechanism by which PTP1B deficiency potentiates MAPK signaling remains unknown, but can be indirect and related to increased inflammation. Last, ER stress is implicated in the pathophysiology of pancreatitis,^{53,67} and attenuation of ER stress by chemical chaperones mitigates the disease in animal models.⁵⁴ Increased PERK and IRE1 α activation in PTP1B knockout mice and inhibitor-treated acinar cells is consistent with previous findings of enhanced PERK and IRE1 α signaling in MIN6 β -cells.³¹ Conceivably, pancreatic PTP1B can regulate ER stress through direct and/or indirect mechanisms. One scenario involves direct interaction and dephosphorylation of component(s) in ER stress signaling, such as PERK. Of note, PERK is a PTP1B substrate in fibroblasts⁶⁸ and adipocytes,⁶⁹ and PERK Tyr615 is a mediator of its interaction with PTP1B. In support of this scenario, we observed increased cerulein-induced PERK phosphorylation in knockout mice and inhibitor-treated acinar cells. However, we cannot exclude the possibility that PTP1B modulates ER stress indirectly. Indeed, PTP1B activates c-Src by dephosphorylating Tyr527,⁷⁰ and Src has been implicated in ER stress response.⁷¹

The salutary metabolic effects of PTP1B deficiency and pharmacological inhibition on insulin sensitivity and energy balance establish this enzyme as a potential therapeutic target for obesity and type 2 diabetes. However, the current findings define a role for pancreatic PTP1B in experimental AP and suggest that PTP1B likely acts in a cell type- and tissue-dependent manner to regulate signaling and the inflammatory response.

Acknowledgments

A.B. designed and performed research, analyzed data, and wrote the manuscript; S.K., S.C., S.B., and S.G. performed research and revised the manuscript; J.S. contributed reagents and intellectual input and revised the manuscript; F.G.H. designed research, analyzed data, and wrote the manuscript.

References

1. Baron TH, Morgan DE: Acute necrotizing pancreatitis. *N Engl J Med* 1999, 340:1412–1417
2. Pavlidis P, Crichton S, Lemmich Smith J, Morrison D, Atkinson S, Wyncoll D, Ostermann M: Improved outcome of severe acute pancreatitis in the intensive care unit. *Crit Care Res Pract* 2013, 2013: 897107

3. Yadav D, Lowenfels AB: The epidemiology of pancreatitis and pancreatic cancer. *Gastroenterology* 2013, 144:1252–1261
4. Roberts SE, Akbari A, Thorne K, Atkinson M, Evans PA: The incidence of acute pancreatitis: impact of social deprivation, alcohol consumption, seasonal and demographic factors. *Aliment Pharmacol Ther* 2013, 38:539–548
5. Singh VK, Moran RA, Afghani E, de-Madaria E: Treating acute pancreatitis: what's new? *Expert Rev Gastroenterol Hepatol* 2015, 9: 901–911
6. Saluja AK, Donovan EA, Yamanaka K, Yamaguchi Y, Hofbauer B, Steer ML: Cerulein-induced in vitro activation of trypsinogen in rat pancreatic acini is mediated by cathepsin B. *Gastroenterology* 1997, 113:304–310
7. Naruse S: Molecular pathophysiology of pancreatitis. *Intern Med* 2003, 42:288–289
8. Lerch MM, Gorelick FS: Models of acute and chronic pancreatitis. *Gastroenterology* 2013, 144:1180–1193
9. Jensen RT, Wank SA, Rowley WH, Sato S, Gardner JD: Interaction of CCK with pancreatic acinar cells. *Trends Pharmacol Sci* 1989, 10: 418–423
10. Willemer S, Elsasser HP, Adler G: Hormone-induced pancreatitis. *Eur Surg Res* 1992, 24(Suppl 1):29–39
11. Gukovsky I, Gukovskaya AS, Blinman TA, Zaninovic V, Pandol SJ: Early NF-kappaB activation is associated with hormone-induced pancreatitis. *Am J Physiol* 1998, 275:G1402–G1414
12. Kim H: Cerulein pancreatitis: oxidative stress, inflammation, and apoptosis. *Gut Liver* 2008, 2:74–80
13. Lutz MP, Sutor SL, Abraham RT, Miller LJ: A role for cholecystokinin-stimulated protein tyrosine phosphorylation in regulated secretion by the pancreatic acinar cell. *J Biol Chem* 1993, 268: 11119–11124
14. Rivard N, Lebel D, Laine J, Morisset J: Regulation of pancreatic tyrosine kinase and phosphatase activities by cholecystokinin and somatostatin. *Am J Physiol* 1994, 266:G1130–G1138
15. Sarmiento N, Sanchez-Bernal C, Ayra M, Perez N, Hernandez-Hernandez A, Calvo JJ, Sanchez-Yague J: Changes in the expression and dynamics of SHP-1 and SHP-2 during cerulein-induced acute pancreatitis in rats. *Biochim Biophys Acta* 2008, 1782:271–279
16. Garcia-Hernandez V, Sarmiento N, Sanchez-Bernal C, Matellan L, Calvo JJ, Sanchez-Yague J: Modulation in the expression of SHP-1, SHP-2 and PTP1B due to the inhibition of MAPKs, cAMP and neutrophils early on in the development of cerulein-induced acute pancreatitis in rats. *Biochim Biophys Acta* 2014, 1842: 192–201
17. Sarmiento N, Sanchez-Bernal C, Perez N, Sardina JL, Mangas A, Calvo JJ, Sanchez-Yague J: Rolipram and SP600125 suppress the early increase in PTP1B expression during cerulein-induced pancreatitis in rats. *Pancreas* 2010, 39:639–645
18. Bettaieb A, Xi Y, Hosein E, Coggins N, Bachaalany S, Wiede F, Perez S, Griffey SM, Sastre J, Tiganis T, Haj FG: Pancreatic T cell protein-tyrosine phosphatase deficiency ameliorates cerulein-induced acute pancreatitis. *Cell Commun Signal* 2014, 12:13
19. Frangioni JV, Beahm PH, Shifrin V, Jost CA, Neel BG: The nontransmembrane tyrosine phosphatase PTP-1B localizes to the endoplasmic reticulum via its 35 amino acid C-terminal sequence. *Cell* 1992, 68:545–560
20. Haj FG, Markova B, Klamann LD, Bohmer FD, Neel BG: Regulation of receptor tyrosine kinase signaling by protein tyrosine phosphatase-1B. *J Biol Chem* 2003, 278:739–744
21. Arregui CO, Balsamo J, Lilien J: Impaired integrin-mediated adhesion and signaling in fibroblasts expressing a dominant-negative mutant PTP1B. *J Cell Biol* 1998, 143:861–873
22. Liu F, Sells MA, Chernoff J: Protein tyrosine phosphatase 1B negatively regulates integrin signaling. *Curr Biol* 1998, 8:173–176
23. Byon JC, Kusari AB, Kusari J: Protein-tyrosine phosphatase-1B acts as a negative regulator of insulin signal transduction. *Mol Cell Biochem* 1998, 182:101–108
24. Elchebly M, Payette P, Michaliszyn E, Cromlish W, Collins S, Loy AL, Normandin D, Cheng A, Himms-Hagen J, Chan CC, Ramachandran C, Gresser MJ, Tremblay ML, Kennedy BP: Increased insulin sensitivity and obesity resistance in mice lacking the protein tyrosine phosphatase-1B gene. *Science* 1999, 283:1544–1548
25. Klamann LD, Boss O, Peroni OD, Kim JK, Martino JL, Zabolotny JM, Moghal N, Lubkin M, Kim YB, Sharpe AH, Stricker-Krongrad A, Shulman GI, Neel BG, Kahn BB: Increased energy expenditure, decreased adiposity, and tissue-specific insulin sensitivity in protein-tyrosine phosphatase 1B-deficient mice. *Mol Cell Biol* 2000, 20: 5479–5489
26. Delibegovic M, Bence KK, Mody N, Hong EG, Ko HJ, Kim JK, Kahn BB, Neel BG: Improved glucose homeostasis in mice with muscle-specific deletion of protein-tyrosine phosphatase 1B. *Mol Cell Biol* 2007, 27:7727–7734
27. Delibegovic M, Zimmer D, Kauffman C, Rak K, Hong EG, Cho YR, Kim JK, Kahn BB, Neel BG, Bence KK: Liver-specific deletion of protein-tyrosine phosphatase 1B (PTP1B) improves metabolic syndrome and attenuates diet-induced ER stress. *Diabetes* 2008, 58: 590–598
28. Bence KK, Delibegovic M, Xue B, Gorgun CZ, Hotamisligil GS, Neel BG, Kahn BB: Neuronal PTP1B regulates body weight, adiposity and leptin action. *Nat Med* 2006, 12:917–924
29. Tsou RC, Rak KS, Zimmer DJ, Bence KK: Improved metabolic phenotype of hypothalamic PTP1B-deficiency is dependent upon the leptin receptor. *Mol Metab* 2014, 3:301–312
30. Kushner JA, Haj FG, Klamann LD, Dow MA, Kahn BB, Neel BG, White MF: Islet-sparing effects of protein tyrosine phosphatase-1b deficiency delays onset of diabetes in IRS2 knockout mice. *Diabetes* 2004, 53:61–66
31. Bettaieb A, Liu S, Xi Y, Nagata N, Matsuo K, Matsuo I, Chahed S, Bakke J, Keilhack H, Tiganis T, Haj FG: Differential regulation of endoplasmic reticulum stress by protein tyrosine phosphatase 1B and T cell protein tyrosine phosphatase. *J Biol Chem* 2011, 286: 9225–9235
32. Liu S, Xi Y, Bettaieb A, Matsuo K, Matsuo I, Kulkarni RN, Haj FG: Disruption of protein-tyrosine phosphatase 1B expression in the pancreas affects beta-cell function. *Endocrinology* 2014, 155: 3329–3338
33. Dawra R, Sharif R, Phillips P, Dudeja V, Dhaulakhandi D, Saluja AK: Development of a new mouse model of acute pancreatitis induced by administration of L-arginine. *Am J Physiol Gastrointest Liver Physiol* 2007, 292:G1009–G1018
34. Ornitz DM, Palmiter RD, Messing A, Hammer RE, Pinkert CA, Brinster RL: Elastase I promoter directs expression of human growth hormone and SV40 T antigen genes to pancreatic acinar cells in transgenic mice. *Cold Spring Harb Symp Quant Biol* 1985, 50: 399–409
35. Wiesmann C, Barr KJ, Kung J, Zhu J, Erlanson DA, Shen W, Fahr BJ, Zhong M, Taylor L, Randal M, McDowell RS, Hansen SK: Allosteric inhibition of protein tyrosine phosphatase 1B. *Nat Struct Mol Biol* 2004, 11:730–737
36. Zhou X, Liu Z, Cheng X, Zheng Y, Zeng F, He Y: Socs1 and Socs3 degrades Traf6 via polyubiquitination in LPS-induced acute necrotizing pancreatitis. *Cell Death Dis* 2015, 6:e2012
37. Bettaieb A, Bakke J, Nagata N, Matsuo K, Xi Y, Liu S, AbouBechara D, Melhem R, Stanhope K, Cummings B, Graham J, Bremer A, Zhang S, Lyssiotis CA, Zhang ZY, Cantley LC, Havel PJ, Haj FG: Protein tyrosine phosphatase 1B regulates pyruvate kinase M2 tyrosine phosphorylation. *J Biol Chem* 2013, 288:17360–17371
38. Dembinski A, Warzecha Z, Ceranowicz P, Warzecha AM, Pawlik WW, Dembinski M, Rembiasz K, Sendur P, Kusnier-Cabala B, Tomaszewska R, Chowaniec E, Konturek PC: Dual, time-dependent deleterious and protective effect of anandamide on the course of cerulein-induced acute pancreatitis: role of sensory nerves. *Eur J Pharmacol* 2008, 591:284–292

39. Bettaieb A, Chahed S, Tabet G, Yang J, Morisseau C, Griffey S, Hammock BD, Haj FG: Effects of soluble epoxide hydrolase deficiency on acute pancreatitis in mice. *PLoS One* 2014, 9:e113019
40. Al-Bahrani AZ, Ammori BJ: Clinical laboratory assessment of acute pancreatitis. *Clin Chim Acta* 2005, 362:26–48
41. Ceranowicz P, Cieszkowski J, Warzecha Z, Dembinski A: Experimental models of acute pancreatitis. *Postepy Hig Med Dosw (Online)* 2015, 69:264–269
42. Czako L, Szabolcs A, Vajda A, Csati S, Venglovecz V, Rakonczay Z Jr, Hegyi P, Tiszlavicz L, Csont T, Posa A, Berko A, Varga C, Varga Ilona S, Boros I, Lonovics J: Hyperlipidemia induced by a cholesterol-rich diet aggravates necrotizing pancreatitis in rats. *Eur J Pharmacol* 2007, 572:74–81
43. Vaquero E, Gukovsky I, Zaninovic V, Gukovskaya AS, Pandol SJ: Localized pancreatic NF-kappaB activation and inflammatory response in taurocholate-induced pancreatitis. *Am J Physiol Gastrointest Liver Physiol* 2001, 280:G1197–G1208
44. Bonizzi G, Karin M: The two NF-kappaB activation pathways and their role in innate and adaptive immunity. *Trends Immunol* 2004, 25: 280–288
45. Yang F, Tang E, Guan K, Wang CY: IKK beta plays an essential role in the phosphorylation of RelA/p65 on serine 536 induced by lipopolysaccharide. *J Immunol* 2003, 170:5630–5635
46. Xu H, An H, Hou J, Han C, Wang P, Yu Y, Cao X: Phosphatase PTP1B negatively regulates MyD88- and TRIF-dependent proinflammatory cytokine and type I interferon production in TLR-triggered macrophages. *Mol Immunol* 2008, 45:3545–3552
47. Heinonen KM, Dube N, Bourdeau A, Lapp WS, Tremblay ML: Protein tyrosine phosphatase 1B negatively regulates macrophage development through CSF-1 signaling. *Proc Natl Acad Sci U S A* 2006, 103:2776–2781
48. Berdnikovs S, Pavlov VI, Abdala-Valencia H, McCary CA, Klumpp DJ, Tremblay ML, Cook-Mills JM: PTP1B deficiency exacerbates inflammation and accelerates leukocyte trafficking in vivo. *J Immunol* 2012, 188:874–884
49. Traves PG, Pardo V, Pimentel-Santillana M, Gonzalez-Rodriguez A, Mojena M, Rico D, Montenegro Y, Cales C, Martin-Sanz P, Valverde AM, Bosca L: Pivotal role of protein tyrosine phosphatase 1B (PTP1B) in the macrophage response to pro-inflammatory and anti-inflammatory challenge. *Cell Death Dis* 2014, 5:e1125
50. Grant L, Shearer KD, Czopek A, Lees EK, Owen C, Agouni A, Workman J, Martin-Granados C, Forrester JV, Wilson HM, Mody N, Delibegovic M: Myeloid-cell protein tyrosine phosphatase-1B deficiency in mice protects against high-fat diet and lipopolysaccharide-induced inflammation, hyperinsulinemia, and endotoxemia through an IL-10 STAT3-dependent mechanism. *Diabetes* 2014, 63:456–470
51. Schafer C, Williams JA: Stress kinases and heat shock proteins in the pancreas: possible roles in normal function and disease. *J Gastroenterol* 2000, 35:1–9
52. Ron D, Walter P: Signal integration in the endoplasmic reticulum unfolded protein response. *Nat Rev Mol Cell Biol* 2007, 8: 519–529
53. Kubisch CH, Sans MD, Arumugam T, Ernst SA, Williams JA, Logsdon CD: Early activation of endoplasmic reticulum stress is associated with arginine-induced acute pancreatitis. *Am J Physiol Gastrointest Liver Physiol* 2006, 291:G238–G245
54. Malo A, Kruger B, Goke B, Kubisch CH: 4-Phenylbutyric acid reduces endoplasmic reticulum stress, trypsin activation, and acinar cell apoptosis while increasing secretion in rat pancreatic acini. *Pancreas* 2013, 42:92–101
55. Bettaieb A, Chahed S, Bachaalany S, Griffey S, Hammock BD, Haj FG: Soluble epoxide hydrolase pharmacological inhibition ameliorates experimental acute pancreatitis in mice. *Mol Pharmacol* 2015, 88:281–290
56. Ji B, Chen XQ, Misek DE, Kuick R, Hanash S, Ernst S, Najarian R, Logsdon CD: Pancreatic gene expression during the initiation of acute pancreatitis: identification of EGR-1 as a key regulator. *Physiol Genomics* 2003, 14:59–72
57. Dadke SS, Li HC, Kusari AB, Begum N, Kusari J: Elevated expression and activity of protein-tyrosine phosphatase 1B in skeletal muscle of insulin-resistant type II diabetic Goto-Kakizaki rats. *Biochem Biophys Res Commun* 2000, 274:583–589
58. Taghibiglou C, Rashid-Kolvear F, Van Iderstine SC, Le-Tien H, Fantus IG, Lewis GF, Adeli K: Hepatic very low density lipoprotein-ApoB overproduction is associated with attenuated hepatic insulin signaling and overexpression of protein-tyrosine phosphatase 1B in a fructose-fed hamster model of insulin resistance. *J Biol Chem* 2002, 277:793–803
59. Bakke J, Haj FG: Protein-tyrosine phosphatase 1B substrates and metabolic regulation. *Semin Cell Dev Biol* 2015, 37C:58–65
60. Ahmad F, Considine RV, Bauer TL, Ohannesian JP, Marco CC, Goldstein BJ: Improved sensitivity to insulin in obese subjects following weight loss is accompanied by reduced protein-tyrosine phosphatases in adipose tissue. *Metabolism* 1997, 46:1140–1145
61. Cheung A, Kusari J, Jansen D, Bandyopadhyay D, Kusari A, Bryer-Ash M: Marked impairment of protein tyrosine phosphatase 1B activity in adipose tissue of obese subjects with and without type 2 diabetes mellitus. *J Lab Clin Med* 1999, 134:115–123
62. Zabolotny JM, Kim YB, Welsh LA, Kershaw EE, Neel BG, Kahn BB: Protein-tyrosine phosphatase 1B expression is induced by inflammation in vivo. *J Biol Chem* 2008, 283:14230–14241
63. Pereda J, Sabater L, Aparisi L, Escobar J, Sandoval J, Vina J, Lopez-Rodas G, Sastre J: Interaction between cytokines and oxidative stress in acute pancreatitis. *Curr Med Chem* 2006, 13:2775–2787
64. Baumann B, Wagner M, Aleksic T, von Wichert G, Weber CK, Adler G, Wirth T: Constitutive IKK2 activation in acinar cells is sufficient to induce pancreatitis in vivo. *J Clin Invest* 2007, 117:1502–1513
65. Chen X, Ji B, Han B, Ernst SA, Simeone D, Logsdon CD: NF-kappaB activation in pancreas induces pancreatic and systemic inflammatory response. *Gastroenterology* 2002, 122:448–457
66. Minutoli L, Altavilla D, Marini H, Passaniti M, Bitto A, Seminara P, Venuti FS, Famulari C, Macri A, Versaci A, Squadrito F: Protective effects of SP600125 a new inhibitor of c-jun N-terminal kinase (JNK) and extracellular-regulated kinase (ERK1/2) in an experimental model of cerulein-induced pancreatitis. *Life Sci* 2004, 75:2853–2866
67. Lugea A, Tischler D, Nguyen J, Gong J, Gukovsky I, French SW, Gorelick FS, Pandol SJ: Adaptive unfolded protein response attenuates alcohol-induced pancreatic damage. *Gastroenterology* 2011, 140: 987–997
68. Krishnan N, Fu C, Pappin DJ, Tonks NK: H2S-induced sulphydration of the phosphatase PTP1B and its role in the endoplasmic reticulum stress response. *Sci Signal* 2011, 4:ra86
69. Bettaieb A, Matsuo K, Matsuo I, Wang S, Melhem R, Koromilas AE, Haj FG: Protein tyrosine phosphatase 1B deficiency potentiates PERK/eIF2alpha signaling in brown adipocytes. *PLoS One* 2012, 7: e34412
70. Bjorge JD, Pang A, Fujita DJ: Identification of protein-tyrosine phosphatase 1B as the major tyrosine phosphatase activity capable of dephosphorylating and activating c-Src in several human breast cancer cell lines. *J Biol Chem* 2000, 275:41439–41446
71. Moon SY, Kim HS, Nho KW, Jang YJ, Lee SK: Endoplasmic reticulum stress induces epithelial-mesenchymal transition through autophagy via activation of c-Src kinase. *Nephron Exp Nephrol* 2014, 126:127–140