Effect of interleukin-10 and platelet-derived growth factor on expressions of matrix metalloproteinases-2 and tissue inhibitor of metalloproteinases-1 in rat fibrotic liver and cultured hepatic stellate cells

Li-Juan Zhang, Yun-Xin Chen, Zhi-Xin Chen, Yue-Hong Huang, Jie-Ping Yu, Xiao-Zhong Wang

INTRODUCTION

Liver fibrosis and its end-stage sequelae cirrhosis represent a major worldwide health problem. By definition progressive fibrosis occurs when the rate of matrix synthesis exceeds matrix degradation¹. Considerable evidence suggests that the hepatic stellate cells (HSCs) are central to the fibrotic process. HSCs are normally located in the perisinusoidal space as quiescent vitamin A-storing cells secreting low levels of extracellular matrix (ECM). Following liver injury, increased synthesis of extracellular matrix constituents occurs in combination with other phenotypic changes (also called activation) of HSCs into myofibroblast-like cells. It has been demonstrated by the analysis of freshly isolated HSCs that a number of these phenotypic changes take place, including increased expression of extracellular matrix constituents and the expression of α-SMA²-⁴. This activated phenotype of HSCs subsequently becomes the major source of the interstitial collagens⁵-⁶. It has been suggested that HSCs are also a source of matrix-degrading metalloproteinases (MMPs), indicating their participation in matrix remodeling⁷-⁸. As a family of neutral proteinases, MMPs act on a variety of substrates¹⁰. Different expression profiles of MMPs influence the outcome of ECM components, resulting in preferential accumulation of interstitial collagens, type I in particular, in the fibrotic liver. MMPs are tightly regulated at the levels of transcription, secretion, and proteolytic activation, and their activities are governed by tissue-derived inhibitors¹¹. The expression of tissue inhibitors of MMPs (TIMPs) has also been demonstrated in human fibrotic liver disease and animal models of liver fibrosis¹²-¹⁵. At present, 4 TIMPs have been characterized¹³, all being low-molecular-weight proteins sharing structural similarities. Individual members of the TIMP family display selective affinities for different members of the MMP family¹⁴-¹⁵. TIMP-1 controls mostly the activity of MMPs, particularly MMP-1, whereas TIMP-2 is the major inhibitor of MMP-2 and MMP-9. MMP/TIMP balance is thought to play a pivotal role in the development of liver fibrosis, but their direct interaction in vivo has not yet been clarified. In the present study, the expressions of MMP-2 and TIMP-1 in rat fibrotic liver and in HSCs were examined and their changes were investigated in the presence of interleukin (IL)-10 and PDGF.

MATERIALS AND METHODS

Materials

One hundred clean male Sprague-Dawley rats weighing 140-180 g...
Preparation of rats
Rats in control group were given intraperitoneal injection with saline at 2 mL/kg twice a week, and those in model and IL-10 treatment groups received intraperitoneal injection with 500 mL/L CCl4 (dissolved in castor oil) at 2 mL/kg twice a week. From the third week, rats in treatment group were given intraperitoneal injection with IL-10 at 4 µg/kg (dissolved in saline) 20 min prior to CCl4 injection. All injections were performed on Mondays and Thursdays after measurement of the rats’ body weight. In the 5th wk, 3 rats in model group and 2 in treatment group died; in the 7th wk, 8 and 4 rats in these two groups died, respectively, and in the 9th week, another 10 and 6 died. At this time point, 3 rats in control group also died. In the 5th, 7th and 9th wk, 7 to 10 rats in each group were sacrificed to collect their liver samples, which were fixed in 40 g/L formaldehyde and embedded with paraffin.

Immunohistochemistry
Rat liver tissues were sectioned at the thickness of 4 µm. After deparaffinization with xylene and dehydration with graded ethanol, the sections were incubated in PBS containing 30 mL/L H2O2 to remove endogenous peroxidases and then in PBS containing 0.1 mol/L citrate to saturate the nonspecific binding sites. After incubation with goat anti-rat MMP-2 and TIMP-1 containing 0.1 mol/L citrate to saturate the nonspecific binding thereafter. The subsequent passages of HSCs were diluted to 1×106/mL in DMEM containing 100 mL/L fetal calf serum (FCS), and incubated at 37 °C with 50 mL/L CO2. The culture medium was replaced 24 h after plating and every 48-72 h thereafter. The subsequent passages of HSCs were diluted to 5×104/mL before seeded into 50 mL culture flask containing DMEM medium supplemented with FCS.

PDGF treatment of HSCs
The cultured HSCs were divided into 6 groups: the first and sixth groups serving as control were cultured in 3 mL DMEM medium for 2 and 24 h, respectively, with the second, third, fourth and fifth groups cultured in 3 mL DMEM medium in the presence of 20 ng/mL PDGF for 2, 4, 8 and 24 h, respectively. The cells were then harvested for reverse transcriptional-PCR (RT-PCR).

Co-treatment of HSCs with IL-10 and PDGF
Cultured HSCs were divided into 8 groups: the first and second groups (blank control) were cultured in 3 mL DMEM medium for 2 and 24 h, respectively, and the third, fifth and seventh groups (negative control) cultured in 3 mL DMEM medium containing 20 ng/mL PDGF for 2, 12 and 24 h, respectively. The fourth, sixth and eighth groups were cultured in 3 mL DMEM medium containing both 20 ng/mL IL-10 and 20 ng/mL PDGF for 2, 12 h and 24 h, respectively. The cells were then harvested for RT-PCR.

RT-PCR for MMP-2 and TIMP-1
Total RNA was isolated from HSCs using Genta reagent (USA) according to the protocol provided by the manufacturer. The A260/A280 of total RNA ranged between 1.8 and 2.0. After treatment with DNase-I (1-2 µg), total RNA was reversely transcribed into complementary DNA (cDNA) with oligo (dT) using cDNA synthesis kit, and 2 µL cDNA product was then used as the template to amplify specific fragments in a 25 µL reaction system. PCRs using Taq polymerase reaction were carried out with an initial denaturation at 95 °C for 5 min, followed by 25 cycles at 94 °C for 45 s, annealing at 60 °C for 30 s, at 72 °C for 60 s, with a final extension at 72 °C for 7 min. The primers sequences used for MMP-2 were 5'-GTGCTGAAGGAACCATCCGCTAAGAGA-3' (sense) and 5'-TGCCGGTCCTTTCAAGTTGTACG-3' (antisense), and those for TIMP-1 were 5'-GCCATGGAGAGCCTCTGTGG-3' (sense) and 5'-GCAGGCAGGCAAAGTGATCG-3' (antisense); the primers for β-actin as the internal control were 5'-GAGCTATGAGCTGCGCTGA-3' (sense) and 5'-AAGCTTGGGGTCCGACGT-3' (antisense).

Electrophoresis and semi-quantitative analysis
PCR products underwent 2% agarose gel electrophoresis and were visualized with ethidium bromide. The expected product sizes were 604 bp for MMP-2, 310 bp for TIMP-1 and 410 bp for β-actin. Bio Imagine System was applied to detect the density of the bands of PCR products. The expression levels of MMP-2 and TIMP-1 were calculated by the ratio of their band densities of PCR products to that of β-actin. Semi-quantitative detection was repeated for 5 times. SPSS 10.0 software was used to analyze the difference between the groups.

RESULTS

MMP-2 and TIMP-1 expressions in liver tissues
The positive rate of MMP-2 in control group, model group and IL-10 treatment group was 9.5%, 84.0% and 39.3%, respectively, and that of TIMP-1 was 23.8%, 92.0% and 71.4%, respectively. The granular positive products were localized in the cytoplasm of hepatocytes and biliary epithelial cells. In control group, the positive expressions of MMP-2 and TIMP-1 were weak and found mainly in endothelial cells and hepatic cells. In model group, positive expressions increased obviously with the development of hepatic fibrosis, distributing in biliary epithelial cells, fibroblasts and muscular cells. In treatment group the
Changes were less pronounced than in model group (Figures 1-4).

**Figure 1** MMP-2-positive cells in the model group (S-P method, ×200).

**Figure 2** MMP-2-positive cells in IL-10 treatment group (S-P method, ×200).

**Figure 3** TIMP-1-positive cells in the model group (S-P method, ×400).

**Figure 4** TIMP-1-positive cells in IL-10 treatment group (S-P method, ×400).

**Figure 5** Effects of PDGF on TIMP-1 and MMP-2 expressions in HSCs: 1: Control group (2 h); 2: PDGF-treated group (2 h); 3: PDGF-treated group (4 h); 4: PDGF-treated group (8 h); 5: PDGF-treated group (24 h); 6: Control group (24 h).

Intensities of MMP-2 and TIMP-1 immunoreactivities
Comparison of MMP-2 and TIMP-1 positive expression levels between the 3 groups is shown in Table 1. Ridit analysis showed significant difference between the 3 groups (P<0.01). Higher expression levels of MMP-2 and TIMP-1 in model group were detected than in control group (P<0.01). In treatment group, IL-10 treatment resulted in decreased immunoreactivities for MMP-2 and TIMP-1 (P<0.01 and P<0.05 respectively). The expression levels of MMP-2 and TIMP-1 in different phases of hepatic fibrosis are listed in Table 2. With the development of hepatic fibrosis, the intensities of MMP-2 and TIMP-1 immunoreactivities increased gradually, and the difference was

| Group | MMP-2 | TIMP-1 |
|-------|-------|--------|
|       | n     | - | + | ++ | Ridit value | - | + | ++ | Ridit value |
| Control | 21 | 19 | 2 | 0 | 0.312 | 16 | 5 | 0 | 0.277<sup>b</sup> |
| Model  | 25 | 4 | 13 | 8 | 0.712<sup>a</sup> | 2 | 14 | 9 | 0.684<sup>g</sup> |
| Treatment | 28 | 17 | 10 | 1 | 0.451<sup>e</sup> | 8 | 18 | 2 | 0.503<sup>g</sup> |

<sup>b</sup>P<0.01 vs treatment group; <sup>a</sup>P<0.05; <sup>g</sup>P<0.01 vs control group; <sup>e</sup>P>0.05 vs control group; <sup>g</sup>P<0.05 vs model group.

| Wk | n | MMP-2 | TIMP-1 |
|----|---|-------|--------|
|    |   | - | + | ++ | Ridit value | - | + | ++ | Ridit value |
| 5  | 8 | 1 | 7 | 0 | 0.378 | 1 | 6 | 1 | 0.36<sup>h</sup> |
| 7  | 8 | 2 | 5 | 1 | 0.388 | 1 | 5 | 2 | 0.418<sup>h</sup> |
| 9  | 9 | 1 | 1 | 7 | 0.709 | 0 | 2 | 7 | 0.698<sup>h</sup> |

<sup>h</sup>P<0.01 vs wk 7 and 9; <sup>g</sup>P<0.05 vs wk 9; <sup>h</sup>P<0.05 vs wk 7; <sup>h</sup>P>0.05 vs wk 5.
Relative quantities of MMP-2 and TIMP-1 mRNA in HSCs

Expression of TIMP-1 in PDGF-treated HSCs was significantly increased time-dependently as compared with that in control cells (P<0.01). There was no difference in MMP-2 expression between PDGF-treated HSCs and control cells (Figure 5). After treatment with IL-10 and PDGF, expressions of TIMP-1 and MMP-2 in HSCs were similar to those in negative control groups (P>0.05), without changes over time (Figure 6).

DISCUSSION

Liver fibrosis is thought to be a progressive pathological process that leads ultimately to deposition of excess matrix proteins in extracellular space[17], and destroys normal liver architecture to finally result in cirrhosis. In extracellular space, matrix degradation occurs predominantly consequent to the action of a family of enzymes known as matrix metalloproteinases[18]. These enzymes are secreted by cells into extracellular space as proenzymes, which are then activated by a number of specific mechanisms. MMP-2 (gelatinase A) produced by activated HSCs, as demonstrated by immunohistochemistry and in situ hybridization[19,20], plays an important role in remodeling the basement membranes as it degrades several of the collagen components including collagen IV, laminin and fibronectin[21]. In other tissues, such as lung, kidney and heart, MMP-2 expression is also increased during fibrogenesis[22-25]. Recent studies showed that inhibition of MMP-2 activity[26] or blockade of MMP-2 synthesis[27] might effectively prevent mesangial cell proliferation and collagen I synthesis in vitro, indicating the possible role of MMP-2 as a growth factor and activator for mesangial cells, performed probably through an autocrine pathway[27]. In human liver fibrosis or in rat models of CCl4-intoxication and CCl4-induced liver fibrosis, the expression of TIMP-1 mRNA was increased by several fold, and HSCs expressed MMP-2 when induced liver fibrosis, the expression of MMP-2 mRNA was increased time-dependently as compared with that in control cells (Figure 5). After treatment with IL-10 and PDGF, expressions of TIMP-1 and MMP-2 in HSCs were similar to those in negative control groups (P>0.05), without changes over time (Figure 6).

Figure 6 Effects of IL-10 and PDGF on TIMP-1 and MMP-2 expressions in HSCs. 1: Blank control group (2 h); 2: Blank control group (24 h); 3: Negative control group (2 h); 4: Treatment group (2 h); 5: Negative control group (12 h); 6: Treatment group (12 h); 7: Negative control group (24 h); 8: Treatment group (24 h).

The best characterized chemotactic factor for HSCs identified so far is PDGF-BB[46-49], known also as the most potent mitogen for HSCs and overexpression during active hepatic fibrogenesis[50]. Our findings in this study showed that after PDGF treatment, the expression of TIMP-1 increased significantly in HSCs possibly because PDGF-promoted HSC proliferation and activation, indicating the importance of PDGF in pathogenesis of liver fibrosis. It has been established that human and rat HSCs are able to migrate according to the concentration gradients of chemotactic factors[46-48]. Recent studies showed that IL-10 down-regulated expression of MMP-2 mRNA in rat fibrotic liver, possibly another way that IL-10 exerts its effect of antifibrogenesis. In our previous study, we found that the expression of MMP-2 did not increase in HSCs after PDGF treatment, which is suggestive of the irrelevance of MMP-2 in fibrogenesis induced by PDGF.

IL-10, originally isolated from mouse helper T cells, is a cytokine that regulates a number of interleukins. It inhibits synthesis of several cytokines by T lymphocytes and activates monocytes, and was therefore originally named cytokine synthesis inhibitory factor[51]. Recent studies showed that IL-10 also acted on connective tissue cells such as fibroblasts, inducing, for instance, transcriptional inhibition of the expression of type I collagen, which is the major component of extracellular matrix[52]. IL-10 also possesses antifibrogenic properties by down-regulating profibrogenic cytokines like TGF-β1 and TNF-α[53]. Our previous studies indicated that IL-10 could produce antifibrogenesis effect on CCl4-induced rat hepatic fibrosis. In this study, we found that IL-10 down-regulated expression of MMP-2 and TIMP-1 in rat fibrotic liver, possibly another way that IL-10 exerts its effect of antifibrogenesis. In our previous study, PDGF was found to markedly promote the contraction and proliferation of HSCs and expressions of collagen type I and III as well as TGF-β, in HSCs, which was significantly inhibited by IL-10[54]. But results of the present experiment showed that at the dose of 20 ng/mL, IL-10 did not inhibit TIMP-1 and MMP-2 expressions in PDGF-treated HSCs, indicating that the inhibitory effects of IL-10 on HSCs may not involve TIMP-1 and MMP-2.

In summary, the present study demonstrates that positive...
expressions of MMP-2 and TIMP-1 in rat liver tissue increase with the development of hepatic fibrosis, and MMP-2 and TIMP-1 play an important role during the development of liver fibrosis. Exogenous IL-10 decreases the expression of MMP-2 and TIMP-1 in liver tissues. PDGF increases the expression of TIMP-1 in HSCs, possibly through promoting HSC proliferation and activation, and this effect is not inhibited by IL-10.

REFERENCES

1 Bedossa P, Paradis V. Liver extracellular matrix in health and disease. J Pathol 2003; 200: 504-515
2 Rockey DC. The cell and molecular biology of hepatic fibrogenesis. Clinical and therapeutic implications. Clin Liver Dis 2000; 4: 319-355
3 Bataller R, Brenner DA. Hepatic stellate cells as a target for the treatment of liver fibrosis. Semin Liver Dis 2001; 21: 437-451
4 Safadi R, Friedman SL. Hepatic fibrosis-role of hepatic stellate cell activation. Med Gen Med 2002; 4: 27
5 Reeves HL, Friedman SL. Activation of hepatic stellate cells-a key issue in liver fibrosis. Front Biosci 2002; 7: d808-826
6 Iredale JP. Hepatic stellate cell behavior during resolution of liver injury. Semin Liver Dis 2001; 21: 427-436
7 Imai K, Sato T, Senoo H. Adhesion between cells and extracellular matrix with special reference to hepatic stellate cell adhesion to three-dimensional collagen fibers. Cell Struct Funct 2000; 25: 329-336
8 Neubauer K, Saile B, Ramadori G. Liver fibrosis and altered matrix synthesis. Can J Gastroenterol 2001; 15: 187-193
9 Benyon RC, Arthur MJ. Extracellular matrix degradation and the role of hepatic stellate cells. Semin Liver Dis 2001; 21: 373-384
10 Okazaki I, Watanabe T, Hozawa S, Arai M, Maruyama K. Molecular mechanism of the reversibility of hepatic fibrosis: with special reference to the role of matrix metalloproteinases. J Gastroenterol Hepatol 2000; 15(Suppl): D26-32
11 Will H, Atkinson SJ, Butler GS, Smith B, Murphy G. The soluble catalytic domain of membrane type 1 matrix metalloproteinase cleaves the propeptide of progelatinase A and initiates autoproteolytic activation. Regulation by TIMP-2 and TIMP-3. J Biol Chem 1996; 271: 17119-17123
12 McCrudden R, Iredale JP. Liver fibrosis, the hepatic stellate cell and tissue inhibitors of metalloproteinases. Histol Histopathol 2000; 15: 1159-1168
13 Gomez DE, Alonso DF, Yoshiji H, Thorgeirsson UP. Tissue inhibitors of metalloproteinases: structure, regulation and biochemical functions. Eur J Cell Biol 1997; 74: 111-122
14 Arthur MJ. Fibrogenesis II. Metalloproteinases and their inhibitors in liver fibrosis. Am J Physiol Gastrointest Liver Physiol 2000; 279: G245-249
15 Senoo H, Imai K, Matano Y, Sato M. Molecular mechanisms in the reversible regulation of morphology, proliferation and collagen metabolism in hepatic stellate cells by the three-dimensional structure of the extracellular matrix. J Gastroenterol Hepatol 1998; 13(Suppl): S19-32
16 Ramm GA. Isolation and culture of rat hepatic stellate cells. J Gastroenterol Hepatol 1998; 13: 846-851
17 Iredale JP. Tissue inhibitors of metalloproteinases in liver fibrosis. Int J Biochem Cell Biol 1997; 29: 43-54
18 Benyon RC, Arthur MJ. Extracellular matrix degradation and the role of hepatic stellate cells. Semin Liver Dis 2001; 21: 373-384
19 Takahara T, Furui K, Yata K, Jin B, Zhang LP, Nambu S, Sato H, Seki M, Watanabe A. Dual expression of matrix metalloproteinase-2 and membrane-type 1-matrix metalloproteinase in fibrotic human livers. Hepatology 1997; 26: 1521-1529
20 Takahara T, Furui K, Funaki J, Nakayama Y, Itoh H, Miiyayashii C, Sato H, Seki M, Ooshima A, Watanabe A. Increased expression of matrix metalloproteinase-II in experimental liver fibrosis in rats. Hepatology 1995; 21: 787-795
21 Tucek J, Polgar G, Lee LK, Marti HP, Lovett DH. Matrix metalloproteinase 2 (gelatinase A) regulates glomerular mesangial cell proliferation and differentiation. J Biol Chem 1996; 271: 15074-15083
22 Swiderski RE, Dencoff JE, Floerchinger CS, Shapiro SD, Hunninghake GW. Differential expression of extracellular matrix remodeling genes in a murine model of bleomycin-induced pulmonary fibrosis. Am J Pathol 1998; 152: 821-828
23 Fukuda Y, Ishizaki M, Kudoh S, Kitachi M, Yamanaka N. Localization of matrix metalloproteinases-1, -2, -9 and tissue inhibitor of metalloproteinase-2 in interstitial lung diseases. Lab Invest 1998; 78: 687-698
24 Shimizu T, Kuroda T, Hata S, Fukagawa M, Margolin SB, Kurokawa K. Pirfenidone improves renal function and fibrosis in the post-obstructed kidney. Kidney Int 1998; 54: 99-109
25 Bakowska J, Adamson IY. Collagenase and gelatinase activities in bronchoalveolar lavage fluids during bleomycin-induced lung injury. J Pathol 1998; 185: 319-323
26 Steinmann-Niggli K, Zwisler R, Kung M, Marti HP. Inhibition of matrix metalloproteinases attenuates anti-Thy1.1 nephritis. J Am Soc Nephrol 1998; 9: 397-407
27 Benyon RC, Hovell CJ, Da Gaca M, Jones EH, Iredale JP, Arthur MJ. Progelatinase A is produced and activated by rat hepatic stellate cells and promotes their proliferation. Hepatology 1999; 30: 977-986
28 Arthur MJ, Stanley A, Iredale JP, Rafferty JA, Hembry RM, Friedman SL. Secretion of 72 kDa type IV collagen/gelatine by cultured human lipocytes. Analysis of gene expression, protein synthesis and proteinase activity. Biochem J 1992; 287(Pt 3): 705-710
29 Arthur MJ, Iredale JP, Mann DA. Tissue inhibitors of metalloproteinases: role in liver fibrosis and alcoholic liver disease. Alcohol Clin Exp Res 1999; 23: 940-943
30 Arthur MJ, Mann DA, Iredale JP. Tissue inhibitors of metalloproteinases, hepatic stellate cells and liver fibrosis. J Gastroenterol Hepatol 1998; 13(Suppl): S33-38
31 Murphy FR, Issa R, Zhou X, Ratnarajah S, Nagase H, Arthur MJ, Benyon C, Iredale JP. Inhibition of apoptosis of activated hepatic stellate cells by tissue inhibitor of metalloproteinase-1 is mediated via effects on matrix metalloproteinase inhibition: implications for reversibility of liver fibrosis. J Biol Chem 2002; 277: 11069-11076
32 Bahr MJ, Vincent KJ, Arthur MJ, Fowler AV, Smart DE, Wright MC, Clark IM, Benyon RC, Iredale JP, Mann DA. Control of the tissue inhibitor of metalloproteinases-1 promoter in culture-activated rat hepatic stellate cells: regulation by activator protein-1 DNA binding proteins. Hepatology 1999; 29: 839-848
33 Tsigarides EP, Yoshiji H, Sinha CG, Gomez DE, Breast cancer; tumor neoangiogenesis and the effect of tissue inhibitor of metalloproteinases-1 (TIMP-1) on angiogenesis. In Vivo 1996; 10: 137-144
34 Zhao WQ, Li H, Yamashita K, Guo XQ, Hoshino T, Yoshida S, Shinya T, Hayakawa T. Cell cycle-associated accumulation of tissue inhibitor of metalloproteinases-1 (TIMP-1) in the nuclei of human gingival fibroblasts. J Cell Sci 1998; 111(Pt 9): 1147-1153
35 Ritter LM, Garfield SH, Thorgeirsson UP. Tissue inhibitor of metalloproteinases-1 inhibits cell growth of human hepatoma cell lines and translocates to the nucleus of human MCF-7 breast carcinoma cells. Biochem Biophys Res Commun 1999; 257: 494-499
36 Iredale JP, Benyon RC, Pickering J, McCullen M, Northrop M, Pawley S, Hovell C, Arthur MJ. Mechanisms of spontaneous resolution of rat liver fibrosis. Hepatic stellate cell apoptosis and reduced hepatic expression of metalloproteinase inhibitors. J Clin Invest 1998; 102: 536-549
37 Herbst H, Weges T, Miltner S, Pellegrini G, Orzechowski HD, Hechstein WO, Neuhaus P, Gressner AM, Schuppan D. Tissue inhibitor of metalloproteinase-1 and -2 RNA expression in rat and human liver fibrosis. Am J Pathol 1997; 150: 1647-1659
38 Sakaida I, Uchida K, Hironaka K, Okita K. Prolyl 4-hydroxylase inhibitor (HOE 077) prevents TIMP-1 gene expression in rat liver fibrosis. Gastroenterology 1999; 34: 376-377
39 Yoshiji H, Kuriyama S, Yoshii J, Ikemura Y, Noguchi R, Nakatani T, Tsujinoue H, Yanase K, Namiskai T, Imaizu T, Fukuji H. Tissue inhibitor of metalloproteinases-1 attenuates spontaneous liver fibrosis resolution in the transgenic mouse. Hepatology 2002; 36(4 Pt): 850-860
Yoshiji H, Kuriyama S, Miyamoto Y, Thorgerisson UP, Gomez DE, Kawata M, Yoshii J, Ikenaka Y, Noguchi R, Tsujinoue H, Nakatani T, Thorgerisson SS, Fukui H. Tissue inhibitor of metalloproteinases-1 promotes liver fibrosis development in a transgenic mouse model. Hepatology 2000; 32: 1248-1254

Zhang BB, Cai WM, Weng HL, Hu ZR, Lu J, Zheng M, Liu RH. Diagnostic value of platelet derived growth factor-BB, transforming growth factor-beta1, matrix metalloproteinase-1, and tissue inhibitor of matrix metalloproteinase-1 in serum and peripheral blood mononuclear cells for hepatic fibrosis. World J Gastroenterol 2003; 9: 2490-2496

Tangkijvanich P, Melton AC, Chitapanarux T, Han J, Yee HF. Platelet-derived growth factor-BB and lysophosphatidic acid distinctly regulate hepatic myofibroblast migration through focal adhesion kinase. Exp Cell Res 2002; 281: 140-147

Di Sario A, Bendia E, Svegliati-Baroni G, Marzioni M, Ridolfi F, Trozzi L, Ugili L, Saccomanno S, Jezequel AM, Benedetti A. Rearrangement of the cytoskeletal network induced by platelet-derived growth factor in rat hepatic stellate cells: role of different intracellular signalling pathways. J Hepatol 2002; 36: 179-190

Kinnman N, Goria O, Wendum D, Gendron MC, Rey C, Poupon R, Houset C. Hepatic stellate cell proliferation is an early platelet-derived growth factor-mediated cellular event in rat cholestatic liver injury. Lab Invest 2003; 81: 1709-1716

Kinnman N, Hultrcrantz R, Barbu V, Rey C, Wendum D, Pou routed S, Houset C. PDGF-mediated chemoattraction of hepatic stellate cells by bile duct segments in cholestatic liver injury. Lab Invest 2000; 80: 697-707

Ikeda K, Wakahara T, Wang YQ, Kadoya H, Kawada N, Kaneda K. In vitro migratory potential of rat quiescent hepatic stellate cells and its augmentation by cell activation. Hepatology 1999; 29: 1760–1767

Marra F, Gentilini A, Pinzani M, Choudhury GG, Parola M, Herbst H, Dianzani MU, Laffi G, Abboud HE, Gentilini P. Phosphatidylinositol 3-kinase is required for platelet-derived growth factor's actions on hepatic stellate cells. Gastroenterology 1997; 112: 1297–1306

Marra F, Romanelli RG, Giannini C, Failli P, Pastacaldi S, Arrighi MC, Pinzani M, Laffi G, Montalto P, Gentilini P. Monocyte chemotactic protein-1 as a chemoattractant for human hepatic stellate cells. Hepatology 1999; 29: 140–148

Carloni V, Romanelli RG, Pinzani M, Laffi G, Gentilini P. Focal adhesion kinase and phospholipase C gamma involvement in adhesion and migration of human hepatic stellate cells. Gastroenterology 1997; 112: 522–531

Pinzani M. PDGF and signal transduction in hepatic stellate cells. Front Biosci 2002; 7: d1720-1726

Nelson DB, Lauwers GY, Lau JY, Davis GL. Interleukin 10 treatment reduces fibrosis in patients with chronic hepatitis C: a pilot trial of interferon nonresponders. Gastroenterology 2000; 118: 655-660

Reitamo S, Remitz A, Tamai K, Uitto J. Interleukin-10 modulates type I collagen and matrix metalloprotease gene expression in cultured human skin fibroblasts. J Clin Invest 1994; 94: 2489-2492

Kovalovich K, DeAngelis RA, Li W, Furth EE, Ciliberto G, Taub R. Increased toxin-induced liver injury and fibrosis in interleukin-6-deficient mice. Hepatology 2000; 31: 149-159

Louis H, Van Laethem JL, Wu W, Quertinmont E, Degraef C, Van den Berg K, Demols A, Goldman M, Le Moine O, Geerts A, Deviere J. Interleukin-10 controls neutrophil infiltration, hepatocyte proliferation, and liver fibrosis induced by carbon tetrachloride in mice. Hepatology 1998; 28: 1607-1615

Chen YY, Wang XZ, Weng SG, Chen ZX, Huang YH, Zhang Lj. Effects of IL-10 and PDGF on expression of TGF-β1 at hepatic stellate cells. Zhongxiyi Jiehe Ganbin Zazhi 2002; 12: 343-345

Edited by Chen WW Proofread by Zhu LH and Xu FM