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The naïve effector cells of collagen type I during acute experimental pancreatitis are acinar cells and not pancreatic stellate cells

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ABSTRACT

Objective: The purpose of this study was to investigate the expression of collagen type I and the mRNA level of its regulatory factor, TGF-β1, in tissue samples of acute pancreatitis and to determine the significance of collagen type I in predisposition to pancreatic fibrosis during acute pancreatitis.
Methods: Sprague–Dawley rats were divided into an experimental group (30 rats) and a control group (12 rats). The rats in the experimental group were intraperitoneally injected with cerulein to induce acute pancreatitis. The distribution and expression of collagen type I in the pancreatic tissues were examined by immunohistochemical staining. The mRNA level of TGF-β1 was determined by real-time polymerase chain reaction (PCR).
Results: (1) Collagen type I was localized in the cytoplasm of pancreatic acinar cells. With pancreatitis progressed, strong positive staining for collagen type I covered whole pancreatic lobules, whereas, the islet tissue, interlobular area, and pancreatic necrotic area were negative for collagen type I. (2) The level of TGF-β1 mRNA in rats from the experimental group increased gradually the establishment of acute pancreatitis, and was significantly higher than that in the control group at every time point.
Conclusions: (1) During acute pancreatitis, pancreatic acinar cells, not pancreatic stellate cells as traditionally believed, were the naïve effector cells of collagen type I. (2) TGF-β1 played a key role in regulating collagen I expression during acute pancreatitis.

1. Introduction

Acute pancreatitis (AP) is commonly seen in clinical practice. Chronic pancreatitis (CP) is usually associated with repeated outbreaks of acute pancreatitis. Fibrosis induced by acute pancreatitis leads to pancreatic tissue fibrosis [1,2]. Excessive production and deposition of collagen also result in tissue fibrosis. Collagen has at least ten subtypes, among which collagen type I is the most abundant and has the most diverse functions [3]. Numerous studies have shown that during the process of pancreatic fibrosis, pancreatic stellate cells (PSCs) are the effector cells of collagen type I [4,5]. PSCs are activated by multiple stimulatory factors during pancreatic injury and synthesize extracellular matrix (ECM) proteins, mainly collagen type I and type III and fibronectin, which play key roles in pancreatic fibrosis [6,7]. PSCs are located at the peripheral area of pancreatic acinar cells, the interlobular area, the pancreatic duct epithelium, and the interlobular vascular epithelial [8,9]. Currently, most studies on pancreatic fibrosis focus on chronic pancreatitis. However, the expression status of collagen type I in acute pancreatitis and whether other types of cells in addition to PSCs also express collagen type I remain unclear. Transforming growth factor β1 (TGF-β1) is a member of the TGF-β superfamily and is the main regulatory factor for collagen type I. TGF-β1 is an active peptide with a molecular weight of 25 kD that plays a role in stimulating cell proliferation and differentiation [10–12]. The expression status of TGF-β1 during acute pancreatitis remains unknown. In this study, we examined the expression of collagen type I and the mRNA level of its regulator TGF-β1 during acute pancreatitis and investigated the role of collagen type I in early pancreatic fibrosis.

2. Materials and methods

2.1. Materials

Rabbit anti-rat collagen type I monoclonal antibody was purchased from Santa Cruz Biotechnology (Dallas, TX, USA). Ready-to-use immunohistochemical staining kits (SABC) were purchased

Abbreviations: AP, acute pancreatitis; CP, chronic pancreatitis; PSCs, pancreatic stellate cells; ECM, synthesize extracellular matrix; TGF, transforming growth factor; PCR, polymerase chain reaction.

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from Wuhan Boster Biological Technology (Wuhan, China). DAB color development solution was purchased from Beijing Zhongshan Biotechnology Co., Ltd. (China). The following reagents and instruments were used in this study: TRIzol (Invitrogen, Grand Island, NY, USA), Ribolock inhibitor (MBI), agarose (Bio-Rad, Hercules, CA, USA), RevertAid™ M-MuLV reverse transcriptase (MBI), SYBR Premix Ex Taq II (Takara, Shiga, Japan), PCR Mixture (SinoBi), low temperature centrifuge (Jacque Fresco, USA), UV spectrophotometer (GE Healthcare, Uppsala, Sweden), PCR machine (Bio-Rad), nucleic acid electrophoresis system (Beijing Baijing, China), and an image analysis system (UVP, Upland, CA, USA).

2.2. Animal grouping and the establishment of a rat acute pancreatitis model

Forty-two Sprague–Dawley (SD) rats (female or male, body weight 120 ± 20 g) were provided by the animal care center of Huaxi Medical University. The rats were housed at a temperature of 18–28 °C and a humidity of 40–70%. The rats were randomly divided into an experimental group containing 30 rats and a control group containing 12 rats. Food uptake of the rats was restricted, but the animals were allowed water for 12 h before acute pancreatitis was induced and within 24 h after the establishment of the acute pancreatitis model. Twenty-four hours after the establishment of acute pancreatitis, the rats were allowed free access to food and water. The rats in the experimental group were intraperitoneally injected with cerulein at a dosage of 50 μg/kg body weight four times. Each injection lasted for 1 h. The rats in the control group were injected with 0.5 mL saline in the same manner and at the same time points. The rats in the experimental group (five rats) and the control group (two rats) were sacrificed by cervical dislocation at 6 h, 1 d, 2 d, 3 d, 5 d, or 7 d after the establishment of the model.

2.3. Immunohistochemistry

Color development of collagen type I staining was performed using an SABC method. Slides were treated with poly-D-lysine to enhance cell attachment. Paraffin-embedded tissue was sectioned with 5 μm thickness. Tissue sections were routinely dehydrated with xylene, dewaxed with an alcohol gradient, washed with 0.01 mol/L PBS for 2 min three times, and exposed to 3 mol/L H_2O_2 solution for 10 min to inactivate peroxidase. The tissue sections were then microwaved at 100 °C for 10 min in citric acid solution to restore the antigen, cooled at room temperature for 25 min, and washed with 0.01 mol/L PBS for 2 min three times. The tissue sections were blocked in goat serum for 10 min at room temperature. Excess liquid was shaken off the slide. Primary antibody with rabbit anti-rat IgG was added to the slide without washing the slide. The slide was incubated at 4 °C overnight and washed with 0.01 mol/L PBS for 2 min three times. The biotin-conjugated secondary antibody goat anti-rabbit IgG was then added. The slide was incubated at 37 °C for 20 min and washed with 0.01 mol/L PBS for 2 min three times. The slide was then exposed to SABC reagent, incubated at 37 °C for 20 min and washed with 0.01 mol/L PBS for 2 min three times. Color was developed using DAB. One drop of reagents A, B, and C from the DAB kit were added to 1 mL of distilled water and mixed well. The solution was then added to the tissue section. The tissue section was lightly stained with hematoxylin for 50 s, dehydrated, permeabilized, and mounted. For a negative control, a tissue section was incubated with PBS buffer instead of primary antibody. Cells exhibiting brown-yellow particles in the cytoplasm and nucleus were considered positive for staining. Positive tissue sections were analyzed with the Motic Images Advanced 3.2 image analysis system.

2.4. Real-time PCR

PCR primers were designed using the software Primer 5.0 and synthesized by Shanghai Sangon Company (China). The forward primer for TGF-β1 was 5′-ACCGCAACAACGCAATCTATG-3′, and the reverse primer was 5′-ATTCCGTCTCCTTGGTTCAG C-3′. β-actin was used as the reference gene. The forward primer for β-actin was 5′-TGACGTGGACATCCGCAAAG-3′, and the reverse primer was 5′-CTGGAAGGTGGACAGCGAGG-3′. Total RNA was extracted using the TRIzol method. The reverse transcription reaction system contained 10 μl 5× buffer, 5 μl 10 mmol/L dNTP, 1 μl RNase inhibitor, 3 μl 100 mg/L Oligo(dT), 1 μl total RNA (2–4 μg), and 2 μl 200 U/μl M-MuLV. The volume was brought to 50 μl by adding RNase-free water. The reverse transcription reaction was incubated at 42 °C for 60 min and then 95 °C for 5 min. The system was cooled rapidly on ice to 10 °C and stored at −40 °C for future use. The PCR reaction mixture contained 12.5 μl 2× ExTaq, 0.8 μl 10 μM forward and reverse primers, and 2 μl cDNA, and the volume was brought to 25 μl with RNase-free water. The reaction condition was optimized to for the following steps: (1) pre-denature at 95 °C for 3 min; (2) denature at 94 °C for 20 s, anneal at 56 °C for 30 s, and extend at 72 °C for 30 s for 40 cycles; (3) denature at 95 °C for 1 min; (4) anneal at 55 °C for 20 s for 80 cycles. Four microliters of PCR product was analyzed by 1.5% agarose gel electrophoresis. DL2000 was used as a molecular weight standard. The gel was photographed with a gel imaging system and analyzed using an absorbance scan.

2.5. Statistical analysis

PCR results were analyzed with the statistical analyzing software SPSS 13.0. Data were analyzed using the one-way ANOVA and LSD tests for inter-group comparison. $P < 0.05$ was considered to be significantly different.

3. Results

3.1. Characteristics of collagen type I protein distribution (Fig. 1)

Collagen type I appeared as granular particles and was localized in the cytoplasm. Positive staining showed as a brown color. Normal pancreatic tissue showed little positive staining. Six hours after the establishment of acute pancreatitis, pancreatic acinar cells exhibited positive staining. This staining, which was pedal and fan shaped, was localized in the peripheral area of the pancreatic gland. The positive staining was relatively weak. The center of the pancreatic gland was negative for the staining. One to three days after the establishment of acute pancreatitis, the area of positive staining progressed to the center of the pancreatic gland. The peripheral area of the pancreatic gland where inflammatory cells accumulated exhibited strong positive staining, and the center of the pancreatic gland showed weak staining. The interlobular area and islets did not contain any cells with positive staining. Five to seven days after the establishment of acute pancreatitis, positive staining covered entire pancreatic lobules. The positive staining was very strong in acinar cells. The interlobular area, islet tissue, and necrotic area did not show positive staining.

3.2. Expression of TGF-β1 mRNA in pancreatic tissue (Fig. 2 and Table 1)

Electrophoresis of the PCR product amplified using the TGF-β1 and β-actin primers and templates from both the control and experimental group at different time points are shown in Fig. 2. shows the comparison of the expression of TGF-β1 mRNA between

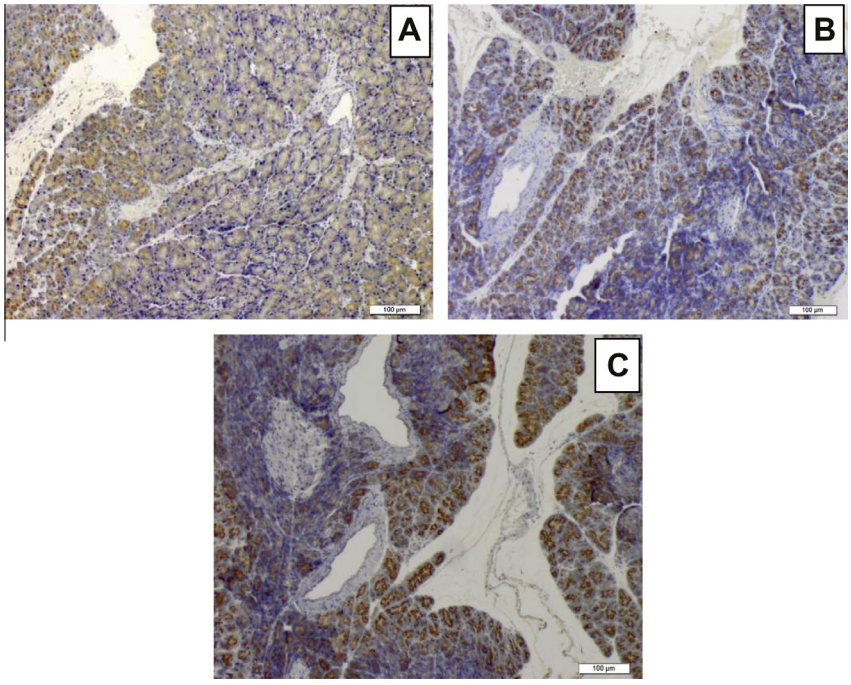

Fig. 1. One day after the establishment of acute pancreatitis, staining for collagen type I in the pancreatic gland was strong, and the staining in the center of pancreatic gland was weak. The interlobular area and islet were negative for staining (A). Five to seven days after the establishment of acute pancreatitis, the positive staining in the gland area was strong and showed a diffuse pattern. The staining appeared as a banded and patchy distribution. The interlobular area, islet tissue, and inflammatory necrosis were negative for staining (B and C. Express of collagen type I in the pancreatic tissue).

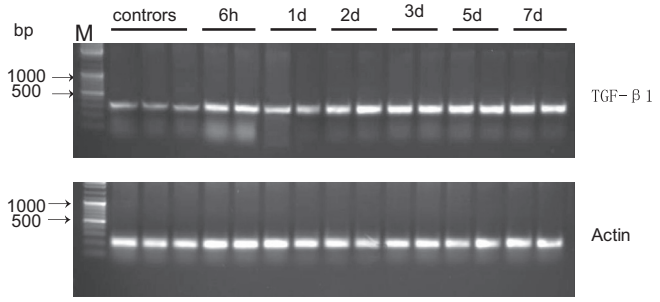

Fig. 2. Show that the relative densitometry of TGF-β1 mRNA in the experimental group increased gradually 6 h after the establishment of acute pancreatitis and reached its peak level at 7 d. There was a significantly higher amount of TGF-β1 mRNA in the experimental group than in the control group at each time point and at all time points ($p < 0.05$. Gelelectrophoresis of TGF-β1 RT-PCR product).

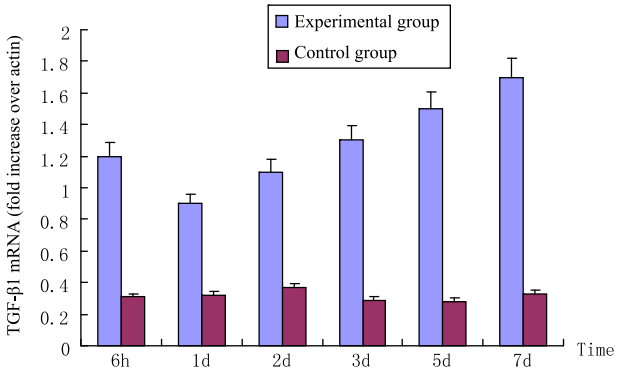

Table 1. Expression of TGF-β1 mRNA.

the experimental and the control group. The PCR products for TGF-β1 and β-actin were 338 bp and 210 bp long, respectively. Normal pancreatic tissue expressed a low level of TGF-β1 mRNA. After the establishment of acute pancreatitis, the expression level of TGF-β1 mRNA gradually increased and reached its peak level at 7 d. Densitometry showed that the level of TGF-β1 mRNA in the experimental group was significantly higher than that in the control group at the same time point.

4. Discussion

4.1. Acinar cells of the pancreatic gland are the cells expressing collagen type I

In this study, we investigated the localization and distribution of collagen type I during the process of damage repair in acute pancreatitis and found that the damaged acinar cells of pancreatic gland were the cells expressing collagen type I. The evidence to support our conclusion is listed as follows: (1) Six hours after the establishment of acute pancreatitis, acinar cells around lobules exhibited weak collagen type I expression. Acinar cells in the center of lobules did not show significant expression. As the disease progressed, inflammatory cells invaded into the center of the pancreatic gland along the interlobular tissue, and the expression of collagen type I in acinar cells gradually increased. At day 7 of the disease, the expression of collagen type I in the acinar cells of the lobules became strong and diffuse. (2) Our previous study demonstrated that pancreatic lobules are the smallest component of pancreatic tissue to react to acute pancreatitis. The cells most damaged initially are acinar cells surrounding pancreatic lobules. The islets in the center of pancreatic lobules are the last to be affected and damaged [13]. This phenomenon may be associated with a self-defensive reaction of acinar cells. At the early stage of acute pancreatitis, an inflammatory cascade is triggered in pancreatic tissue, and large amounts of inflammatory factors are released into interstitial fluid that subsequently induce acinar cells to initiate self-defensive reactions such as synthesizing and depositing larges

amount of collagen into the interstitial area. The accumulated collagen forms a protective barrier for the pancreatic gland that prevents inflammatory factors from damaging gland cells and the islet. (3) Except for acinar cells, all the other areas of pancreatic tissue were negative for collagen I expression. As the disease progressed, pancreatic tissue exhibited areas of local necrosis, and large amounts of inflammatory cells invaded the interlobular area; these areas and the islet were negative for the expression of collagen I.

The traditional view is that PSCs play a key role in the development of pancreatic fibrosis and that PSCs are the cells producing collagen type I [14,15]. Based on our study, we believe that the collagen type I-positive cells are not PSCs for the following reasons: (1) PSCs are mainly localized in the peripheral area of acinar cells and also in the lobular duct epithelium, lobular vascular epithelium, and interlobular area [8,9]. In this study, we found that only acinar cells expressed collagen type I, whereas other areas did not show any positive cells. (2) It has been shown that α-smooth muscle actin (α-SMA) is specifically expressed in PSCs during the early stage of acute pancreatitis [3]; These α-SMA positive cells are mainly localized at the peripheral area of acinar cells and can also be found in the lobular duct epithelium and lobular vascular epithelium [16,17]. In this study, collagen type I expression was not detected in the lobular duct epithelium or the lobular vascular epithelium. (3) Acinar cells have been recognized to be the cells that respond the earliest to inflammatory factors in pancreatic tissue. Collagen type I was expressed at 6 h after the establishment of acute pancreatitis, and PSCs are activated by inflammatory factors. In vitro studies demonstrated that the expression of collagen is delayed in PSCs [18,19]. (4) Some studies have shown that PSCs express stem cell factors such as nestin and are migratory [20]. Our previous study demonstrated that at the early stage of inflammation, nestin was mainly expressed in the interlobular vascular epithelium. As the disease progressed, the cells expressing nestin migrated to the acinar tissue [21]. If the nestin-expressing cells were PSCs, However, our study showed that the expression of collagen type I was mainly in the area of acinar cells but not in the interlobular vascular epithelium.

Acharya et al. analyzed the histology of pancreatic tissue from acute and chronic pancreatitis patients and also performed in vitro experiments [22]. They found that fat necrosis associated peri-fat acinar necrosis contributed to most of the necrosis observed in AP samples; However, peri-fat acinar necrosis and total necrosis were significantly lower in samples from patients with CP and AP-on-CP. Fibrosis appeared to wall off the fat necrosis and limit peri-fat acinar necrosis, reducing acinar necrosis. In vitro, collagen type I limited the lipolytic flux between acinar cells and adipocytes and prevented increases in adipokines in the acinar compartment. Thus, collagen fiber hyperplasia can induce the benign reversion of acute pancreatitis. Other studies showed that collagen expression in the area of the pancreatic gland is gradually reduced when pancreatic inflammation is relieved [23]. So, we propose that the high expression of collagen type I in acinar cells is a defensive reaction to acute damage during pancreatic acute injury.

4.2. TGF-β1 plays a key role in regulating the expression of collagen type I

TGF-β1 is mainly synthesized and secreted by inflammatory cells such as lymphocytes, monocyte-macrophages, and platelets. As a cytokine with multiple functions, TGF-β1 regulates proliferation, differentiation, and the expression of extracellular matrix proteins [24]. Recently, it has been increasingly recognized that TGF-β1 plays a key role in wound healing [25]. TGF-β1 has been found to be the most relevant and most representative growth factor associated with scar formation. It is also the key cytokine causing fibrosis [11,26]. TGF-β1 stimulates ECM production and inhibits the activity of proteinases and matrix metalloproteinases to promote ECM deposition, substantially inducing the synthesis of pre-collagen type I and stimulating the synthesis of cellulose-binding factor. TGF-β1 also provides a scaffold for collagen deposition, induces the formation of granulation tissue, and stimulates repair after tissue damage [27]. It has been demonstrated that the expression of TGF-β1 mRNA and collagen fiber deposition are positively correlated in chronic pancreatitis [28]. PSCs play a key role in the process of tissue fibrosis.

We believe that high expression of collagen type I is a defensive response of acinar cells to acute pancreatitis. Whether the high expression is also related to the regulation of the mRNA level of TGF-β1 is unclear. In our study, the level of TGF-β1 mRNA was low in the control group but constantly highly expressed during inflammation. This result may be associated with the continuous effects of large amounts of inflammatory cells in the pancreatic inflammatory area. At the early stage of acute inflammation, TGF-β1, as the predominant mediator, either alone or synergistically and either directly or indirectly plays a key role in the interaction between cells and the ECM. TGF-β1 causes acinar cells in the peripheral area of pancreatic lobules to highly express collagen type I, which then protects the acinar cells and islet tissue in the center of the gland and maximally maintains the normal function of the gland. It has been demonstrated that the injection of a TGF-β1 neutralizing antibody can significantly reduce collagen expression [29]. An in vitro study has shown that pre-treatment of PSCs with the TGF-β1 antagonists bone morphogenetic proteins (BMPs) significantly downregulates the expression of collagen type I [30]. These findings suggest that TGF-β1 effectively regulates the expression of collagen type I during acute pancreatitis.

References

[1] M. Apte, R. Pirola, J. Wilson, The fibrosis of chronic pancreatitis: new insights into the role of pancreatic stellate cells, Antioxid. Redox Signal. 15 (2011) 2711–2722.

[2] M. Pasca, C. Forsmark, S. Freedman, et al., Advances in acute and chronic pancreatitis: from development to inflammation and repair, Gastroenterology 144 (2013) 1–4.

[3] A. Rudnick, Advances in tissue engineering and use of type 1 bovine collagen particles in wound bed preparation, J. Wound Care 15 (2006) 402–404.

[4] D. Long, J. Lu, L. Luo, et al., Effects of octreotide on activated pancreatic stellate cell-induced pancreas graft fibrosis in rats, J. Surg. Res. 176 (2012) 248–259.

[5] L. Li, D. Bimmler, R. Graf, et al., PSP/reg inhibits cultured pancreatic stellate cell and regulates MMP/TIMP ratio, Eur. J. Clin. Invest. 41 (2011) 151–158.

[6] L. Li, M.G. Bachem, S. Zhou, et al., Pancreatitis-associated protein inhibits human pancreatic stellate cell MMP-1 and -2, TIMP-1 and -2 secretion and RECK expression, Pancreatology 9 (2009) 99–110.

[7] S. Hisada, K. Shimizu, K. Shiratori, et al., Peroxisome proliferator-activated receptor gamma ligand prevents the development of chronic pancreatitis through modulating NF-kappaB-dependent proinflammatorycytokine production and pancreatic stellate cell activation, Rocz. Akad. Med. Bialymst. 50 (2005) 142–147.

[8] M. Erkan, G. Adler, M.V. Apte, et al., StellaTUM: current consensus and discussion on pancreatic stellate cell research, Gut 61 (2012) 172–178.

[9] M.B. Omary, A. Lugea, A.W. Lowe, et al., The pancreatic stellate cell: a star on the rise in pancreatic diseases, J. Clin. Invest. 117 (2007) 50–59.

[10] X. Pan, Z. Chen, R. Huang, et al., Transforming growth factor β1 induces the expression of collagen type I by DNA methylation in cardiac fibroblasts, PLoS One 8 (2013) e60335.

[11] E.C. Chan, H.M. Peshavariya, G.S. Liu, et al., Nox4 modulates collagen production stimulated by transforming growth factor β1 in vivo and in vitro, Biochem. Biophys. Res. Commun. 430 (2013) 918–925.

[12] B.R. Klass, A.O. Grobbelaar, K.J. Rolfe, Transforming growth factor beta1 signalling, wound healing and repair: a multifunctional cytokine with clinical implications for wound repair, a delicate balance, Postgrad. Med. J. 85 (2009) 9–14.

[13] J.Q. Gong, G.H. Zhang, F.Z. Tian, et al., Islet-derived stem cells from adult rats participate in the repair of islet damage, J. Mol. Histol. 43 (2012) 745–750.

[14] J.A. Paulo, R. Urrutia, P.A. Banks, et al., Proteomic analysis of a rat pancreatic stellate cell line using liquid chromatography tandem mass spectrometry (LC–MS/MS), J. Proteomics 75 (2011) 708–717.

[15] A. Mathison, A. Liebl, J. Bharucha, et al., Pancreatic stellate cell models for transcriptional studies ofdesmoplasia-associated genes, Pancreatology 10 (2010) 505–516.

[16] A.L. Charrier, D.R. Brigstock, Connective tissue growth factor production by activated pancreatic stellate cells in mouse alcoholic chronic pancreatitis, Lab. Invest. 90 (2010) 1179–1188.

[17] A. Masamune, T. Watanabe, K. Kikuta, et al., Roles of pancreatic stellate cells in pancreatic inflammation and fibrosis, Clin. Gastroenterol. Hepatol. 7 (2009) S48–S54.

[18] M.V. Apte, P.S. Haber, T.L. Applegate, et al., Periacinar stellate shaped cells in rat pancreas: identification, isolation, and culture, Gut 43 (1998) 128–133.

[19] A. Vonlaufen, P.A. Phillips, L. Yang, et al., Isolation of quiescent human pancreatic stellate cells: a promising in vitro tool for studies of human pancreatic stellate cell biology, Pancreatology 10 (2010) 434–443.

[20] J. Lardon, I. Rooman, L. Bouwens, Nestin expression in pancreatic stellate cells and angiogenic endothelial cells, Histochem. Cell Biol. 117 (2002) 535–540.

[21] J.Q. Gong, F.Z. Tian, J.D. Ren, et al., Experimental evidence supporting the lack of primary stem cells in adult pancreatic tissue, Pancreatology 10 (2010) 620–630.

[22] C. Acharya, R.A. Cline, D. Jaligama, et al., Fibrosis reduces severity of acute-on-chronic pancreatitis in humans, Gastroenterology 145 (2013) 466–475.

[23] L. Yang, J. Shen, S. He, et al., L-Cysteine administration attenuates pancreatic fibrosis induced by TNBS in rats by inhibiting the activation of pancreatic stellate cell, PLoS One 7 (2012) e31807.

[24] Y.C. Yang, N. Zhang, C.K. Van, et al., Transforming growth factor-beta1 in inflammatory airway disease: a key for understanding inflammation and remodeling, Allergy 67 (2012) 1193–1202.

[25] A.C. Midgley, M. Rogers, M.B. Hallett, et al., Transforming growth factor-β1 (TGF-β1)-stimulated fibroblast to myofibroblast differentiation is mediated by hyaluronan (HA)-facilitated epidermal growth factor receptor (EGFR) and CD44 Co-localization in Lipid Rafts, J. Biol. Chem. 288 (2013) 14824–14838.

[26] L. Wang, C.Y. Ko, E.E. Meyers, et al., Concentration-dependent effects of transforming growth factor β1 on corneal wound healing, Mol. Vis. 17 (2011) 2835–2846.

[27] T.A. Wynn, Common and unique mechanisms regulate fibrosis in various fibroproliferative disease, J. Clin. Invest. 117 (2007) 524–529.

[28] K. Shimizu, Mechanisms of pancreatic fibrosis and applications to the treatment of chronic pancreatitis, J. Gastroenterol. 43 (2008) 823–832.

[29] H. Ye, P.C. Cai, Q. Zhou, et al., Transforming growth factor-β1 suppresses the up-regulation of matrix metalloproteinase-2 by lung fibroblasts in response to tumor necrosis factor-α, Wound Repair Regen. 19 (2011) 392–399.

[30] X. Gao, Y. Cao, W. Yang, et al., BMP2 inhibits TGF-β-induced pancreatic stellate cell activation and extracellular matrix formation, Am. J. Physiol. Gastrointest. Liver Physiol. 304 (2013) G804–G813.