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Cholestatic liver fibrosis and toxin-induced fibrosis are exacerbated in matrix metalloproteinase-2 deficient mice

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ABSTRACT

Matrix metalloproteinase (MMP) plays an important role in homeostatic regulation of the extracellular environment and degradation of matrix. During liver fibrosis, several MMPs, including MMP-2, are up-regulated in activated hepatic stellate cells, which are responsible for exacerbation of liver cirrhosis. However, it remains unclear how loss of MMP-2 influences molecular dynamics associated with fibrogenesis in the liver. To explore the role of MMP-2 in hepatic fibrogenesis, we employed two fibrosis models in mice; toxin (carbon tetrachloride, CCl4)-induced and cholestasis-induced fibrosis. In the chronic CCl4 administration model, MMP-2 deficient mice exhibited extensive liver fibrosis as compared with wild-type mice. Several molecules related to activation of hepatic stellate cells were up-regulated in MMP-2 deficient liver, suggesting that myofibroblastic change of hepatic stellate cells was promoted in MMP-2 deficient liver. In the cholestasis model, fibrosis in MMP-2 deficient liver was also accelerated as compared with wild type liver. Production of tissue inhibitor of metalloproteinase 1 increased in MMP-2 deficient liver in both models, while transforming growth factor β, platelet-derived growth factor receptor and MMP-14 were up-regulated only in the CCl4 model. Our study demonstrated, using 2 experimental murine models, that loss of MMP-2 exacerbates liver fibrosis, and suggested that MMP-2 suppresses tissue inhibitor of metalloproteinase 1 up-regulation during liver fibrosis.

1. Introduction

The incidence of liver cirrhosis and cirrhosis-related cancer has steadily increased. Insight into the pathophysiology of hepatic fibrogenesis and fibrolysis is of particular importance. Accumulating evidences has indicated that matrix metalloproteinases (MMPs) play an essential role in hepatic fibrogenesis [1]. Traditional substrates of MMPs are components of the extracellular matrix (ECM), such as collagen, laminin, and fibronectin [2]. More than 20 enzymes have been identified as MMPs in mammals. In healthy liver, homeostasis of the ECM is maintained by precisely regulated turnover controlled by MMPs and tissue inhibitors of metalloproteinases (TIMPs) [1]. Hepatic stellate cells activated in chronically damaged liver disrupt this regulation by synthesizing a large amount of ECM, including collagen and proteoglycan, followed by production of MMPs and TIMPs [3]. It has been reported that the hepatic expression of MMP-1, -2, -7 and -14 is steadily increased with disease progression, and that the expression of MMP-9, -11 and -13 is transiently elevated in hepatitis C virus-induced cirrhosis [4]. Up-regulation of MMPs and TIMPs during liver fibrogenesis has been observed in rodent models of liver fibrosis. In chronic toxin (carbon tetrachloride, CCl4)-induced rat liver fibrosis, production of MMP-2, -9, and -14 is increased during the accumulation of fibrotic scarring in the liver, and hepatic stellate cells

Abbreviations: ALT, alanine aminotransferase; αSMA, α smooth muscle actin; AST, aspartate aminotransferase; BDL, bile duct ligation; CCl4, carbon tetrachloride; ECM, extracellular matrix; GGT, γ-glutamyltranspeptidase; MMP, matrix metalloproteinase; MMP-2 KO, MMP-2 deficient; PDGF, platelet-derived growth factor; T-Bil, total bilirubin; TIMP, tissue inhibitor of metalloproteinase; TGF, transforming growth factor; WT, wild type .

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appear to be the predominant source [5–7]. MMPs degrade the ECM, and yet loss of MMP activity does not always induce fiborogenesis *in vivo*, because MMP functions are associated with regulation of several inflammatory cytokines [8].

MMP-2 is a 72-kDa zinc-dependent type IV collagenase, and the activation of proMMP-2 is controlled by MMP-14 and TIMP2 [9]. In the rat CCl4-induced fibrosis model, expression of MMP-2 increase up to 7–12 times as compared with that of controls, with the expression rate being maximal at an intermediate stage of fibrosis [5]. During the aggressive phase of CCl4-induced liver fibrosis, MMP-2 is predominantly produced from myofibroblasts derived from hepatic stellate cells [7]. MMP-2 expression has been shown to increase during spontaneous recovery from cirrhosis in a rat toxin-induced fibrosis model [10]. The proteolytic activity of MMP-2 is increased in cholestasis-induced liver fibrosis models in rats and mice [6,11]. These studies indicate that MMP-2 regulates both liver fibrogensis and fibrolysis. However, it remains unclear how MMP-2 functions during hepatic fibrogenesis in the liver *in vivo*, and the functional correlations between MMP and TIMP expression with liver fibrogenesis has not yet been fully elucidated.

In this study, to clarify the pivotal role of MMP-2 during liver fibrosis, we studied fibrotic changes in the liver of MMP-2 deficient (MMP-2 KO) mice using two types of fibrosis models; toxin (CCl4)-induced fibrosis and cholestasis-induced fibrosis. In both models, liver fibrosis was accelerated in MMP-2 KO mice as compared with wild type (WT) littermates. Type I collagen expression and TIMP1 production were up-regulated in MMP-2 KO livers in both models. Our data indicated that MMP-2 plays an essential role in suppressing fibrotic changes in the liver arising from chemical or cholestatic damage.

2. Materials and methods

2.1. Animals

MMP-2 deficient (MMP-2 KO) mice on C57BL/6J background were originally generated by Itoh et al. [12]. MMP-2 KO mice and wild type (WT) littermates were produced by crossbreeding MMP-2 heterozygous mice. All animals were treated under the guidelines of the Institute of Medical Science, University of Tokyo and those of Tokyo Medical and Dental University.

2.2. Liver fibrosis induced by chronic CCl4 administration

To induce hepatic fibrosis in mice, 6-week-old female MMP-2 KO and WT mice ($n = 4$ per group) were intraperitoneally injected with 10% CCl4 (2 ml/kg body weight, Wako Pure Chemical Industries, Osaka, Japan) dissolved in olive oil (Wako) twice weekly for 12 weeks. Negative control mice were injected with 100% olive oil alone twice weekly for 12 weeks.

2.3. Liver fibrosis induced by bile duct ligation (BDL)

To induce fibrosis by cholestasis, 8-week-old female MMP-2 KO and WT mice ($n = 4$ per group) underwent common bile duct ligation under anesthesia as previously described [13]. Mice were sacrificed 2 weeks after the operation.

2.4. Blood biochemical tests

Peripheral blood of MMP-2 KO and WT mice in the BDL group was collected and assayed for total bilirubin (T-Bil), alanine aminotransferase (ALT), aspartate aminotransferase (AST), and γ-glutamyltranspeptidase (GGT) using a Fuji DRI-Chem 3000 system (Fujifilm, Tokyo, Japan).

2.5. Immunohistological analysis

Fresh livers were snap-frozen in OCT compound and sectioned 8-μm thick. The protocols for immunostaining has been described previously [14]. Immunohistological analysis was performed using mouse anti-α smooth muscle actin (αSMA) antibody (Sigma, St. Louis, MO, USA). For each analysis, addition of an appropriate immune serum provided a negative control.

2.6. Western blot analyses

Samples of liver tissue were homogenized in RIPA buffer. The protocols for western blot analyses have been described previously [15]. Western blot analyses were performed with liver homogenates (50 μg protein) using anti-αSMA Ab (Sigma, 1:2000 dilution), anti-MMP9 antibody (Chemicon, Billerica, MA, USA, 1:1000 dilution), anti-MMP14 antibody (Abcam, Cambridge, MA, USA, 1:500 dilution), anti-TIMP1 antibody (Abcam, 1:1000 dilution), anti-TIMP2 antibody (Cell Signaling, Danvers, MA, USA, 1:500 dilution), or anti-β-actin antibody. Membrane pictures of immunoblots were quantified using ImageJ software (http://rsb.info.nih.gov/ij/).

Supplementary materials and methods are described in supplementary file.

3. Results

3.1. CCl4-induced hepatic fibrosis in MMP-2 KO mice

We initially studied liver fibrosis induced by chronic CCl4 administration in WT and MMP-2 KO mice. To induce extensive liver fibrosis, CCl4 was injected twice weekly for 12 weeks. Damage of hepatocytes in MMP-2 KO livers was very severe (Fig. S1). Sirius red staining of KO livers demonstrated that CCl4-induced fibrosis was severe in comparison with WT livers (Fig. 1A). Marked porto-central and porto-portal fibrosis was observed in the liver of MMP-2 KO mice treated with CCl4. The fibrosis areas, quantified using an image analysis system, were significantly increased in MMP-2 KO livers as compared with WT livers (Fig. 1B). Following chronic CCl4 administration, a 2-fold increase in fibrosis area was observed in MMP-2 KO livers as compared with WT livers.

3.2. Increased hepatic production of αSMA in MMP-2 KO mice treated with CCl4

To assess whether the activation of hepatic stellate cells is promoted in MMP-2 KO mice with CCl4-induced liver fibrosis, we examined hepatic αSMA production, which is a representative marker for myofibroblasts. Immunohistological analysis showed that αSMA-positive areas in the liver were much larger in CC14-treated MMP-2 KO mice than in CC14-treated WT mice (Fig. 1C). αSMA-positive areas quantified using an image analysis system were significantly increased in MMP-2 KO livers (Fig. 1D). Immunoblot analysis indicated that αSMA production was markedly increased in the livers of CCl4-treated WT and MMP-2 KO mice as compared with vehicle (olive oil)-treated WT and MMP-2 KO mice, respectively. Hepatic αSMA production was higher in CCl4-treated MMP-2 KO mice as compared with CCl4-treated WT mice (Fig. 1E). Quantitative analysis using densitometry demonstrated that hepatic level of αSMA protein was significantly higher in CCl4-treated MMP-2 KO mice than in CCl4-treated WT mice (Fig. 1F). These data suggest that the activation of hepatic stellate cells was promoted in MMP-2 KO liver as compared with WT liver during liver fibrosis induced by chronic CCl4 administration.

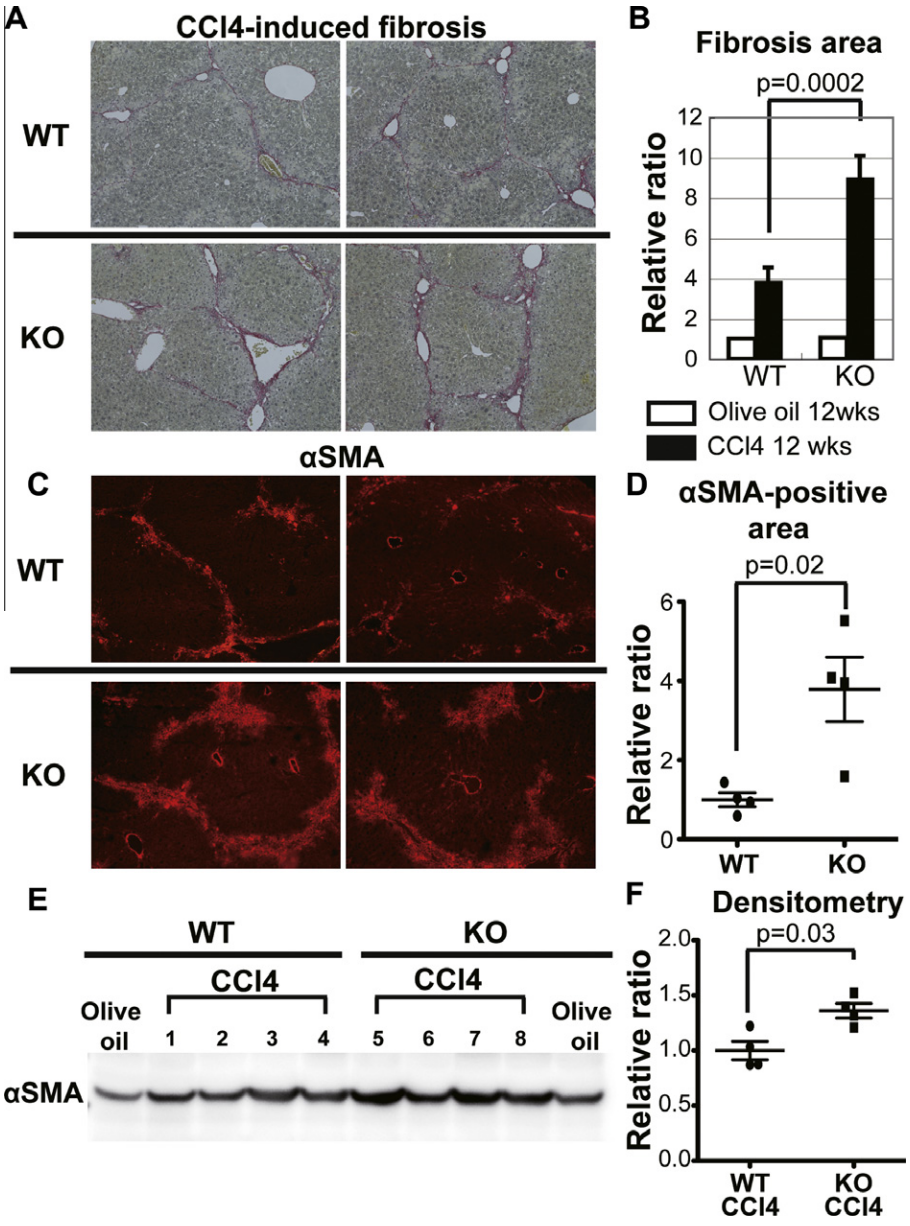

Fig. 1. CCl4-induced liver fibrosis is promoted in MMP-2 deficient (KO) mice. (A) Representative photographs of Sirius red staining in the livers of CCl4-treated mice. (B) Ratio of overall fibrosis area (red areas in Sirius red staining) relative to olive oil-treated WT mice. Data represent means ± SD. (C) Representative images of αSMA immunostaining in the livers of CCl4-treated mice. (D) Areas immunostained with αSMA antibody, quantified using an image analysis system, were significantly increased in CCl4-treated MMP-2 KO mice as compared with WT mice. Values represent the ratio relative to the mean for CCl4-treated WT mice. Bars represent means ± SEM. (E) Immunoblot analysis of αSMA in the liver of MMP-2 KO and WT mice. Mice 1, 2, 3, and 4 were CCl4-treated WT. Mice 5, 6, 7, and 8 were CCl4-treated MMP-2 KOs. (F) Hepatic αSMA protein quantified using densitometry was significantly increased in CCl4-treated MMP-2 KO mice as compared with WT mice. Values represent the ratio relative to the mean for CCl4-treated WT mice. Bars represent means ± SEM.

3.3. Hepatic expression of fibrosis-related molecules in CCl4-treated MMP-2 KO mice

To explore the molecular mechanism underlying the differences between WT and MMP-2 KO mice in response to CCl4-induced fibrosis, we analyzed the expression of molecules related to the promotion of liver fibrosis. Quantitative RT-PCR analysis showed that the hepatic expression of type I collagen in CCl4-treated MMP-2 KO mice was 4-fold higher than that in CCl4-treated WT mice (Fig. 2A). In addition, hepatic expression of transforming growth factor (TGF) β1 and platelet-derived growth factor (PDGF) receptor was up-regulated in CCl4-treated MMP-2 KO mice, whereas that of TGFβ receptor was comparable between CCl4-treated MMP-2 KO mice and CCl4-treated WT mice (Fig. 2A).

Immunoblot analysis showed that hepatic levels of MMP-9, MMP-14, TIMP1, and TIMP2 protein were increased in CCl4-treated WT mice relative to vehicle-treated mice, indicating that production of fibrosis-related molecules was up-regulated in our experimental model (Fig. 2B). Production of MMP-9, MMP-14, TIMP1 and TIMP2 in the liver was comparable between vehicle-treated WT mice and vehicle-treated KO mice (Fig. 2B). Quantitative analysis using densitometry also showed that hepatic production of MMP-14 and TIMP1 was significantly higher in CCl4-treated MMP-2 KO mice than in CCl4-treated WT mice (Fig. 2C). Hepatic

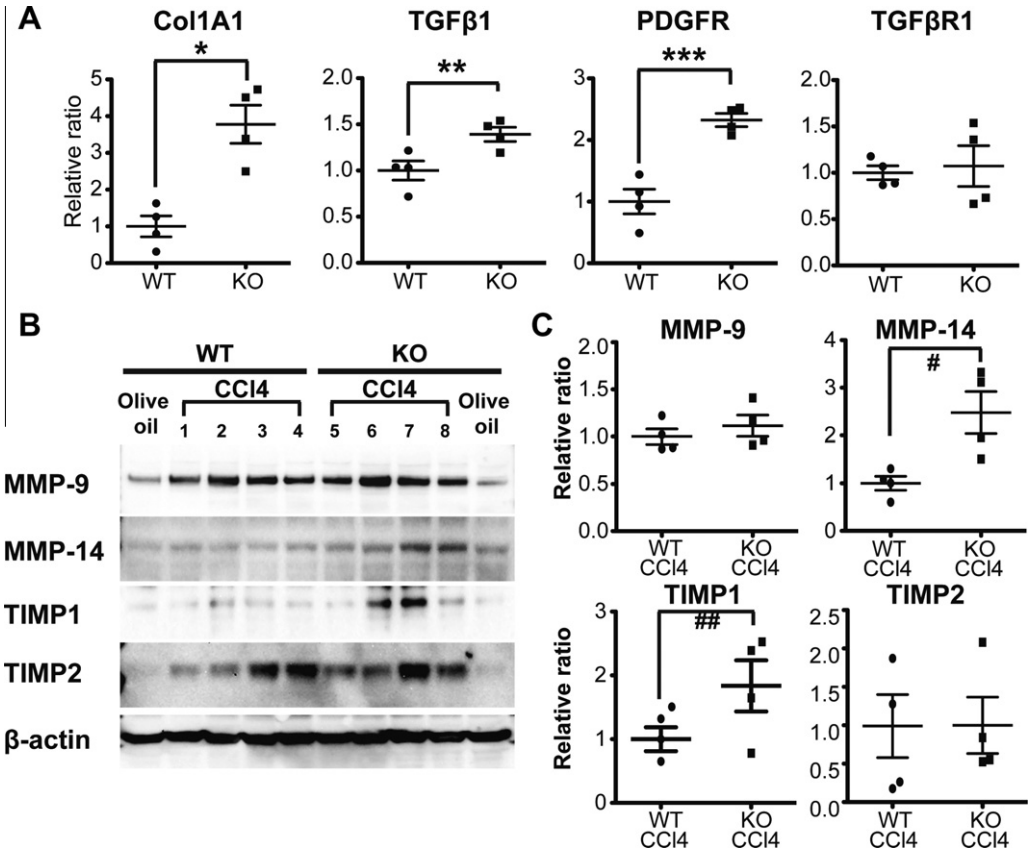

Fig. 2. Analysis of fibrosis-related molecules in the liver of CCl4-treated MMP-2 KO and WT mice. (A) Relative expression of type I collagen (Col1A1), TGFβ1, PDGF receptor (PDGFR), and TGFβ receptor 1 (TGFβR1). Test samples were normalized to the copy number for β-actin, with equal copies applied as templates. Col1A1, TGFβ1, and PDGFR were significantly up-regulated in MMP-2 KO liver as compared with WT liver. $^{*}P = 0.008$. $^{**}P = 0.02$. $^{***}P = 0.006$. (B) Immunoblot assays of MMP-9, MMP-14, TIMP1, and TIMP2. (C) Quantitative analysis using densitometry. Production of MMP-14 and TIMP1 in the liver was increased in CCl4-treated MMP-2 KO mice as compared with CCl4-treated WT mice. $^{\#}P = 0.01$. $^{\#\#}P = 0.03$. Values represent the ratio relative to the mean for CCl4-treated WT mice. Bars represent means ± SEM. The result is representative of 3 independent experiments.

production of MMP-9 and TIMP2 in CCl4-treated KO mice was almost equal to that in CCl4-treated WT mice (Fig. 2C). These data indicated that molecules related to stellate cell activation and fibrosis were up-regulated in CCl4-treated MMP-2 KO livers as compared with CCl4-treated WT livers, suggesting that loss of MMP-2 promoted the activation of hepatic stellate cells during CCl4-induced liver fibrosis.

3.4. Enhanced cholestatic fibrosis in MMP-2 KO mouse liver

Cholestatic fibrosis induced by BDL in MMP-2 KO and WT mice was analyzed at 2-weeks post-induction. Blood biochemical testing showed severe hyperbilirubinemia in mice treated with BDL (Table S2). The serum level of GGT was significantly higher in BDL-treated MMP-2 KO mice as compared with BDL-treated WT mice, whereas no significant change in T-Bil, AST, and ALT was found in either group (Table S2). Microscopic analysis of liver specimens showed extensive bile ductule proliferation and peribiliary fibrosis in BDL-treated mice (Fig. 3A). Bile ductule proliferation was more severe in BDL-treated MMP-2 KO mice than in BDL-treated WT mice (Fig. 3A). Sirius red staining analysis demonstrated more severe BDL-induced peribiliary fibrosis in KO mice than in WT mice (Fig. 3B). The fibrosis areas, quantified using an image analysis system, were significantly increased in BDL-treated KO mice as compared with BDL-treated WT mice (Fig. 3C). There was no difference in hepatic fibrosis area between WT and MMP-2 KO sham-operated mice (Fig. S2). This data indicated that cholestasis-induced liver fibrosis was exacerbated in MMP-2 KO mice as compared with WT mice.

3.5. Hepatic αSMA production in MMP-2 KO mice with BDL-induced fibrosis

Hepatic αSMA production was assessed to determine changes in hepatic myofibroblasts associated with cholestasis-induced fibrosis. The size of αSMA-positive areas in the liver did not differ significantly between WT and MMP-2 KO sham-operated mice (Fig. S3A). Hepatic αSMA in BDL-treated mice was detected in periportal regions only. The αSMA-positive area in the liver of BDL-treated MMP-2 KO mice was almost equal to that of BDL-treated WT mice (Fig. S3A). As well as immunostaining analysis, immunoblot analysis showed that hepatic αSMA production in BDL-treated MMP-2 KO mice was almost equal to that in BDL-treated WT mice (Fig. 3D). Quantitative analyses using an image analysis system and densitometry also showed that hepatic αSMA production in BDL-treated MMP-2 KO mice was not significantly increased relative to WT mice (Figs. S3B and 3E).

3.6. Expression of fibrosis-related molecules in MMP-2 KO mice with cholestasis-induced fibrosis

The hepatic expression of molecules related to the promotion of liver fibrosis was studied in MMP-2 KO and WT mice with BDL-treated fibrosis. Quantitative RT-PCR analysis showed that hepatic

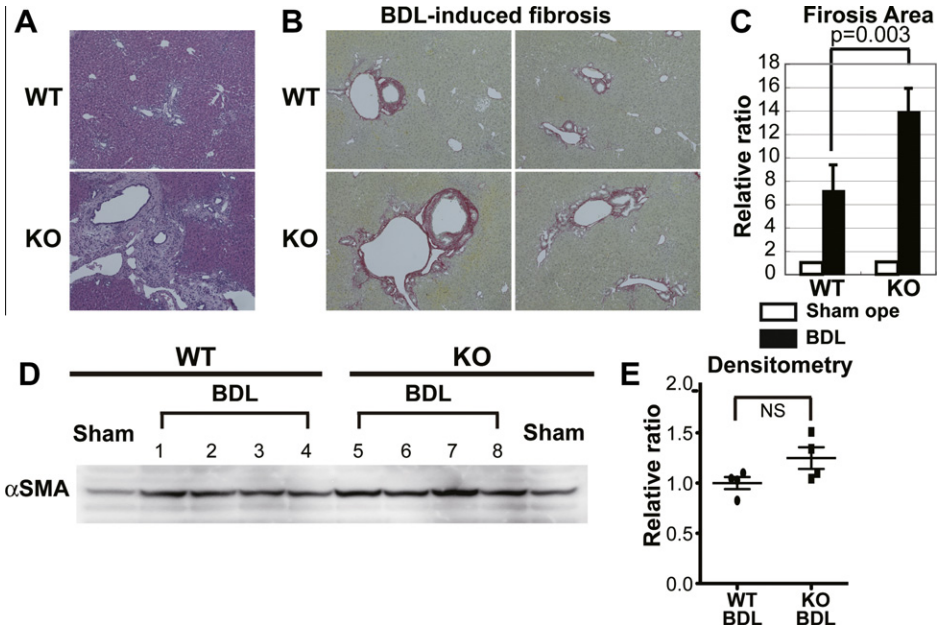

Fig. 3. Cholestasis-induced liver fibrosis is accelerated in MMP-2 KO mice. MMP-2 KO mice and WT littermates ($n = 4$ per group) were subjected to bile duct ligation (BDL). Negative control mice were sham operated (Sham). (A) Hematoxylin eosin staining of liver specimens of BDL-treated mice. (B) Representative photographs of Sirius red staining in the liver of BDL-treated mice. (C) Ratio of overall fibrosis area relative to sham-operated WT mice. Data represent means ± SD. (D) Immunoblot analysis of αSMA in the liver of MMP-2 KO and WT mice. (E) Quantitative analysis of αSMA using densitometry. The αSMA protein in the immunoblot analysis was unchanged. Values represent the ratio relative to the mean for BDL-treated WT mice. Bars represent means ± SEM.

expression of type I collagen, TIMP1, MMP-9, MMP-14, PDGF receptor, and TGFβ was up-regulated in BDL-treated mice as compared with sham-operated mice (Figs. 4A and S4). Hepatic expression of type I collagen in BDL-treated KO mice was 2-fold higher than that in BDL-treated WT mice (Fig. 4A). The hepatic expression of TIMP1 was also up-regulated in BDL-treated KO mice (Fig. 4A). However, there was no significant difference in the hepatic expression of MMP-9, MMP-14, TGFβ, TGFβ receptor, or PDGF receptor between BDL-treated KO and BDL-treated WT mice (Figs. 4A and S4).

Immunoblot analysis showed that the levels of MMP-9, MMP-14, TIMP1 and TIMP2 protein in the liver were higher in BDL-treated mice as compared with sham-operated mice, indicating that production of fibrosis-related molecules was up-regulated in our experimental model (Fig. 4B). Quantitative analysis using densitometry showed that hepatic TIMP1 production was significantly higher in BDL-treated KO mice than BDL-treated WT mice (Fig. 4C). There was no significant difference in the production of MMP-9, MMP-14, or TIMP2 between BDL-treated KO mice and BDL-treated WT mice. Our data indicated that type I collagen and TIMP1 was up-regulated in BDL-treated MMP-2 KO mice as compared with WT mice, suggesting that TIMP1 production is related to acceleration of cholestasis-induced fibrosis in the liver of MMP-2 KO mice.

4. Discussion

In the present study, we analyzed liver fibrosis and fibrosis-related molecules in MMP-2 KO mice in models of toxin- and cholestasis-induced fibrosis. This is the first study to show that BDL-induced hepatic fibrosis is accelerated in MMP-2 KO mice, and that the expression of type I collagen is increased (Figs. 3C and 4A). BDL duplicates the hepatocytic damage, activation of hepatic stellate cells, and liver fibrosis observed in human liver disease. In the early phase of BDL, there is a marked and transient proliferation of bile duct epithelial cells associated with proliferation of portal periductular fibroblasts which rapidly express αSMA [16]. This supports the hypothesis that portal myofibroblasts play an important role in BDL-induced fibrogenesis. Myofibroblastic differentiation of portal fibroblasts is mediated by several signaling pathways including the TGFβ [17] and PDGF [18] pathways. Our data indicate that these molecules were up-regulated in the liver of BDL-treated mice as compared with sham-operated mice, in consistent with previous reports. Hepatic αSMA production in MMP-2 KO mice was almost equal to that of WT mice (Fig. 3E), and the hepatic expression of TGFβ1, TGFβ receptor, and PDGF receptor in BDL-treated MMP-2 KO liver was unchanged in comparison with BDL-treated WT liver (Figs. 4A and S4). Taken together, our data suggest that the myofibroblastic differentiation of portal fibroblasts in KO liver was almost equal to that of WT liver.

Hepatic TIMP1 was up-regulated in BDL-treated MMP-2 KO mice as compared with BDL-treated WT mice (Figs. 4A and 4C), indicating that loss of MMP-2 in cholestatic liver induces the TIMP1 production. In BDL-induced liver fibrosis, TIMP1 is expressed in myofibroblasts. However, loss of TIMP1 activity does not result in improvement of liver fibrosis as indicated by a previous study in TIMP1/TIMP2 double-KO mice, which showed that TIMP1 and TIMP2 do not play an essential role in experimental hepatic fibrogenesis by schistosomiasis [19]. The mechanism of TIMP1 up-regulation in MMP-2 KO liver remains unclear; however, it is possible that the ability of MMP-2-deficient portal fibroblasts to suppress TIMP1 production is impaired due to lack of MMP-2-mediated signaling. Further study is necessary to address this issue.

Previous studies have indicated that hepatic stellate cells undergo an activation process, acquire a fibroblastic phenotype, express αSMA, and play a major role in collagen deposition in CCl4-induced liver fibrosis [1,20]. TGFβ induces the production of type I collagen in activated hepatic stellate cells. PDGF receptor is up-regulated in activated hepatic stellate cells proliferating in response to PDGF. Progression of CCl4-induced liver fibrosis is

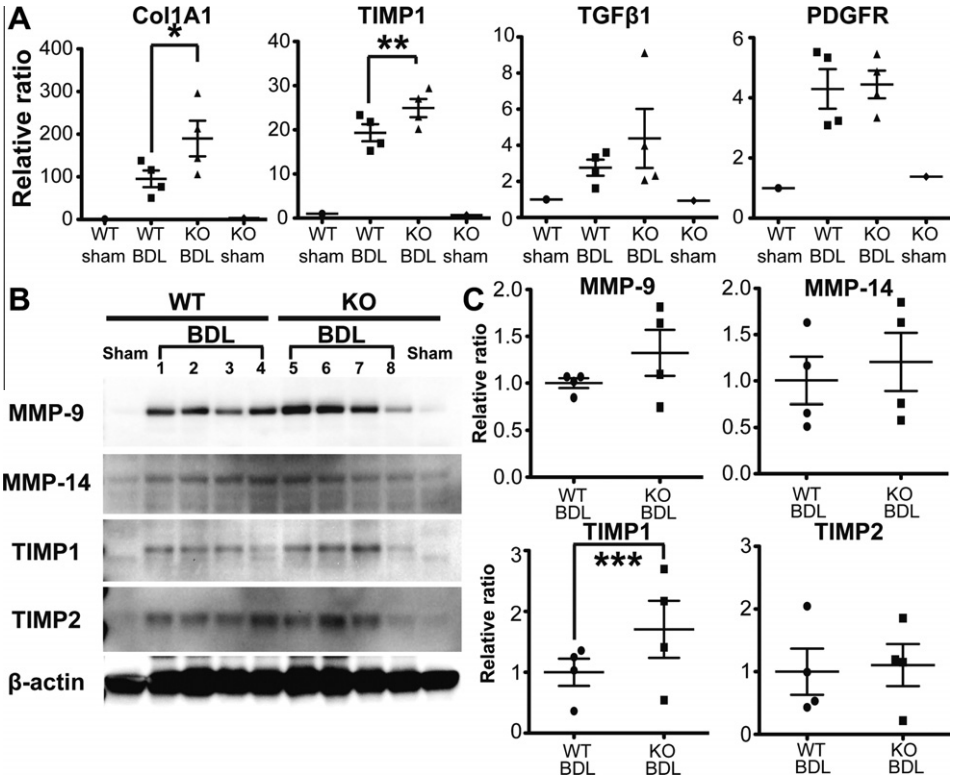

Fig. 4. Analysis of fibrosis-related molecules in the liver of BDL-treated mice. (A) Relative expression of Col1A1, TIMP1, TGFβ1, and PDGFR. Test samples were normalized to the copy number for β-actin, with equal copies applied as templates. Hepatic expression of Col1A1 and TIMP1 was significantly up-regulated in BDL-treated MMP-2 KO mice as compared with BDL-treated WT mice. Values represent the ratio relative to the mean for sham-operated WT mice. (B) Immunoblot assays of MMP-9, MMP-14, TIMP1, and TIMP2. (C) Quantitative analysis using densitometry. Hepatic production of TIMP1 in BDL-treated MMP-2 KO mice was increased as compared with BDL-treated WT mice. Values represent the ratio relative to the mean for BDL-treated WT mice. Bars represent means ± SEM. $^{*}P = 0.02$. $^{**}P = 0.003$. $^{***}P = 0.04$. The result is representative of 3 independent experiments.

mediated by these molecules. Our data suggest that activation of hepatic stellate cells was promoted in MMP-2 KO mice as compared with WT mice. This is supported by the observation that the production of αSMA and TIMP1 was increased in MMP-2 KO liver (Figs. 1 and 2), because the activated hepatic stellate cells (myofibroblasts) are a major source of TIMP1 [21]. Thus, our data suggest the activation of hepatic stellate cells was promoted by loss of MMP-2 activity *in vivo*. Previous research has shown that loss of MMP-2 amplifies CCl4-induced liver fibrosis, but no increase was seen in the *in vivo* levels of several molecules related to activation of hepatic stellate cells [22]. This discrepancy may be due to the duration of chronic CCl4 administration, which was twice as long in our study. Increased hepatic levels of MMP-14 protein in CCl4-treated MMP-2 KO mice may be due to a compensatory effect for MMP-2 deficiency, which is generally activated by MMP-14.

Classical substrates of MMPs are components of the ECM; however, the list of substrates has been extended to include various receptors, ligands and adhesion molecules [2]. Several studies have demonstrated changes in the hepatic fibrotic response in MMP-deficient mice. Madala et al. reported that chronic helminth-induced liver fibrosis was attenuated in MMP-12 deficient mice. MMP-12 deficiency is associated with up-regulation of ECM-degrading enzymes such as MMP-2, -9, and -13 in Th2 cytokine-driven fibrosis [23]. Furthermore, loss of ECM-degrading MMPs does not always lead to progression of liver fibrosis. MMP-13 is one of the representative ECM-degrading enzymes in mice. In the liver of BDL-treated MMP-13 KO mice, activation of hepatic stellate cells was paradoxically suppressed in comparison with WT mice [8], suggesting that MMP-13 probably contributes to accelerating fibrogenesis in cholestatic livers by mediating inflammation rather than promoting fibrolysis. On the other hand, our data showed that MMP-2 contributes to suppression of fibrogenesis in both toxin- and cholestasis-induced liver damage. One possible interpretation is that MMP-2-mediated signaling is associated with the suppression of inflammation or fibrosis, which influence the production of type I collagen and TIMP1 derived from myofibroblasts. Another is that MMP-2 simply contributes to acceleration of fibrolysis. Radbill et al. showed that the expression of type I collagen in cultured hepatic stellate cells was decreased by knockdown of MMP-2 [22], suggesting that MMP-2-mediated signals directly regulate the expression of type I collagen. Thus, development of specific activators of MMP-2, but not of other ECM-degrading enzymes, could be promising as a new therapeutic strategy for the treatment of liver cirrhosis.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bbrc.2011.02.012.

References

[1] S. Hemmann, J. Graf, M. Roderfeld, et al., Expression of MMPs and TIMPs in liver fibrosis – a systematic review with special emphasis on anti-fibrotic strategies, J. Hepatol. 46 (2007) 955–975.
[2] M. Seiki, The cell surface: the stage for matrix metalloproteinase regulation of migration, Curr. Opin. Cell Biol. 14 (2002) 624–632.
[3] S.L. Friedman, Evolving challenges in hepatic fibrosis, Nat. Rev. Gastroenterol. Hepatol. 7 (2010) 425–436.
[4] R. Lichtinghagen, M.J. Bahr, M. Wehmeier, et al., Expression and coordinated regulation of matrix metalloproteinases in chronic hepatitis C and hepatitis C virus-induced liver cirrhosis, Clin. Sci. 105 (2003) 373–382.
[5] T. Takahara, K. Furui, J. Funaki, et al., Increased expression of matrix metalloproteinase-II in experimental liver fibrosis in rats, Hepatology 21 (1995) 787–795.
[6] A.E. Kossakowska, D.R. Edwards, S.S. Lee, et al., Altered balance between matrix metalloproteinases and their inhibitors in experimental biliary fibrosis, Am. J. Pathol. 153 (1998) 1895–1902.
[7] T. Watanabe, M. Niioka, A. Ishikawa, et al., Dynamic change of cells expressing MMP-2 mRNA and MT1-MMP mRNA in the recovery from liver fibrosis in the rat, J. Hepatol. 35 (2001) 465–473.
[8] H. Uchinami, E. Seki, D.A. Brenner, et al., Loss of MMP 13 attenuates murine hepatic injury and fibrosis during cholestasis, Hepatology 44 (2006) 420–429.
[9] M. Seiki, N. Koshikawa, I. Yana, Role of pericellular proteolysis by membrane-type 1 matrix metalloproteinase in cancer invasion and angiogenesis, Cancer Metastasis Rev. 22 (2003) 129–143.
[10] R. Issa, X. Zhou, C.M. Constandinou, et al., Spontaneous recovery from micronodular cirrhosis: evidence for incomplete resolution associated with matrix cross-linking, Gastroenterology 126 (2004) 1795–1808.
[11] Y. Osawa, Y.A. Hannun, R.L. Proia, et al., Roles of AKT and sphingosine kinase in the antiapoptotic effects of bile duct ligation in mouse liver, Hepatology 42 (2005) 1320–1328.
[12] T. Itoh, T. Ikeda, H. Gomi, et al., Unaltered secretion of beta-amyloid precursor protein in gelatinase A (matrix metalloproteinase 2)-deficient mice, J. Biol. Chem. 272 (1997) 22389–22392.
[13] E.L. Opie, M.B. Rothbard, Osmotic homeostasis maintained by mammalian liver, kidney, and other tissues, J. Exp. Med. 97 (1953) 483–497.
[14] S. Kakinuma, H. Ohta, A. Kamiya, et al., Analyses of cell surface molecules on hepatic stem/progenitor cells in mouse fetal liver, J. Hepatol. 51 (2009) 127–138.
[15] S. Kakinuma, Y. Tanaka, R. Chinzei, et al., Human umbilical cord blood as a source of transplantable hepatic progenitor cells, Stem Cells 21 (2003) 217–227.
[16] J.A. Dranoff, R.G. Wells, Portal fibroblasts: underappreciated mediators of biliary fibrosis, Hepatology 51 (2010) 1438–1444.
[17] Z. Li, J.A. Dranoff, E.P. Chan, et al., Transforming growth factor-beta and substrate stiffness regulate portal fibroblast activation in culture, Hepatology 46 (2007) 1246–1256.
[18] N. Kinnman, C. Francoz, V. Barbu, et al., The myofibroblastic conversion of peribiliary fibrogenic cells distinct from hepatic stellate cells is stimulated by platelet-derived growth factor during liver fibrogenesis, Lab. Invest. 83 (2003) 163–173.
[19] B. Vaillant, M.G. Chiaramonte, A.W. Cheever, et al., Regulation of hepatic fibrosis and extracellular matrix genes by the response: new insight into the role of tissue inhibitors of matrix metalloproteinases, J. Immunol. 167 (2001) 7017–7026.
[20] K. Wake, "Sternzellen" in the liver: perisinusoidal cells with special reference to storage of vitamin A, Am. J. Anat. 132 (1971) 429–462.
[21] E. Roeb, E. Purucker, B. Breuer, et al., TIMP expression in toxic and cholestatic liver injury in rat, J. Hepatol. 27 (1997) 535–544.
[22] B.D. Radbill, R. Gupta, M.C. Ramirez, et al., Loss of matrix metalloproteinase-2 amplifies murine toxin-induced liver fibrosis by upregulating collagen I expression, Dig. Dis. Sci. 56 (2011) 406–416.
[23] S.K. Madala, J.T. Pesce, T.R. Ramalingam, et al., Matrix metalloproteinase 12-deficiency augments extracellular matrix degrading metalloproteinases and attenuates IL-13-dependent fibrosis, J. Immunol. 184 (2010) 3955–3963.