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LIVER PATHOBIOLOGY

Matrix metalloproteinase-10 expression is induced during hepatic injury and plays a fundamental role in liver tissue repair

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Abstract

Background & Aims: Upon tissue injury, the liver mounts a potent reparative and regenerative response. A role for proteases, including serine and matrix metalloproteinases (MMPs), in this process is increasingly recognized. We have evaluated the expression and function of MMP10 (stromelysin-2) in liver wound healing and regeneration. *Methods:* The hepatic expression of MMP10 was examined in two murine models: liver regeneration after two-thirds partial hepatectomy (PH) and bile duct ligation (BDL). MMP10 was detected in liver tissues by qPCR, western blotting and immunohistochemistry. The effect of growth factors and toll-like receptor 4 (TLR4) agonists on MMP10 expression was studied in cultured parenchymal and biliary epithelial cells and macrophages respectively. The role of MMP10 was evaluated by comparing the response of *Mmp10*$^{+/+}$ and *Mmp10*$^{-/-}$ mice to PH and BDL. The intrahepatic turnover of the extracellular matrix proteins fibrin (ogen) and fibronectin was examined. *Results:* MMP10 mRNA was readily induced after PH and BDL. MMP10 protein was detected in hepatocytes, cholangiocytes and macrophages. In cultured liver epithelial cells, MMP10 expression was additively induced by transforming growth factor-β and epidermal growth factor receptor ligands. TLR4 ligands also stimulated MMP10 expression in macrophages. Lack of MMP10 resulted in increased liver injury upon PH and BDL. Resolution of necrotic areas was impaired, and *Mmp10*$^{-/-}$ mice showed increased fibrogenesis and defective turnover of fibrin (ogen) and fibronectin. *Conclusions:* MMP10 expression is induced during mouse liver injury and participates in the hepatic wound healing response. The profibrinolytic activity of MMP10 may be essential in this novel hepatoprotective role.

The liver displays an extraordinary regenerative capacity upon parenchymal tissue loss as a result of partial resection or hepatocellular injury (1). This regenerative response is tightly regulated, involving an array of cytokines, growth factors and metabolites, whose fluctuations contribute to initiate the proliferation of liver cells as well as the arrest of cell replication once the optimal hepatic functional mass is restored (2–4). An essential additional component of the regenerative response of the liver is remodelling of the extracellular matrix (ECM), which also entails the release of matrix-bound growth-regulatory molecules (5, 6). This reaction is part of a wound healing process aimed at the

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restoration of liver architecture, in which a provisional matrix composed of various macromolecules, such as fibrin (ogen) and fibronectin is transiently formed to stabilize wound areas and provide support to the regenerating liver cells (7–9). This controlled ECM remodelling process, as well as the necessary removal of necrotic cells debris, is mediated by two major classes of proteases, serine proteases and matrix metalloproteinases (MMPs), acting in a complex and interlinked fashion (6). An important role for serine proteases such as urokinase-type plasminogen activator (uPA), tissue-type plasminogen activator (tPA) and plasmin has been well established in experimental liver regeneration and repair (6, 7, 10–12). Up-regulation of the expression of several MMPs has been broadly demonstrated during liver injury and regeneration (13–15). However, little is known about the function of specific MMPs in the hepatic response to injury. Among the best characterized is the role of MMP13 (the interstitial collagenase in rodents) in cholestasis-induced liver injury and fibrosis resolution, and that of MMP9 in acute liver failure and liver regeneration, as demonstrated in the corresponding knockout mouse models (16–19).

Within the MMPs gene family, stromelysin-2 (MMP10) has been demonstrated to have critical roles in normal wound healing in skin and colonic tissue (20, 21). However, apart from an early report describing MMP10 mRNA up-regulation in rat liver upon acute injury (14), its potential involvement in liver tissue repair is unknown. MMP10 has a broad substrate spectrum, and besides being able to process a wide range of ECM components, it can activate other MMPs such as proMMP-1, -7, -8 and -9 (22, 23). Interestingly, MMP10 has been recently reported to have profibrinolytic effects *in vivo* by enhancing tissue plasminogen activator fibrinolytic activity (24). These observations, together with the fundamental role of the plasminogen system in liver tissue repair (7, 10–12, 25), prompted us to evaluate the significance of MMP10 in liver injury and regeneration. Our data point to a fundamental role for this metalloproteinase in the hepatic wound healing response.

Material and methods

Animal models

Eight to twelve weeks old *Mmp10*$^{-/-}$ mice (C57BL/6 background) (24, 26) and their corresponding wild-type littermates were used throughout this study. Two-thirds partial hepatectomy (PH) and sham operations were performed as previously reported (4, 27). Essentially, a transverse subxiphoid incision was made to allow for the extrusion and extra-abdominal ligation of the left lateral and median lobes, which are subsequently removed together. Bile duct ligation (BDL) was performed essentially as previously described (28). Mice were sacrificed at the indicated time points, serum was collected and liver tissue samples were either snap-frozen in liquid nitrogen or formalin fixed and paraffin embedded for subsequent analysis. At least four mice were used per condition and time point.

Histological and immunohistochemical studies

Liver tissues were stained with H&E or with picro-Sirius Red to visualize collagen deposition as described (4, 29). Fibrosis and the degree of tissue necrosis were scored by two morphologists (SC and SM) blinded to the identity of the samples. For morphometric analyses, individual slides were digitized using the Aperio ScanScope (Aperio Technologies, Vista, CA, USA), and digital images were analysed using Image Analysis System software (Delta Sistemi, Rome, Italy) to measure the surface area of collagen coverage per ×40 field. Values are the means of 10 fields taken from different tissue sections per mouse. The degree of tissue necrosis was evaluated and scored as previously reported (4, 30). BrdU administration to mice and liver tissue staining for BrdU incorporated into DNA were performed as reported previously (4, 31). Immunohistochemical detection of MMP10 was performed on 3- to 5-μm-thick sections obtained from formalin-fixed tissue embedded in paraffin. Antigen retrieval was performed with Citrate Buffer (pH 6) (Dako, Glostrup, Denmark). Immunohistochemical staining was performed with two different anti-MMP10 antibodies with similar results: rabbit polyclonal ab38930 from Abcam (Cambridge, UK) at 1/200 dilution; and rabbit polyclonal AP07210PU-N from Acris (San Diego, CA, USA) at 1/25 dilution. Incubations with primary antibodies were carried out for 1.5 h. Controls immunohistochemical stainings were performed by omitting the primary antibodies. The immunohistochemical procedure was performed in a Thermo Autostainer 360-2D System (Bio-Optica, Milan, Italy), using the MACH1 detection system (Biocare, Concord, CA, USA) and Betazoid DAB (Biocare, Concord, CA, USA) as a chromogen. Light microscopy micrographs were captured by a Videocam (SPOT Insight; Diagnostic Instrument, Inc., Sterling Heights, MI, USA) connected to an Olympus BX-51 light microscope (Olympus, Tokyo, Japan) and processed with an Image Analysis System (Delta Sistemi, Rome, Italy).

Cell culture and treatments

Normal mouse cholangiocytes (NMCs) and mouse Kupffer cells were isolated and cultured as we previously described (4, 29). The non-transformed mouse hepatocyte cell line AML12 and the murine monocyte/macrophage RAW 264.7 cells, both from the American Type Culture Collection (ATCC), were cultured as we described in previous studies (4, 32). The human cholangiocarcinoma cell line Huh28 was from the Health Science Research Resources Bank (HSRRB, Tokyo, Japan), the human hepatocellular carcinoma cell line

Huh7 was from the ATCC and were, respectively, grown in RPMI1640 or DMEM supplemented with 10% fetal calf serum. The human fibronectin extra domain A (EDA) was cloned, bacterially expressed and purified as previously described (33). To demonstrate that cellular effects of fibronectin-EDA were specific, and not attributable to potential LPS contamination, cells were also incubated with fibronectin-EDA protein previously treated with proteinase K (Sigma, St. Louis, MO, USA) (2.5 mg/ml proteinase K, 20 min at 25°C). During growth factor, bacterial lipopolysaccharide (LPS) (Sigma) and fibronectin-EDA domain treatments cells were kept in serum-free medium.

Western blot analysis

For the determination of MMP10 and α-smooth muscle actin (αSMA), liver tissues were homogenized and proteins were resolved by SDS-PAGE and blotted as described (31, 32, 34). For the analysis of MMP10 protein levels in the conditioned media of Huh7 and Huh28 cells were incubated in serum-free medium with the indicated treatments. After 24 h, media were collected and concentrated 40-fold using Amicon Ultra Centrifugal devices with 10 kDa cut-off membranes (Merck-Millipore). Concentrated conditioned media were resolved by SDS-PAGE and blotted as described (32). For the analysis of insoluble fibrin and fibronectin deposits in liver tissue, samples were homogenized and proteins were extracted exactly as previously described (35). For the detection of fibronectin fragments, liver tissue samples were homogenized in a urea-containing buffer and proteins were solubilized as reported (36). Primary antibodies used were as follows: anti-MMP10 from Acris (AP07210PU-N); antifibrinogen from Abcam (ab62527) (Cambridge, UK); full length fibronectin was detected with an antibody from Abcam (ab23751) and fibronectin cleavage products were detected with an antibody from Santa Cruz Biotechnology (sc8422) (Santa Cruz, CA, USA); α-SMA was detected with an antibody from Sigma (A2547). Blots were stained with Ponceau or probed with anti-GAPDH antibodies (2118S) (Cell Signaling Technology, Danvers, MA, USA) to show equal loading. Densitometric analysis of the films was carried out with the ImageJ Image Processing Program (NIH, Bethesda, MD, USA). The blots shown are representative of all the tissue samples included in this study, and the Western blot procedure was repeated at least twice for each analysis.

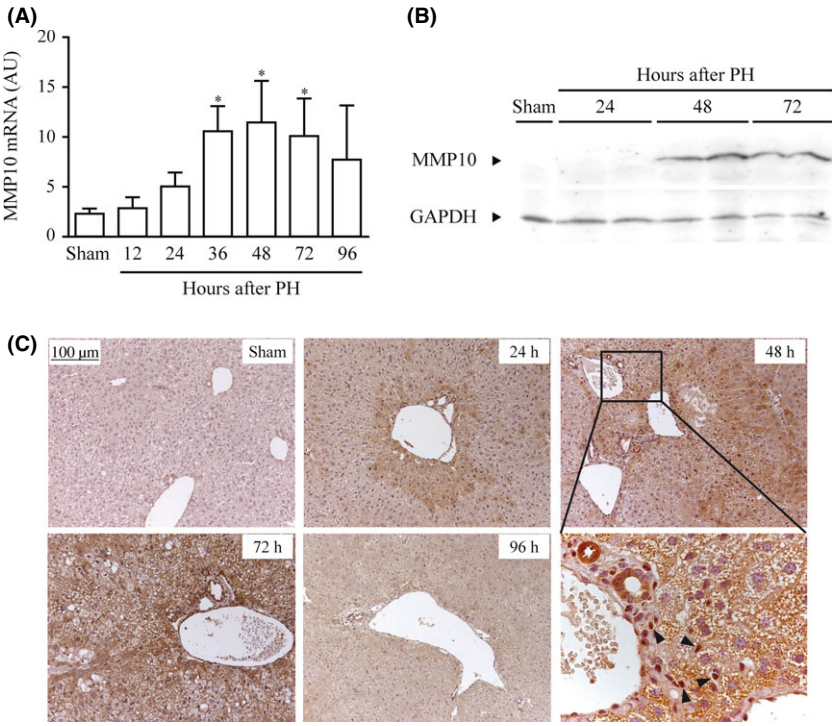

Fig. 1. Expression of MMP10 during mouse liver regeneration after two-thirds partial hepatectomy (PH). (A) MMP10 mRNA expression as analysed by qPCR at the indicated time points after PH ($n = 5$ mice per point). Asterisk indicates $P < 0.05$ vs Sham. (B) Western blot analysis of MMP10 protein levels at the indicated time points after PH. (C) Representative images of MMP10 immunostaining in regenerating mouse liver tissues at different time points after PH (× 100). Bottom right image is an amplification of the area indicated in the above photomicrograph (× 400). Arrowheads indicate monocytes/macrophages. Values shown for sham-operated animals correspond to mice culled 48 h after the intervention. AU, arbitrary units.

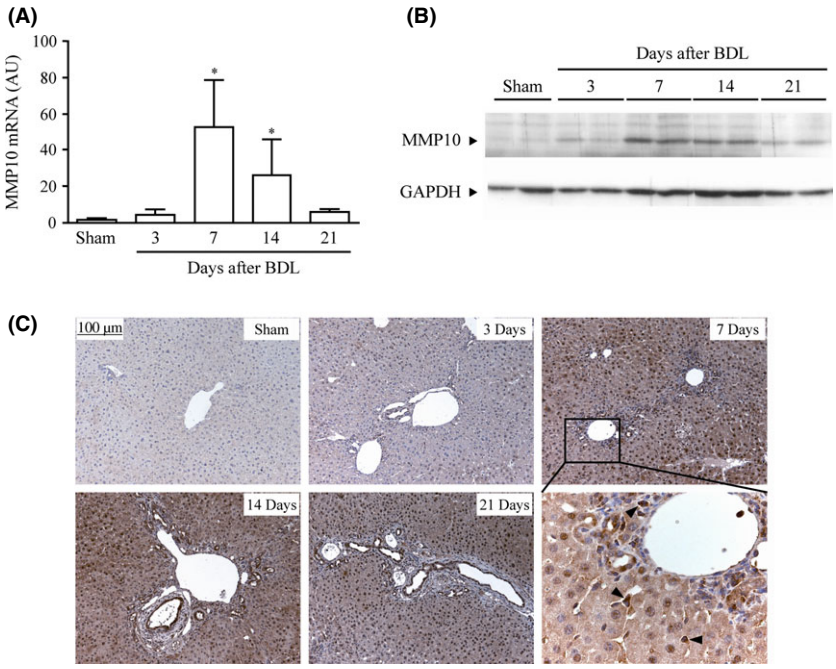

Fig. 2. Expression of MMP10 in mouse liver after bile duct ligation (BDL). (A) MMP10 mRNA expression as analysed by qPCR at the indicated days after BDL (n = 5 mice per point). Asterisk indicates $P < 0.05$ vs Sham. (B) Western blot analysis of MMP10 protein levels at the indicated time points after BDL. (C) Representative images of MMP10 immunostaining in mouse liver tissues at different days after BDL (× 100). Bottom right image is an amplification of the area indicated in the above photomicrograph (× 400). Arrowheads indicate monocytes/macrophages. Values shown for sham-operated animals correspond to mice culled 7 days after the intervention. AU, arbitrary units.

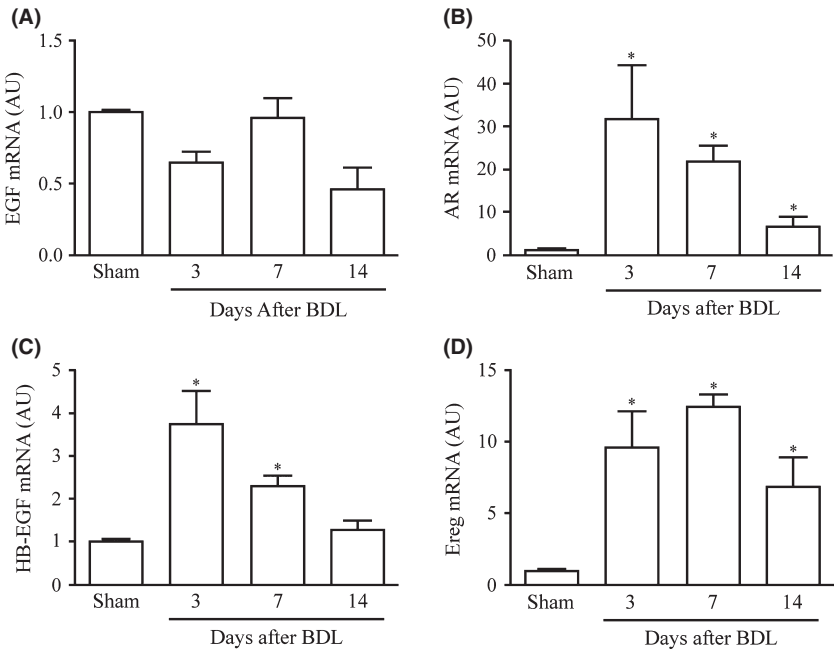

Fig. 3. Expression of EGFR ligands in mouse liver upon bile duct ligation (BDL). The expression of epidermal growth factor (EGF) (A), amphiregulin (AR) (B), heparin-binding EGF (HB-EGF) (C) and epiregulin (Ereg) (D) was measured by qPCR in mouse liver tissues at the indicated days after BDL (n = 5 mice per point). Asterisk indicates $P < 0.05$ vs Sham. Values shown for sham-operated animals correspond to mice culled 7 days after the intervention. AU, arbitrary units.

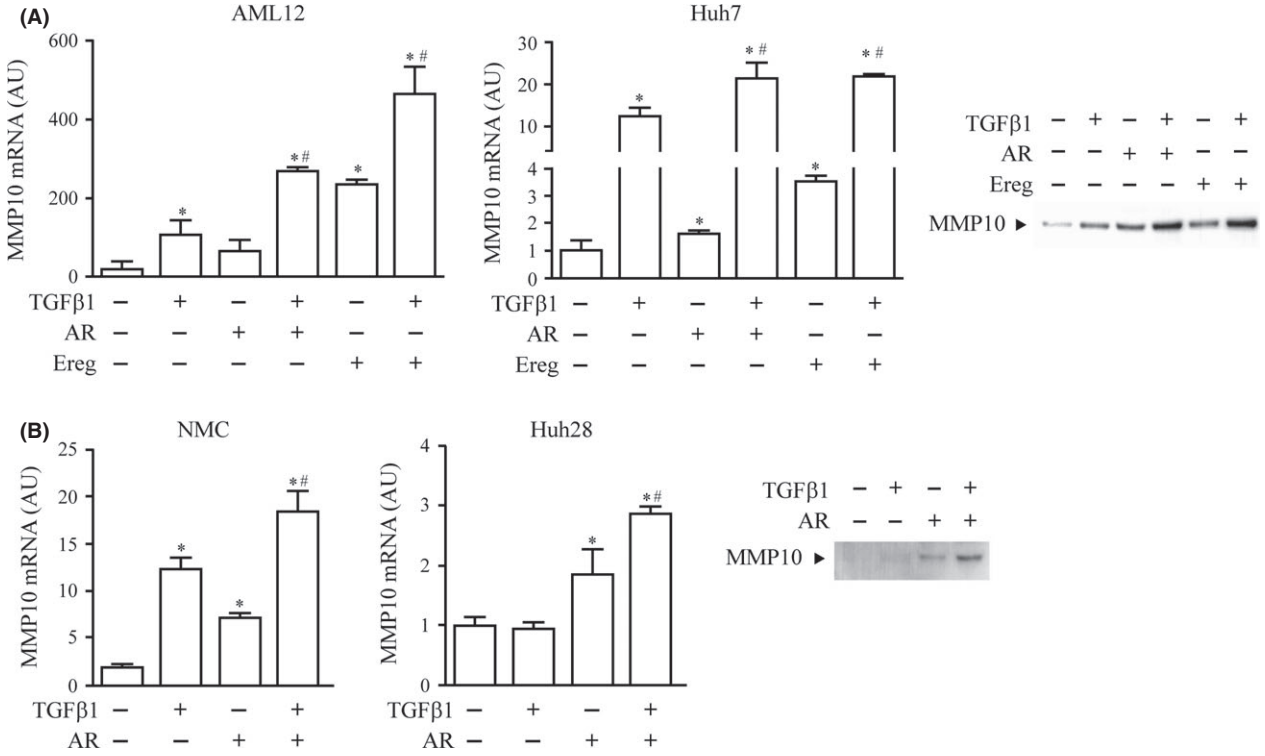

Fig. 4. Expression of MMP10 in mouse and human liver cells in response to growth factors. MMP10 mRNA levels analysed by qPCR in mouse AML12 hepatocytes (24-h treatment) and Huh7 human hepatocellular carcinoma cells (12-h treatment) (A), cultured mouse cholangiocytes (NMC) (12-h treatment) and human cholangiocarcinoma cells (Huh28) (24-h treatment) (B), stimulated with TGFβ1 (80 pM), AR (50 nM) and Ereg (50 nM), as indicated. Asterisks indicate $P < 0.05$ vs control cells; #$P < 0.05$ vs cells treated with TGFβ1 alone. AU, arbitrary units. Westerns blots included in panels A and B show the levels of MMP10 protein accumulated in the culture media of Huh7 and Huh28 cells after 24 h of incubation with the indicated growth factors. Representative blots of two experiments performed in triplicates are shown.

RNA isolation and quantitative real-time PCR (qPCR)

Total RNA was extracted using the TRI Reagent (Sigma). Real-time PCR was performed using an iCycler (BioRad, Hercules, CA, USA) and the iQ SYBR Green Supermix (BioRad) as reported (29). Gene expression was determined using the ΔΔCT calculation as described (37). We designed all primers to distinguish between genomic and cDNA amplification and sequenced all PCR products to confirm the specificity. Primers for amphiregulin (AR), epidermal growth factor (EGF), epiregulin (Ereg), monocyte chemotactic protein-1 (MCP-1), tumour necrosis factor-α (TNFα) and transforming growth factor β-1 (TGFβ1) have been previously described (32, 34). Primer sequences are included in Supplementary Table 1.

Statistical analysis

Data are means ± SEM. Analyses were performed using GraphPad Prism version 5.00 (GraphPad Software, San Diego, CA, USA). Data were compared between pairs of groups using the Student *t*-test. A *P* value of <0.05 was considered significant. *In vitro* experiments were performed at least two times in triplicates.

Results

MMP10 expression during mouse liver regeneration after two-thirds partial hepatectomy (PH)

To address the potential role of MMP10 in liver tissue regeneration, we first examined its expression during hepatic regeneration after two-thirds partial hepatectomy (PH) (2). Although the expression of this MMP is very low in control liver tissues, we found a strong increase in MMP10 mRNA levels between 24 and 36 h post-resection that was accompanied by the accumulation of MMP10 protein (Fig. 1A and B). As shown in Fig. 1C, MMP10 was detected by immunohistochemical analysis in hepatocytes, biliary cells as well as in inflammatory cells (monocytes/macrophages), showing a patchy pattern involving clusters of hepatocytes.

MMP10 expression in cholestatic liver injury

To further understand the role of MMP10 in the hepatic response to injury, we evaluated its expression in mouse liver after BDL, a prototypic model of inflammation, hepatocellular injury and tissue remodelling (38, 39). Similar to the PH model, MMP10 mRNA expression increased significantly by day 7 after BDL and remained

high 2 weeks after the intervention (Fig. 2A). MMP10 protein levels as analysed by western blotting showed a similar expression pattern (Fig. 2B). By tissue immunostaining, increased MMP10 reactivity was detected in biliary epithelial cells and hepatocytes, as well as scattered inflammatory cells, with maximal intensity by 14 days after BDL (Fig. 2C). The expression of MMP10 among liver cells was similar in both the PH and BDL injury models. Interestingly, in addition to cytoplasmic staining, nuclear MMP10 positivity was also observed in hepatocytes and biliary cells.

Identification of potential signals that regulate MMP10 expression in liver injury

Previous reports have shown that MMP10 gene transcription is markedly induced in various tumour and epithelial cell lines by growth factors, such as EGF and TGFβ1 (40–42). Expression of TGFβ1 and the EGF receptor (EGFR) ligands heparin-binding EGF (HB-EGF), AR and Ereg is induced in respone to liver injury and regeneration (2, 3, 34, 43, 44). Here, we show that expression of AR, Ereg and HB-EGF, but not that of EGF, was up-regulated in experimental cholestatic liver injury (Fig. 3). In view of these observations, we assessed if EGFR ligands, AR and Ereg, alone and in combination with TGFβ1, affected the expression of MMP10 in normal mouse hepatocytes (AML12 cells) and the human liver cancer cell line Huh7. As shown in Fig. 4A, we found that these growth factors induced MMP10 expression in liver cell of parenchymal origin and displayed an additive effect when applied in combination. Similar observations were made in cultured normal mouse cholangiocytes (NMC) and in human cholangiocarcinoma cells Huh28, although TGFβ1 alone had no effect on MMP10 mRNA levels in Huh28 cells at the time point tested (Fig. 4B). In accordance with the stimulation of MMP10 gene expression, treatments with these growth factors resulted in the accumulation of MMP10 protein in the conditioned media of Huh7 and Huh28 cells (Fig. 4 A and B).

Bacterial lipopolysaccharide (LPS)-mediated activation of macrophages has been identified as an important determinant in the hepatic responses to experimental cholestasis (45). Recent reports have described that MMP10 expression can be triggered in macrophages upon toll-like receptor 4 (TLR4) activation by LPS (46, 47). Accordingly, we found that LPS stimulation of isolated mouse Kupffer cells resulted in increased MMP10 expression, in parallel to that of the prototypic LPS/TLR4 target gene tumour necrosis factor-α (TNFα) (Fig. 5A). In addition to LPS, TLR4 can be bound and activated by other endogenous ligands that are also up-regulated during liver injury (48). One of these TLR4 agonists that caught our attention was the extra domain A (EDA) of fibronectin, included in a splice variant of fibronectin that arises in cholestatic liver injury and also a potent TLR4 activator (33, 49, 50). This knowledge, together with the fact that fibronectin is a potential substrate of MMP10 (51), prompted us to examine if EDA could also trigger MMP10 expression in macrophages. As shown in Fig. 5B, recombinant EDA markedly induced TNFα and MMP10 expression in RAW 264.7 mouse macrophages, an effect mirrored by LPS stimulation.

Role of MMP10 liver regeneration after two-thirds partial hepatectomy (PH)

To elucidate the significance of MMP10 expression during liver regeneration, we compared the response of wild-type and *Mmp10*$^{-/-}$ mice with two-thirds PH. Mice survival and the recovery of liver mass were followed up to 7 days post-resection. The survival of our *Mmp10*$^{+/+}$ mice colony after 2/3 PH did not differ significantly from that of other wild-type mice colonies (ca. 85% survival) undergoing this experimental protocol (52, 53). However, survival of *Mmp10*$^{-/-}$ mice after PH was remarkably reduced (Fig. 6A). In agreement with this impaired survival, we observed extensive areas of necrosis that accumulated throughout the liver parenchyma of *Mmp10*$^{-/-}$ mice at different time points after PH (Fig. 6B). Restoration of liver mass was slightly

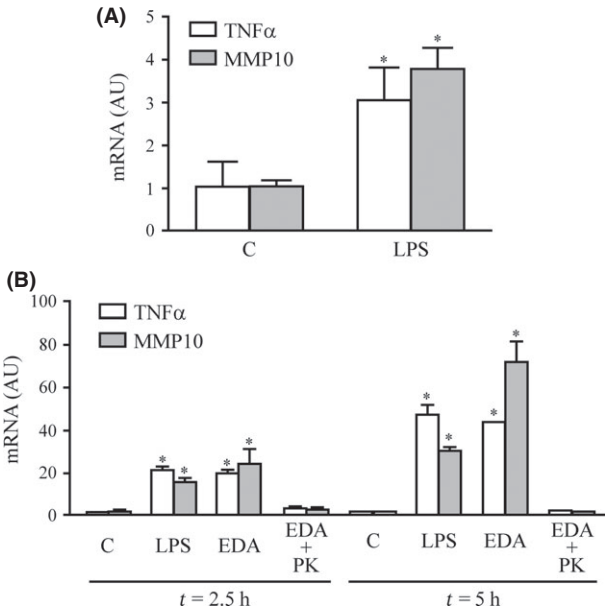

Fig. 5. Expression of MMP10 in mouse Kupffer cells and RAW 264.7 macrophages treated with toll-like receptor 4 (TLR4) agonists. (A) Mouse Kupffer cells were treated with bacterial lipopolysaccharide (LPS) (100 ng/ml) for 3 h, MMP10 and tumour necrosis factor-α (TNFα) mRNA levels were measured by qPCR. Asterisks indicate $P < 0.05$ vs control cells (C). (B) Mouse RAW 264.7 macrophages were treated with LPS (100 ng/ml), the recombinant EDA domain of fibronectin (500 nM) or recombinant EDA previously treated with proteinase K (PK), for the indicated periods of time and MMP10 mRNA levels were measured by qPCR. Asterisks indicate $P < 0.05$ vs control cells (C). AU, arbitrary units.

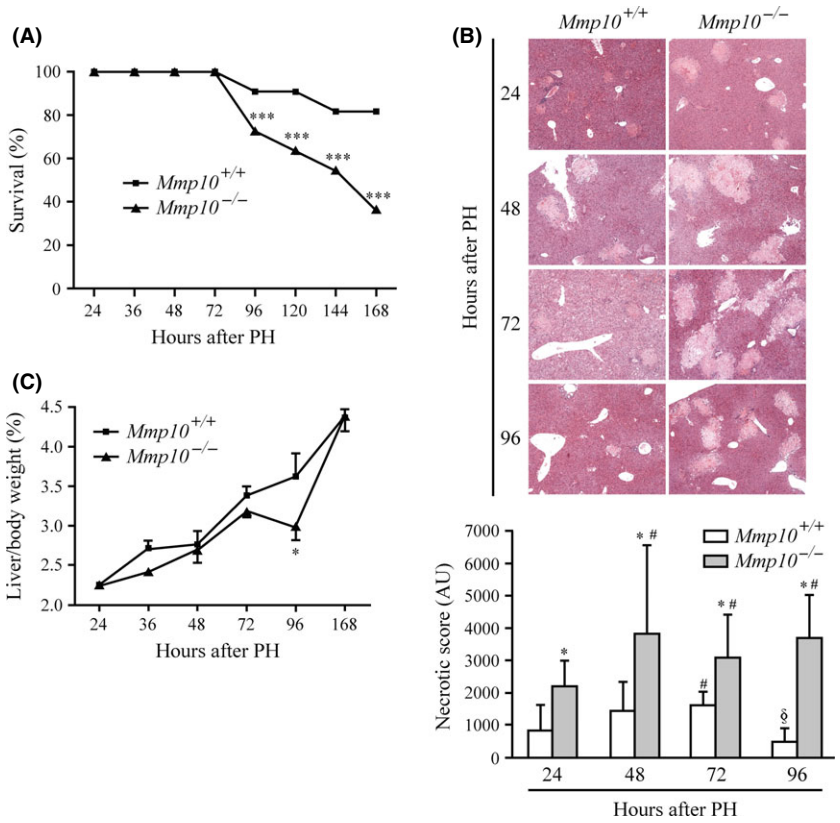

Fig. 6. Liver regeneration in Mmp10$^{+/+}$ and Mmp10$^{-/-}$ mice after two-thirds partial hepatectomy (PH). (A) Two-thirds PH was performed in wild-type and MMP10-deficient mice (n = 15 per genotype) and survival was monitored up to 7 days. Asterisks indicate $P < 0.001$ vs wild-type mice. (B) Representative images of liver tissue sections (H&E staining) from *Mmp10*$^{+/+}$ and *Mmp10*$^{-/-}$ mice at different time points after PH. Graph shows the quantitative analysis of necrotic lesions. Asterisks indicate $P < 0.05$ vs wild-type mice; # indicates $P < 0.05$ vs values found at 24 h for each genotype; § indicates $P < 0.05$ vs values found at 48 and 72 h. (C) The extent of liver regeneration was assessed at different time points by measuring liver regrowth. Asterisk indicates $P < 0.05$ vs wild-type mice. AU: arbitrary units.

delayed in *Mmp10*$^{-/-}$ mice between 72 and 96 h post-resection, but those animals that survived eventually recovered the liver weight (Fig. 6C). Circulating transaminases levels tended to be higher in *Mmp10*$^{-/-}$ mice at 24 and 48 h post-PH, and remained elevated compared with values found in wild-type animals at later time points (Supplementary Fig. 1). However, we saw no differences between the two genotypes in hepatocyte DNA synthesis, as evaluated by BrdU incorporation into nuclei (data not shown). Together, these observations indicate that MMP10 expression is necessary for liver regeneration, possibly being involved in the restoration of parenchymal architecture in the face of tissue injury.

Role of MMP10 in cholestatic liver injury

To gain better insight into the role of MMP10 in liver injury and repair, we examined the response of *Mmp10*$^{+/+}$ and *Mmp10*$^{-/-}$ mice at different time points after BDL. Parenchymal injury in this experimental model of cholestasis is characterized by the appearance of prominent areas of hepatocellular necrosis known as 'bile infarcts', accompanied by an inflammatory response and the development of fibrosis (16, 43, 54, 55). Upon histological examination, we found necrotic lesions developing in both genotypes of mice. However, necrotic areas became more prominent in MMP10-deficient animals starting at 7 days after BDL (Fig. 7), when MMP10 expression had increased in wild-type mice (Fig. 2A, B). Serum transaminases increased similarly in both genotypes, but at later time points, *Mmp10*$^{-/-}$ mice showed higher levels of AST compared with normal mice (Fig. S2). As observed in the PH model, no significant differences in hepatocyte DNA synthesis were found between the two strains of mice (data not shown). The enhanced necrotic injury found in *Mmp10*$^{-/-}$ mice was accompanied by increased deposition of ECM, as estimated by Sirius-red staining. Morphometric quantification of stained areas yielded higher values in *Mmp10*$^{-/-}$ mice at 7 and 14 days after BDL (Fig. 8). Consistently, the expression of α-smooth muscle actin (αSMA), a marker of activated ECM-producing cells (29), and of the chemokine monocyte chemoattractant protein 1 (MCP-1) involved in BDL-induced

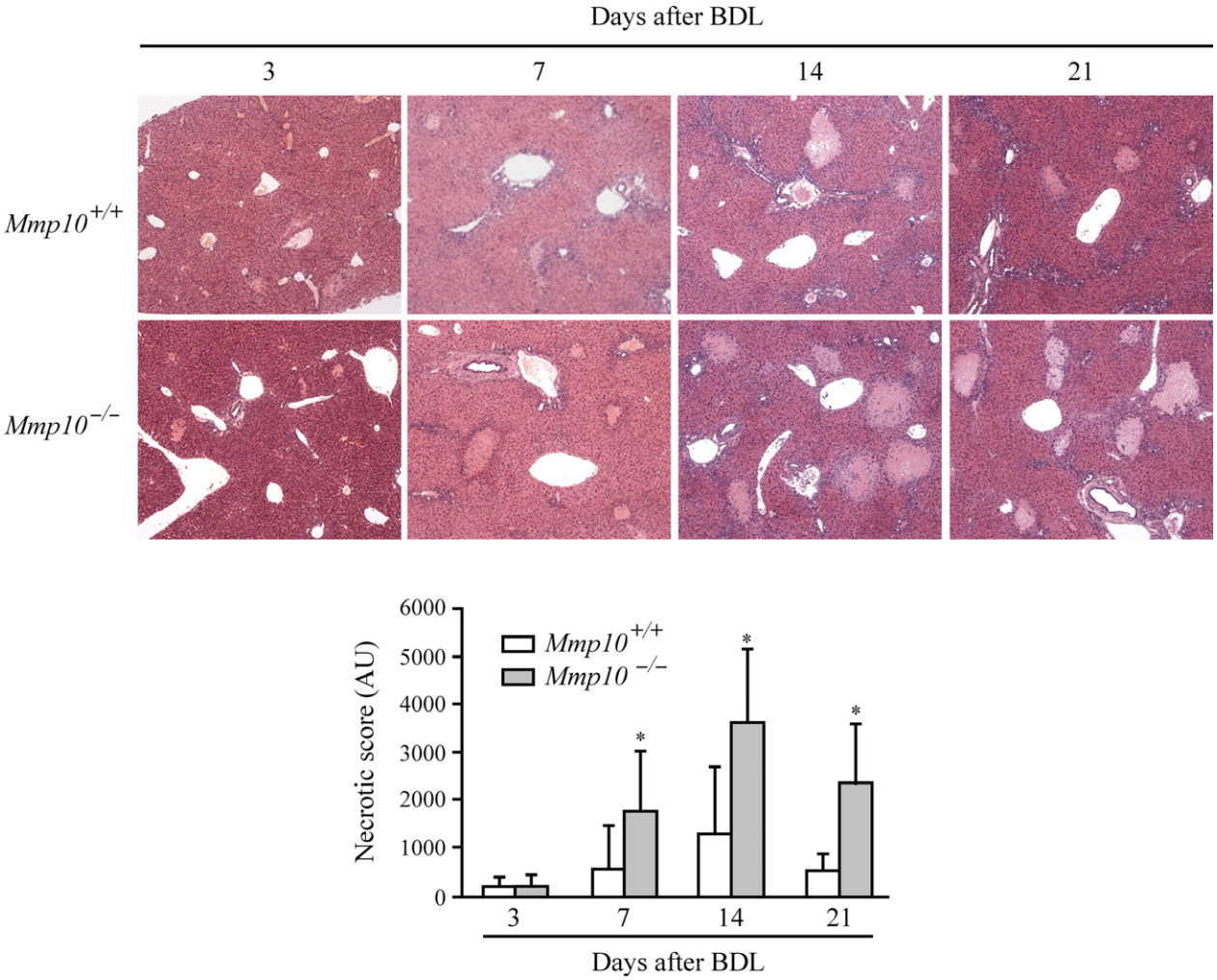

Fig. 7. Liver necrosis in Mmp10$^{+/+}$ and Mmp10$^{-/-}$ mice after bile duct ligation (BDL). Representative images of liver tissue sections (H&E staining) from *Mmp10*$^{+/+}$ and *Mmp10*$^{-/-}$mice at different time points after BDL. Graph shows the quantitative analysis of necrotic lesions. Asterisks indicate $P < 0.05$ vs wild-type mice. AU, arbitrary units.

fibrosis (43) were increased in MMP10-deficient animals (Fig. 9A and B).

Potential mechanisms underlying MMP10 contribution to liver tissue repair

After showing that MMP10 expression is necessary for liver tissue regeneration and repair, one important question would be the identification of the mechanisms relevant to this protective function. MMP10 has many potential target substrates, although few of them have been well characterized *in vivo*. In this context, the recently described profibrinolytic activity of MMP-10 could be of functional significance (24), given the relevance of an adequate turnover of the fibrinogen-rich provisional ECM formed during hepatic injury and wound healing (7–9, 25, 56, 57). Therefore, we determined the intrahepatic levels of fibrinogen in the insoluble protein fraction obtained from liver tissues at different time points after PH and BDL (35). In agreement with previous reports (9, 57), we detected in both experimental models a significant increase in the ca. 100 kDa band that corresponds to insoluble fibrinogen-γ dimers that are formed upon liver injury (Fig. 10). Interestingly, *Mmp10*$^{-/-}$ mice displayed enhanced accumulation of insoluble fibrinogen-γ dimers in the liver when compared with wild-type mice both after PH and upon BDL (Fig. 10). Another important component of the provisional ECM formed during liver repair is fibronectin (9, 58). In addition to the 250-kDa fibronectin monomer, higher molecular weight cross-linked complexes can be found in the insoluble protein fraction of liver tissue during injury and repair (9). Accordingly, we found increased levels of 250-kDa fibronectin and higher molecular weight complexes in the insoluble liver protein fraction of mouse livers at different times after

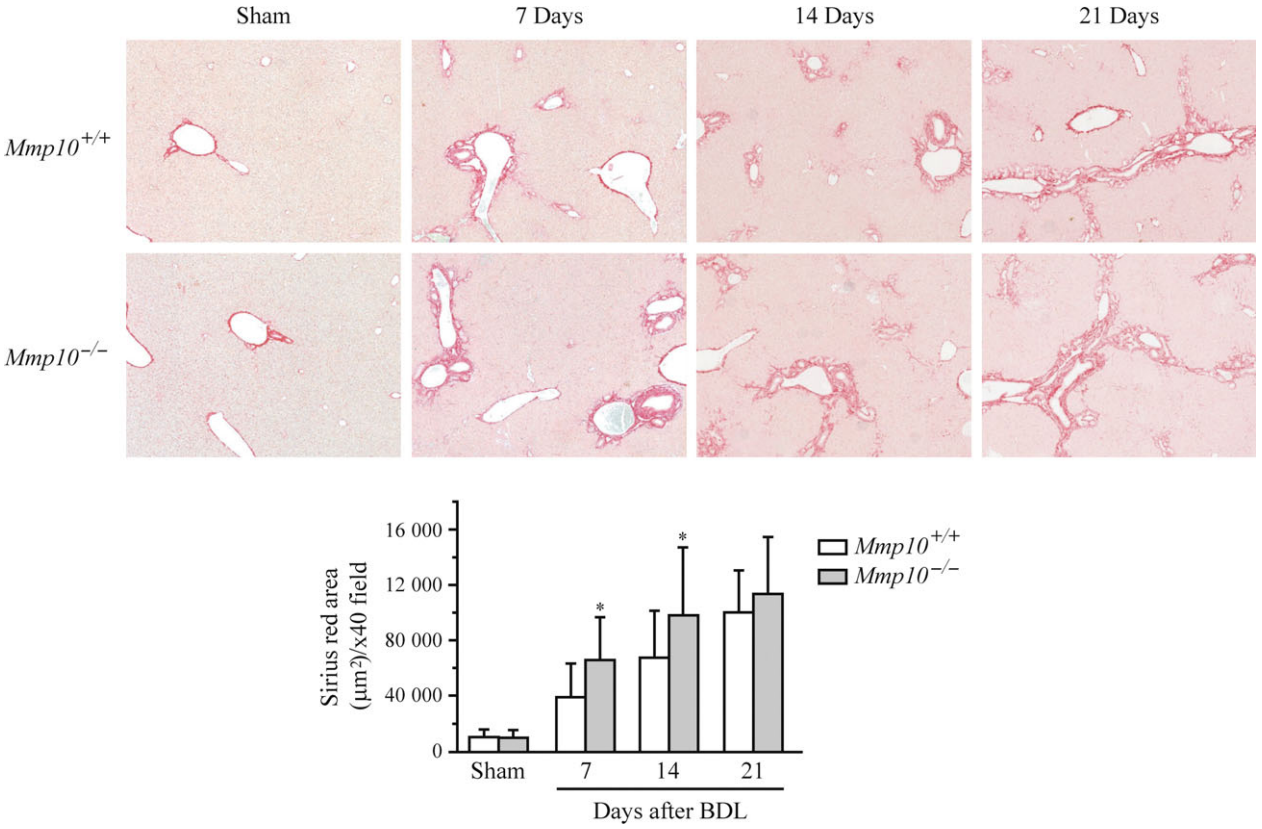

Fig. 8. Liver fibrosis after bile duct ligation (BDL) in Mmp10$^{+/+}$ and Mmp10$^{-/-}$ mice. Representative Sirius-Red stained liver tissue sections from wild-type and MMP10-deficient mice taken at the indicated days after BDL. Graph shows the quantitative analysis of stained area. Asterisks indicate $P < 0.05$ vs wild-type mice.

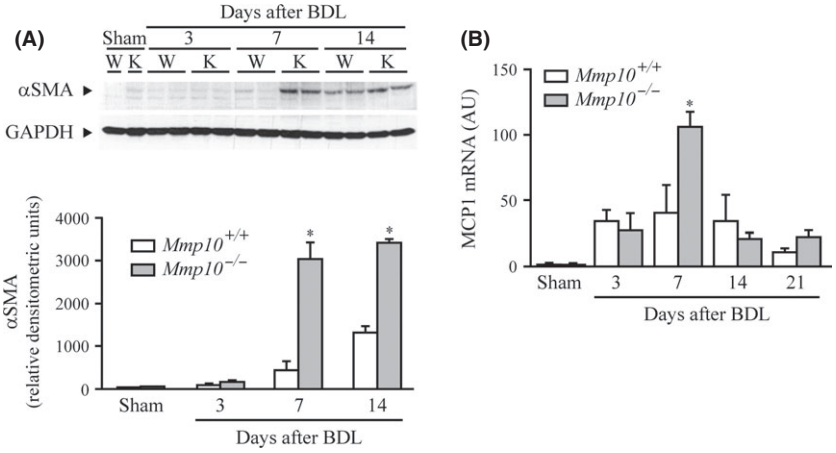

Fig. 9. Hepatic expression of α-smooth muscle actin (αSMA) and monocyte chemoattractant protein 1 (MCP-1) in Mmp10$^{+/+}$ and Mmp10$^{-/-}$ mice after bile duct ligation (BDL). (A) Western blot analysis of αSMA protein in liver extracts from *Mmp10*$^{+/+}$ (W) and *Mmp10*$^{-/-}$ (K) mice obtained at the indicated days after BDL. Representative blots are shown. Graph shows the quantification of αSMA bands ($n = 4$ samples per time point). Asterisks indicate $P < 0.05$ vs wild-type mice. Tissue samples from sham-operated mice were obtained 7 days after the intervention. (B) MCP-1 mRNA levels were measured by qPCR in liver tissue samples taken at the indicated days after BDL. Asterisk indicates $P < 0.05$ vs wild-type mice. AU, arbitrary units.

BDL (Fig. 11A). The abundance of these high molecular weight complexes was increased in extracts from *Mmp10*$^{-/-}$ mice (Fig. 11A). To assess if the expression of MMP10 could influence the turnover of fibronectin and its large complexes, we examined the levels of fibronectin proteolytic fragments previously identified *in vivo*

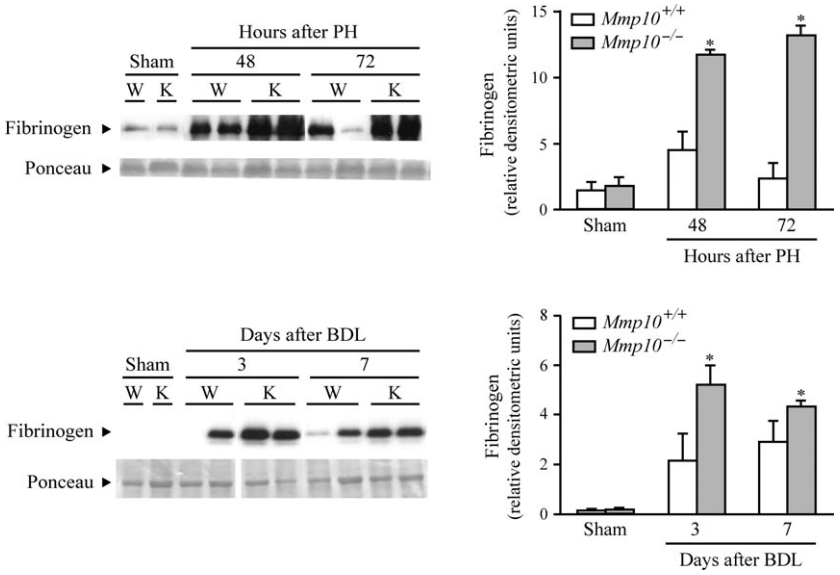

Fig. 10. Fibrinogen levels in the insoluble protein fraction of liver tissues from Mmp10$^{+/+}$ and Mmp10$^{-/-}$ mice after partial hepatectomy (PH) and bile duct ligation (BDL). Fibrinogen (fibrinogen-γ dimer) was detected by western blot in extracts from the insoluble protein fraction of liver tissues. Liver samples were obtained at the indicated time points after PH (upper panel) or BDL (lower panel) from wild-type mice (W) or MMP10-deficient mice (K). Control samples (sham) were obtained at 48 h for PH and 3 days for BDL after sham operations. Representative blots are shown (n = 4 mice per time point). Ponceau staining of a representative band in the blots is shown as loading control. Graphs show the quantification of fibrinogen band. Asterisks indicate $P < 0.05$ vs values found in wild-type (W) mice.

and in cultured cells (59, 60). As shown in Fig. 11B, compared with control animals, we clearly detected augmented levels of fibronectin fragments (around 50 kDa) in extracts of soluble proteins from livers of *Mmp10*$^{+/+}$ mice after BDL. These fragments were much less abundant when tissue extracts from MMP10-deficient mice were examined (Fig. 11B).

Discussion

In this study, we identified a previously unrecognized role for MMP10 during the hepatic wound healing. We found that MMP10 expression is readily induced upon liver injury in different cell types, including hepatocytes, biliary epithelium and inflammatory cells. This response is consistent with previous observations on MMP10 expression in inflamed or injured in other tissues and cells, such as keratinocytes in skin wounds, tracheal epithelial cells upon *Pseudomonas* infection, vascular endothelial cells and colonic epithelial and inflammatory cells in inflammatory bowel diseases (20, 21, 26, 61, 62). In the two experimental models of hepatic damage and repair tested, we observed a transient pattern of MMP10 expression, which onset overlapped with the early inflammatory response occurring during liver injury and regeneration (1–3, 43, 52). MMP10 expression is known to be promoted by growth factors such as EGF and TGFβ1 in different epithelial cell types and by inflammatory mediators like C-reactive protein in vascular endothelial cells or bacterial LPS in macrophages (41, 42, 46, 47, 61). The expression of EGFR ligands, and TGFβ1, is induced early during liver damage and regeneration as part of the endogenous reparative response to liver injury, in close interaction with inflammatory signals (1–3, 27, 34, 44, 63). We also show that the EGFR ligands AR, HB-EGF and Ereg were markedly up-regulated in mouse liver upon BDL. These findings, together with our *in vitro* observations on the additive effects of TGFβ1 and EGFR ligands on MMP10 expression in parenchymal and biliary cells, point to the combined activity of these growth factors as important determinants of MMP10 induction in liver injury.

MMP10 was also detected in inflammatory cells in the liver. Gut-derived LPS plays a significant role in liver regeneration after PH, as well as in experimental cholestatic liver disease, being monocytes/macrophages primary cell targets for the endotoxin (45, 52). We show here that LPS can induce MMP10 expression in liver macrophages. Moreover, we provide evidence demonstrating that the EDA domain of fibronectin, an endogenous TLR4 ligand that is produced during liver injury (49, 50), also triggered MMP10 expression in mouse macrophages. These findings suggest that *Mmp10* gene transcription also responds to molecular changes in the ECM composition of the liver and parallels the transcriptional activation of *MMP10* in response to type I collagen recently observed in human fibroblasts (64).

Upon experimental liver resection and BDL, lack of MMP10 resulted in persistent parenchymal injury, which, in the case of PH, was associated with significant

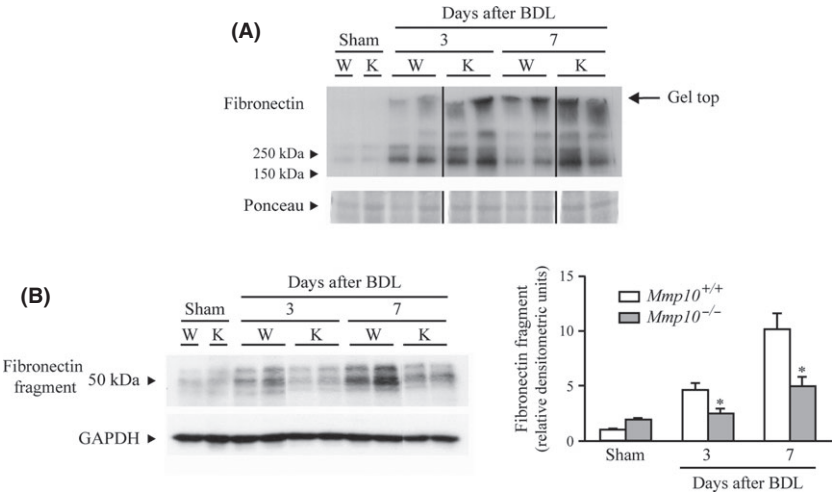

Fig. 11. Analysis of high molecular weight fibronectin complexes and fibronectin fragments in liver extracts from Mmp10$^{+/+}$ and Mmp10$^{-/-}$ mice after bile duct ligation (BDL). (A) Representative western blot analysis of high molecular weight complexes (above 250 kDa) and full length fibronectin (250 kDa) protein levels in extracts from the insoluble protein fraction of wild-type (W) and MMP10-deficient (K) mouse liver tissues at the indicated days after BDL. Ponceau staining of a representative band in the blots is shown as loading control. (B) Representative western blot analysis of low molecular weight fibronectin fragments in soluble extracts of liver tissues from wild-type (W) and MMP10-deficient (K) mouse liver tissues at the indicated days after BDL. Graph shows the quantification of the 50 kDa fibronectin fragment ($n = 4$ mice per time point). Asterisks indicate $P < 0.05$ vs values found in wild-type (W) mice.

mortality and in cholestatic damage was accompanied by increased fibrosis. Therefore, our findings indicate that MMP10 would participate in liver injury resolution rather than in disease progression, at least in the experimental models tested. This hepatoprotective activity is in agreement with previous observations in lung and colonic injury and inflammation models, in which *Mmp10*$^{-/-}$ mice displayed increased damage and an impaired wound healing response (21, 26). The restoration of liver tissue architecture involves cell proliferation as well as the removal of necrotic debris and the transient matrix involved in tissue repair. We did not find significant differences between normal and MMP10-deficient mice in terms of hepatocellular proliferation. These findings suggest that MMP10 would not be essential for the mobilization of growth factors, as shown for other proteases (6). However, liver injury was increased and clearance of necrotic areas was markedly impaired in the absence of MMP10. This phenotype is reminiscent of that found in mice deficient in plasminogen or plasminogen activators (7, 11, 25), yet contrary to responses of plasminogen activator inhibitor-1 knockout mice, which are protected from cholestatic liver injury (65).

The critical activities of these proteases relevant to their beneficial effects in liver wound healing are not completely known, but may range from the correct turnover of wound-associated matrix components and debris (7, 11, 25) to the activation of hepatoprotective growth factors (6, 12). Particular attention has been recently focused on the role of the coagulation cascade in liver injury (56, 57). For instance, fibrin (ogen)-deficient mice, as well as mice with reduced tissue factor expression, show less liver damage in models of cholestasis (35, 66). Intrahepatic deposition of fibrin complexes with reduced solubility has been recently reported in different relevant mouse models of acute liver injury (57). It has been proposed that excess deposition of insoluble fibrin, and fibronectin-containing non-fibrin matrix, leads to a vicious cycle of impaired liver perfusion, hepatocellular injury and activation of fibrogenic cells (11, 57). In line with these observations, it was found that pharmacological interference with coagulation confers liver protection and attenuates the onset of fibrosis (57, 67). Our current experiments show that in the absence of MMP10, the dynamics of fibrinogen and fibronectin turnover during liver injury are dysregulated, with enhanced intrahepatic accumulation of insoluble aggregates of these proteins. These findings are consistent with the recently reported profibrinolytic role of MMP10 (24). It is tempting to propose that the influence of MMP10 on clot resolution may be mechanistically relevant to its role in the liver wound healing response, although MMP10-mediated release of pro-survival factors from the hepatic extracellular matrix cannot be completely ruled out.

Finally, it is important to highlight that we could detect the presence of MMP10 protein in the nuclei of hepatocytes and biliary cells of cholestatic mice. Although putative nuclear localization signals can be found in MMP10 sequence (68), to our knowledge, nuclear localization of this MMP has not been previously reported. Interestingly, the highly related MMP3 (stromelysin-1) has been found in the nucleus of hepatocytes, hepatocarcinoma cells and chondrocytes, where it is thought to be involved in apoptosis and gene

expression regulation (68, 69). The nuclear localization of MMP10 in liver cells suggests the existence of additional biological roles for this MMP. These may be important for the beneficial effects of MMP10 in liver repair described in this study, and are worth of further investigations.

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Conflict of interest: The authors do not have any disclosures to report.

References

1. Taub R. Liver regeneration: from myth to mechanism. *Nat Rev Mol Cell Biol* 2004; **5**: 836–47.
2. Michalopoulos GK. Liver regeneration after partial hepatectomy: critical analysis of mechanistic dilemmas. *Am J Pathol* 2010; **176**: 2–13.
3. Böhm F, Köhler UA, Speicher T, Werner S. Regulation of liver regeneration by growth factors and cytokines. *EMBO Mol Med* 2010; **2**: 294–305.
4. Uriarte I, Fernandez-Barrena MG, Monte MJ, *et al.* Identification of fibroblast growth factor 15 as a novel mediator of liver regeneration and its application in the prevention of post-resection liver failure in mice. *Gut* 2013; **62**: 899–910.
5. Kim TH, Mars WM, Stolz DB, Petersen BE, Michalopoulos GK. Extracellular matrix remodelling at the early stages of liver regeneration in the rat. *Hepatology* 1997; **26**: 896–904.
6. Mohammed FF, Khokha R. Thinking outside the cell: proteases regulate hepatocyte division. *Trends Cell Biol* 2005; **15**: 555–63.
7. Bezerra JA, Bugge TH, Melin-Aldana H, *et al.* Plasminogen deficiency leads to impaired remodelling after a toxic injury to the liver. *Proc Natl Acad Sci USA* 1999; **96**: 15143–8.
8. Neubauer K, Knittel T, Armbrust T, Ramadori G. Accumulation and cellular localization of fibrinogen/fibrin during short-term and long-term rat liver injury. *Gastroenterology* 1995; **108**: 1124–36.
9. Tsujimoto I, Moriya K, Sakai K, Dickneite G, Sakai T. Critical role of factor XIII in the initial stages of carbon tetrachloride-induced adult liver remodelling. *Am J Pathol* 2011; **179**: 3011–9.
10. Roselli HT, Su M, Washington K, *et al.* Liver regeneration is transiently impaired in urokinase-deficient mice. *Am J Physiol Gastrointest Liver Physiol* 1998; **275**: G1472–9.
11. Ng VL, Sabla G, Melin-Aldana H, *et al.* Plasminogen deficiency results in poor clearance of non-fibrin matrix and persistent activation of hepatic stellate cells after an acute injury. *J Hepatol* 2001; **35**: 781–9.
12. Shanmukhappa K, Matte U, Degen JL, Bezerra JA. Plasmin-mediated proteolysis is required for hepatocyte growth factor activation during liver repair. *J Biol Chem* 2009; **284**: 12917–23.
13. Kim TH, Mars WM, Stolz DB, Michalopoulos GK. Expression and activation of pro-MMP-2 and pro-MMP-9 during rat liver regeneration. *Hepatology* 2000; **30**: 75–82.
14. Knittel T, Mehde M, Grundmann A, *et al.* Expression of matrix metalloproteinases and their inhibitors during hepatic tissue repair in the rat. *Histochem Cell Biol* 2000; **113**: 443–53.
15. Hemmann S, Graf J, Roderfeld M, Roeb E. Expression of MMPs and TIMPs in liver fibrosis – a systematic review with special emphasis on anti-fibrotic strategies. *J Hepatol* 2007; **46**: 955–75.
16. Uchinami H, Seki E, Brenner DA, D'Armiento J. Loss of MMP 13 attenuates murine hepatic injury and fibrosis during cholestasis. *Hepatology* 2006; **44**: 420–9.
17. Fallowfield JA, Mizuno M, Kendall TJ, *et al.* Scar-associated macrophages are a major source of hepatic matrix metalloproteinase-13 and facilitate the resolution of murine fibrosis. *J Immunol* 2007; **178**: 5288–95.
18. Olle EW, Ren X, McClintock SD, *et al.* Matrix metalloproteinase-9 is an important factor in hepatic regeneration after partial hepatectomy in mice. *Hepatology* 2006; **44**: 540–9.
19. Yan C, Zhou L, Han YP. Contribution of hepatic stellate cell and matrix metalloproteinase 9 in acute liver failure. *Liver Int* 2008; **28**: 959–71.
20. Krampert M, Bloch W, Sasaki T, *et al.* Activities of the matrix metalloproteinase stromelysin-2 (MMP-10) in matrix degradation and keratinocyte organization in wounded skin. *Mol Biol Cell* 2004; **15**: 5242–54.
21. Koller FL, Dozier EA, Nam KT, *et al.* Lack of MMP10 exacerbates experimental colitis and promotes development of inflammation-associated colonic dysplasia. *Lab Invest* 2012; **92**: 1749–59.
22. Rodriguez JA, Orbe J, Martinez de Lizarrondo s, *et al.* Metalloproteinases and atherothrombosis: MMP-10 mediates vascular remodeling promoted by inflammatory stimuli. *Front Biosci* 2008; **13**: 2916–21.
23. Nakamura H, Fujii Y, Ohuchi E, Yamamoto E, Okada Y. Activation of the precursor of human stromelysin 2 and its interactions with other matrix metalloproteinases. *Eur J Biochem* 1998; **253**: 67–75.
24. Orbe J, Barrenetxe J, Rodriguez JA, *et al.* Matrix metalloproteinase-10 effectively reduces infarct size in experimental stroke by enhancing fibrinolysis via a thrombin-activatable fibrinolysis inhibitor-mediated mechanism. *Circulation* 2011; **124**: 2909–19.

25. Bezerra JA, Currier AR, Melin-Aldana H, *et al.* Plasminogen activators direct reorganization of the liver lobule alter acute injury. *Am J Pathol* 2001; **158**: 921–9.
26. Kassim SY, Gharib SA, Mecham BH, *et al.* Individual matrix metalloproteinases control distinct transcriptional responses in airway epithelial cells infected with Pseudomonas aeruginosa. *Infect Immun* 2007; **75**: 564050.
27. Berasain C, García-Trevijano ER, Castillo J, *et al.* Amphiregulin: an early trigger of liver regeneration. *Gastroenterology* 2005; **128**: 424–32.
28. Mitchell C, Mahrouf-Yorgov M, Mayeuf A, *et al.* Overexpression of Bcl-2 in hepatocytes protects against injury but does not attenuate fibrosis in a mouse model of chronic cholestatic liver disease. *Lab Invest* 2011; **91**: 273–82.
29. Perugorría MJ, Latasa MU, Nicou A, *et al.* The epidermal growth factor receptor ligand amphiregulin participates in the development of mouse liver fibrosis. *Hepatology* 2008; **48**: 1251–61.
30. Rudich N, Zamir G, Pappo O, *et al.* Focal liver necrosis appears early after partial hepatectomy and is dependent on T cells and antigen delivery from the gut. *Liver Int* 2009; **29**: 1273–84.
31. Fernández-Barrena MG, Monte MJ, Latasa MU, *et al.* Lack of Abcc3 expression impairs bile-acid induced liver growth and delays hepatic regeneration after partial hepatectomy in mice. *J Hepatol* 2012; **56**: 367–73.
32. Latasa MU, Gil-Puig C, Fernández-Barrena MG, *et al.* Oral methylthioadenosine administration attenuates fibrosis and chronic liver disease progression in Mdr2-/- mice. *PLoS ONE* 2010; **5**: e15690.
33. Lasarte JJ, Casares N, Gorraiz M, *et al.* The extra domain A from fibronectin targets antigens to TLR4-expressing cells and induces cytotoxic T cell responses *in vivo*. *J Immunol* 2007; **178**: 748–56.
34. Berasain C, García-Trevijano ER, Castillo J, *et al.* Novel role for amphiregulin in protection from liver injury. *J Biol Chem* 2005; **280**: 19012–20.
35. Luyendyk JP, Cantor GH, Kirchofer D, *et al.* Tissue factor-dependent coagulation contributes to α-naphthylisothiocyanate-induced cholestatic liver injury in mice. *Am J Physiol Gastrointest Liver Physiol* 2009; **296**: G840–9.
36. Rabilloud T, Adessi C, Giraudel A, Lunardi J. Improvement of the solubilization of proteins in two-dimensional electrophoresis with immobilized pH gradients. *Electrophoresis* 1997; **18**: 307–16.
37. Berasain C, Hevia H, Fernández-Irigoyen J, *et al.* Methylthioadenosine phosphorylase gene expression is impaired in human liver cirrhosis and hepatocarcinoma. *Biochim Biophys Acta* 2004; **1690**: 276–84.
38. Gujral JS, Farhood A, Bajt ML, Jaeschke H. Neutrophils aggravate acute liver injury during obstructive cholestasis. *Hepatology* 2003; **38**: 355–63.
39. Canbay A, Guicciardi ME, Higuchi H, *et al.* Cathepsin B inactivation attenuates hepatic injury and fibrosis during cholestasis. *J Clin Invest* 2003; **112**: 152–9.
40. Österreicher CH, Trauner M. Animal models of biliary tract injury. *Curr Opin Gastroenterol* 2012; **28**: 239–43.
41. Wilkins-Port C, Ye Q, Mazurkiewicz JE, Higgins PJ. TGF-β1 + EGF-initiated invasive potential in transformed human keratinocytes is coupled to a plasmin/MMP-10/MMP-1-dependent collagen remodelling axis: role for PAI-1. *Cancer Res* 2009; **69**: 4081–91.
42. Ishikawa F, Miyoshi H, Nose K, Shibanuma M. Transcriptional induction of MMP-10 by TGF-β, mediated by activation of MEF2A and downregulation of class IIa HDACs. *Oncogene* 2009; **29**: 909–19.
43. Seki E, De Minicis S, Inokuchi S, *et al.* CCR2 promotes hepatic fibrosis in mice. *Hepatology* 2009; **56**: 185–97.
44. Berasasin C, Castillo J, Prieto J, Avila MA. New molecular targets for hepatocellular carcinoma: the ErbB1 signaling system. *Liver Int* 2007; **27**: 174–85.
45. Isayama F, Hines IN, Kremer M, *et al.* LPS signalling enhances hepatic fibrogenesis caused by experimental cholestasis in mice. *Am J Physiol Gastrointest Liver Physiol* 2006; **290**: G1318–28.
46. Huang WC, Sala-Newby GB, Susana A, Johnson JI, Newby AC. Classical macrophage activation up-regulates several matrix metalloproteinases through mitogen activated protein kinases and nuclear factor-kappa B. *PLoS One* 2012; **7**: e42507.
47. Murray MY, Birkland TP, Howe JD, *et al.* Macrophage migration and invasion is regulated by MMP10 expression. *PLoS One* 2013; **8**: e63555.
48. Kubes P, Mehal WZ. Sterile inflammation in the liver. *Gastroenterology* 2012; **143**: 1158–72.
49. Chang ML, Chen JC, Alonso CR, Kornblihtt AR, Bissell DM. Regulation of fibronectin splicing in sinusoidal endothelial cells from normal or injured liver. *Proc Natl Acad Sci USA* 2004; **101**: 18093–8.
50. Okamura Y, Watari M, Jerud ES, *et al.* The extra domain A of fibronectin activates toll-like receptor 4. *J Biol Chem* 2001; **276**: 10229–33.
51. Treiber M, Neuhöfer P, Anetsberger E, *et al.* Myeloid, but not pancreatic, RelA/p65 is requiered for fibrosis in a mouse model of chronic pancreatitis. *Gastroenterology* 2011; **141**: 1473–85.
52. Strey CW, Markiewski M, Mastellos D, *et al.* The proinflammatory mediators C3a and C5a are essential for liver regeneration. *J Exp Med* 2003; **198**: 913–23.
53. Mitchell C, Willenbring H. A reproducible and well-tolerated method for 2/3 partial hepatectomy in mice. *Nat Protocols* 2008; **3**: 1167–70.
54. Fickert P, Trauner M, Fuchsbichler A, *et al.* Oncosis represents the main type of cell death in mouse models of cholestasis. *J Hepatol* 2005; **42**: 378–89.
55. Wagner M, Fickert P, Zollner G, *et al.* Role of farnesoid X receptor in determining hepatic ABC transporter expression and liver injury in bile duct-ligated mice. *Gastroenterology* 2003; **125**: 825–38.
56. Beier JI, Luyendyk JP, Guo L, *et al.* Fibrin accumulation plays a critical role in the sensitization to lipopolysaccharide-induced liver injury caused by ethanol in mice. *Hepatology* 2009; **49**: 1545–53.
57. Weeerasinghe SVW, Moons D, Altshuler PJ, Shah YM, Omary MB. Fibrinogen-γ proteolysis and solubility dynamics during apoptotic mouse liver injury: heparin prevents and treats liver damage. *Hepatology* 2011; **53**: 1323–32.
58. Moriya K, Sakai K, Yan MH, Sakai T. Fibronectin is essential for survival but is dispensable for proliferation of hepatocytes in acute liver injury in mice. *Hepatology* 2012; **56**: 311–21.
59. Vierkotten S, Muether PS, Fauser S. Overexpression of HTRA1 leads to ultrastructural changes in the elastic layer

of Bruch's membrane via cleavage of extracellular matrix components. *PLoS One* 2011; **6**: e22959.
60. Horowitz JC, Rogers DS, Simon RS, Sisson TH, Thannickal VJ. Plasminogen activation-induced pericellular fibronectin proteolysis promotes fibroblast apoptosis. *Am J Respir Cell Mol Biol* 2008; **38**: 78–87.
61. Montero I, Orbe J, Varo N, *et al.* C-Reactive protein induces matrix metalloproteinase-1 and -10 in human endothelial cells. *J Am Coll Cardiol* 2006; **47**: 1369–78.
62. Gill SE, Parks WC. Metalloproteinases and their inhibitors: regulators of wound healing. *Int J Biochem Cell Biol* 2008; **40**: 1334–47.
63. Berasain C, Perugorria MJ, Latasa MU, *et al.* The epidermal growth factor receptor: a link between inflammation and liver cancer. *Exp Biol Med* 2009; **234**: 713–25.
64. Ruiz PA, Jarai G. Collagen I induces discoidin domain receptor (DDR) 1 expression through DDR2 and JAK2-ERK1/2-mediated mechanism in primary human lung fibroblasts. *J Biol Chem* 2011; **286**: 12912–23.
65. Bergheim I, Guo L, Davis MA, Duveau I, Arteel GE. Critical role of plasminogen activator inhibitor-1 in cholestatic liver injury and fibrosis. *J Pharmacol Exp Ther* 2006; **316**: 592–600.
66. Luyendyk JP, Mackman N, Sullivan BP. Role of fibrinogen and protease-activated receptors in acute xenobiotic-induced cholestatic liver injury. *Toxicol Sci* 2011; **119**: 233–43.
67. Anstee QM, Goldin RD, Wright M, *et al.* Coagulation status modulates murine hepatic fibrogenesis: implications for the development of novel therapies. *J Thromb Haemost* 2008; **6**: 1336–43.
68. Si-Tayeb K, Monvoisin A, Mazzocco C, *et al.* Matrix metalloproteinase 3 is present in the cell nucleus and is involved in apoptosis. *Am J Pathol* 2006; **169**: 1390–401.
69. Eguchi T, Kubota S, Kawata K, *et al.* Novel transcription factor-like function of human matrix metalloproteinase 3 regulating the *CTGF/CCN2* gene. *Mol Cell Biol* 2008; **28**: 2391–413.

Supporting information

Additional Supporting Information may be found in the online version of this article:

Fig. S1. Circulating transaminases levels in wild type and $Mmp10^{-/-}$ mice after partial hepatectomy.

Fig. S2. Circulating transaminases levels in wild type and $Mmp10^{-/-}$ mice after bile duct ligation.

Table S1. Primers used for qPCR.