

# Metalloproteinases in liver fibrosis: current insights

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**Abstract:** Liver fibrosis is defined by excessive deposition of extracellular matrix. The fibrotic conditions result from the imbalance between synthesis and deposition of fibrous tissues and decomposition of these matrix proteins. This process can be reversed as a regular part of the healing process after hepatic damage or can become chronic. When the protein matrix synthesis predominates and the decomposition is suppressed, fibrosis will progress into irreversible cirrhosis or steatosis. Among the molecular players involved in fibrotic liver diseases, metalloproteinases of matrix metalloproteinase (MMP) and a disintegrin and metalloproteinase (ADAM) families are critical in the development of liver fibrosis and its resolution. Previously, MMPs were recognized as extracellular matrix degrading enzymes. Currently, they are also known as mediators in a variety of processes related to immunity and tissue repair. In this article, we have reviewed the models of liver fibrosis and findings on MMPs and ADAMs in hepatic fibrosis conditions.

**Keywords:** matrix metalloproteinase, ADAM, liver, fibrosis

## Introduction

Studies on metalloproteinases have mainly focused on their role in the extracellular matrix decomposition and cell migration. More recently, it has come to light that the function of metalloproteinases is not limited to the degradation of extracellular matrix.<sup>1,2</sup> This is exemplified by the family of a disintegrin and metalloproteinase (ADAM), which executes very specific proteolytic activities known as shedding.<sup>1,3</sup>

Metalloproteinases are involved in many distinct processes in the liver, the central organ for metabolism of carbohydrates, proteins, and lipids; removal of different pharmaceutical compounds and toxins from the blood; and for regulation of immune responses. The liver consists of different types of cells, mainly hepatocytes, endothelial cells, hepatic stellate cells (HSCs), bile duct cells (cholangiocytes), and Kupffer cells (liver specific resident macrophages). Hepatocytes are responsible for the majority of metabolic functions in the liver and are the most numerous cells of the liver, building a front line of detoxification and regeneration.<sup>4</sup>

The liver can develop a range of pathological conditions such as viral hepatitis, classic fibrosis, alcoholic and nonalcoholic fatty liver disease (NAFLD), steatosis, cirrhosis, biliary obstruction, and others. However, the liver also has a vast regenerative capacity as seen following a hepatectomy, toxic or ischemic injury, or acute infection.<sup>5</sup> All the mentioned diseases involve multiple cellular and molecular players,<sup>6</sup> including metalloproteinases, especially matrix metalloproteinase (MMP) and ADAM families. MMPs and ADAMs

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are facilitating extracellular matrix remodeling, cell migration and adhesion, as well as immune responses and regeneration.<sup>4,7,8</sup>

In all fibrotic models as well as human diseases, the induction of metalloproteinases expression and/or activity is documented. This is accompanied by elevated expression of their inhibitors, the tissue inhibitor of metalloproteinases (TIMPs), especially TIMP-1 and TIMP-2.<sup>9,10</sup> Expression of MMPs in the liver is not restricted to a certain cell type; they are produced by Kupffer cells, all immune cells,<sup>11,12</sup> HSCs, cholangiocytes,<sup>13</sup> as well as hepatocytes.<sup>14</sup> MMPs act as a part of a proteolytic network, in which proteases activate each other by processing inactive proforms. For instance, it has been shown that proMMP-2 is activated by MT-MMP-1 (MMP-14) in complex with TIMP-2,<sup>15</sup> and further MMP-2 and MT-MMP-1 are activated by proMMP-13.<sup>16</sup> In general, MMPs are important for initiation and establishment of the liver fibrosis and they are involved in the activation and invasiveness of HSCs, the producers of fibrotic matrix.<sup>17,18</sup> Overall, the MMP and ADAM families of metalloproteinases orchestrate not only the processes of fibrosis development and its resolution but also the inflammatory and regeneration of the liver.

## Liver models

### Animal models of liver fibrosis

The liver has a complex architecture with many specialized cell types and niches. Moreover, the liver is extremely well interconnected with the whole body, maintaining body's homeostasis and controlling central immune responses. The complex of many functionally different cell types and their different reactions to body conditions are the main reasons why it is impossible to accurately mimic liver function and cell-cell interaction in vitro and why animal models are needed to study liver pathophysiology.

Fibrosis in animal models can be induced by administration of hepatotoxins, bile duct ligation (BDL), and using genetic models. To date, several reviews describing animal

models for fibrosis induction have been published.<sup>4,8,19–21</sup> In this review, we recapitulate their findings regarding metalloproteinases of MMP and ADAM families on the background of function of various liver cell types.

### Models for centrilobular hepatocyte damage

These models primarily target hepatocytes in the area around the central vein where the first hepatocytes with the highest concentration of hydroxylating cytochrome P450 involved in detoxification are located.<sup>22</sup> The models include chemically induced fibrosis with toxic compounds, mostly carbon tetrachloride (CCl<sub>4</sub>), thioacetamide (TAA), dialkylated nitrosamines, and others (Table 1). These chemicals are usually applied intraperitoneally and their major advantage is that after the acute phase caused by the single-dose application, a full recovery of liver tissue can be achieved. Repeated applications of these substances result in pericentral fibrosis, later bridging pericentral zones, which further develop into irreversible cirrhosis and carcinoma.<sup>4,19</sup>

The next group of models is based on special dietary conditions, under which the damaging compounds target the liver via blood stream. These models are mainly mimicking the chronic liver damage, which is more reminiscent of liver injury in humans. Modified mouse diet is mainly used to induce nonalcoholic steatohepatitis (NASH) and NAFLD.<sup>23,24</sup> In general, NASH and NAFLD are called silent diseases with few or no symptoms during their progression but resulting in irreversible liver damage after years in humans. Since NASH and NAFLD manifest mostly in adults and the only cure is liver transplantation, extensive efforts have been made to reveal the mechanisms involved in the development of these disorders and their treatment.

NAFLD is a multiorgan disease, which also affects the cardiovascular system, kidney, and pancreas. Accumulation of excess fat in the liver cells, which initiates NAFLD, is the

**Table 1** Hepatic toxins used in animal models

Inducer	Toxic metabolite	Mechanism	References
CCl <sub>4</sub>	Carbon trichloride radical	Lipid peroxidation and membrane damage	Weber et al <sup>11</sup>
TAA	Thioacetamide-S-oxide radical	Carbonyl moiety of TAA is bound to protein, protein damage	Porter and Neal <sup>112</sup>
Nitrosamines: DEN DMN	Diazonium ions	DNA alkylation	Kroeger-Koepke et al <sup>113</sup>
APAP	N-acetyl-p-benzoquinone imine	Oxidative conjugation with proteins and DNA	Jaeschke et al <sup>114</sup>
HFD	–	Accumulation of fat in liver	Lieber et al <sup>25</sup>
MCD diet	–	Low beta-oxidation, oxidative stress and inflammation	Anstee and Goldin <sup>115</sup>

**Abbreviations:** CCl<sub>4</sub>, carbon tetrachloride; TAA, thioacetamide; DEN, diethylnitrosamine; DMN, dimethylnitrosamine; APAP, acetaminophen; HFD, high-fat diet; MCD diet, a diet deprived of methionine and choline.

first clinical sign. Thus, high-fat diet is used to induce fatty liver progression in rodents,<sup>25</sup> which then exhibits obesity, high insulin levels, and insulin resistance.<sup>23</sup> Upon this diet, steatosis develops not only in pericentral zones but also in periportal zones, which are specialized in gluconeogenesis.<sup>26</sup>

In the NASH studies, a diet deprived of methionine and choline (MCD diet), which are needed for beta-oxidation, is used. Rodents fed with MCD develop pericentral fibrosis with necrosis. As NASH is characterized by liver damage involving inflammation paralleled with oxidative stress, this model also displays metabolic syndrome-like features.<sup>24,27</sup> MCD-induced NASH is considered to be a more severe NAFLD model, which develops irreversible damage of the liver in a short time.

Liver injury induced by *Schistosoma* sp. is another model that could be used to study periportal fibrosis. Parasite eggs are deposited in an area with high blood flow – small portal venules.<sup>28</sup> Deposition of parasite eggs triggers accumulation of collagen that initially blocks the release of antigen from parasites and later causes periportal fibrosis, liver cirrhosis, and organ failure.<sup>29</sup> *Schistosoma mansoni* infection of mice mimics the pathological observations in humans.<sup>30</sup>

## Models to target cholangiocytes

Cholangiocytes are epithelial cells of bile ducts that bring the bile, a toxic product of liver, into the gall bladder. BDL is a well-known model of biliary fibrosis where the bile outflow from the liver is interrupted and the bile accumulates in the bile ducts.<sup>13</sup> The backpressure and toxicity of bile acids are the major causes of proliferation of the duct cells and their spreading into liver parenchyma. Subsequently, Kupffer cells migrate to the bile ducts causing portal inflammation and necrosis, thus leading to fibrosis. This model is irreversible in mice; however, it is possible to reconnect the bile duct in rat models.<sup>31</sup> Due to wound healing after surgery, this model is usually performed in studies focused on chronic liver injury. Probably, the most important modification of the BDL model is partial BDL (pBDL).<sup>32</sup> Decreased necrosis and proliferation rates have been reported in pBDL model, which makes pBDL more relevant to human bile duct obstruction than classical BDL. In addition, pBDL is used to simulate acute cholestasis, whereas BDL is considered to be the model for chronic stage. Another nonsurgical model where obstruction of bile ducts and proliferation of cholangiocytes occur is based on dietary model using 3,5-diethoxycarbonyl-1,4-dihydrocollidine-enriched diet.<sup>33</sup> In this model, porphyrin crystals block small intrahepatic bile ducts and large bile ducts remain usually unaffected.

## Targeting Kupffer cells

Kupffer cells are liver-specific macrophages responsible for the release of many cytokines during liver injury, especially tumor necrosis factor alpha (TNF $\alpha$ ). Experimental treatment of animals with antibodies specific to TNF $\alpha$  reduced induced liver injury.<sup>34</sup> Similarly, dexamethasone, coupled to mannosylated albumin (Dexa(5)-Man(10)-HSA), specifically delivered to Kupffer cells, reduced inflammation and liver damage.<sup>35</sup> On the contrary, nonspecific activation of all immune cells including Kupffer cells is a hallmark of autoimmune human diseases. To stimulate immune cells in the liver, concanavalin A can be applied intraperitoneally.<sup>36</sup> High levels of cytokines generated during the immune response cause acute hepatocyte damage. Such an acute immune cell activation may serve as a model of autoimmune hepatitis<sup>37</sup> and for studying the role of immune cells in liver injury and regeneration.<sup>38</sup>

## Unspecific overall liver damage

During viral hepatitis, no primary cell target can be determined in the liver. Human and chimpanzee are the only two species susceptible to human hepatitis B virus (HBV) infection; murine hepatocytes cannot be infected by human hepatitis as mice lack receptors for human viral particles.<sup>39</sup> The first model of HBV is transgenic mouse, in which the surface antigen (HBsAg) and HBV e antigen were found in the serum.<sup>40</sup> Another model is based on injection of high amounts of plasmid bearing the viral genome into the blood stream.<sup>41</sup> Similar to toxic models, where the liver is the first affected organ, high replication rate is preserved up to 80 days in immunodeficient mice. Infection with adenovirus containing HBV genome is a model consisting of both adaptive and innate systems.<sup>42</sup> An alternative to this model is human liver chimeric mouse model,<sup>43</sup> in which the mouse hepatocytes are replaced by transplanted human hepatocytes; however, preparation of this chimeric mouse is challenging. Finally, humanized mice with human immune system and hepatocytes is the only model, which upon infection triggers responses similar to humans, i.e., hepatitis and fibrosis.<sup>44-47</sup>

## Genetically modified mice

Genetically modified animals are becoming a more frequent tool to study liver pathophysiology as well as the roles of metalloproteinases. The liver consists of several cell types and it is not easy to target them specifically. To study specific functions in hepatocytes, the albumin promoter is often used in combination with Cre recombinase, although it partially affects cholangiocytes.<sup>48</sup> The transgenic

mouse with the Cre-driven lecithin-retinol acyltransferase promoter targets 99% of HSCs. This recent model of cell-specific transgene has been used to demonstrate that HSCs give rise to 82%–96% of myofibroblasts in models of toxic, cholestatic, and fatty liver disease.<sup>49</sup> The Mx1-Cre model<sup>50</sup> expresses Cre recombinase under the control of an inducible Mx1 promoter, which is silent in healthy mice and active in the liver and in lymphocytes after stimulation with interferon alpha or polyinosinic:polycytidylic acid. Thus, mice with gene deficiencies in both hepatocytes and immune cells can be studied. To target myeloid cell lineage (monocytes, mature macrophages, and granulocytes) including the Kupffer cells, a model with Cre recombinase activity under the control of *Lyz2* promoter/enhancer elements is often used.<sup>51</sup> However, since *Lyz2* promoter-based recombinase is not specific only for Kupffer cells but also affects floxed alleles in all myeloid cells, targeting the immune compartment cannot be completely restricted just to the liver or to Kupffer cells only.

Regarding the ablation of gene functions in the liver, only a few genetically modified mice develop spontaneous liver injury. Interestingly, metalloproteinase-deficient mice do not display any obvious liver damage; ubiquitous deletion is either lethal or mostly with minor or no effects.<sup>6</sup> The reason for that is likely the redundancy of metalloproteinases and relatively large substrate promiscuity. Genes whose deletion leads to spontaneous liver phenotype are listed in Table 2.

## Metalloproteinases in the liver

### Metalloproteinases in the development of liver fibrosis

All fibrotic models are accompanied by elevated expression of MMP-2 and MMP-9 (Table 3). These two MMPs are called gelatinases as they cleave collagen-IV besides other basal

**Table 2** List of genes involved in spontaneous liver damage

Gene	Disease/pathology	Reference
<i>NOD.c3c4</i>	Primary biliary cirrhosis	Koarada et al, <sup>116</sup> Mauad et al <sup>117</sup>
<i>Mrd2</i>	Cholestasis	Mauad et al, <sup>117</sup> Oude Elferink et al, <sup>118</sup> Smit et al, <sup>119</sup> Fickert et al <sup>120</sup>
<i>IL-2R<math>\alpha</math></i>	Primary biliary cirrhosis	Wakabayashi et al <sup>121</sup>
<i>dnTGF-<math>\beta</math>RII</i>	Primary biliary cirrhosis	Oertelt et al <sup>122</sup>
<i>Ae2a,b</i>	Primary biliary cirrhosis	Salas et al <sup>123</sup>
<i>KK Ay/a</i>	Steatosis/NAFLD	Okumura et al <sup>124</sup>
<i>AlbCre Adam 10</i>	Cholestasis/Fibrosis	Müller et al <sup>78</sup>
<i>PTEN</i>	Steatosis/NASH	Horie et al <sup>125</sup>

**Abbreviations:** NAFLD, nonalcoholic fatty liver disease; NASH, nonalcoholic steatohepatitis.

**Table 3** MMPs and TIMPs involved in mouse fibrosis

Treatment	Metalloproteinases and TIMP involved	Reference
Carbon tetrachloride	MMP-2, MMP-8, MMP-9, MMP-13, MMP-19, TIMP-1, TIMP-2	Jirouskova et al, <sup>65</sup> Chung et al, <sup>126</sup> Jiang et al, <sup>127</sup> Marsillach et al <sup>128</sup>
Thioacetamide	MMP-2, MMP-12, TIMP-1, TIMP-2	Park et al, <sup>129</sup> Chen et al <sup>130</sup>
Nitrosamines	MMP-2, MMP-9, MMP-13, TIMP-1, TIMP-2	Chen et al, <sup>130</sup> Subramanian and Arul, <sup>131</sup> Prakobwong et al, <sup>132</sup> Mandal et al <sup>133</sup>
Acetaminophen	MMP-2, MMP-9	Ito et al <sup>134</sup>
High-fat diet	MMP-9, MMP-12, MMP-13, TIMP-1, ADAM17	Kato et al, <sup>135</sup> Stanton et al, <sup>136</sup> de Meijer et al, <sup>137</sup> Wang et al <sup>138</sup>
MCD diet	MMP-2, MMP-9, MMP-13	Lee et al, <sup>139</sup> Wang et al, <sup>140</sup> Velayudham et al <sup>141</sup>
BDL	MMP-2, MMP-8, MMP-9, MMP-13, ADAM17, TIMP-1	Uchinami et al, <sup>142</sup> Buryova et al, <sup>143</sup> Popov et al, <sup>144</sup> Schradung et al, <sup>145</sup> Diaz-Gil et al, <sup>146</sup> Kossakowska et al <sup>147</sup>
DDC diet	MMP-2, MMP-9, ADAM17, ADAM10	Chalupsky et al, <sup>106</sup> Ishikawa et al <sup>148</sup>
Concanavalin A	MMP-2, MMP-9, ADAM17	Arthur, <sup>7</sup> Wang et al, <sup>37</sup> Maeda et al, <sup>149</sup> Okazaki et al <sup>150</sup>

**Abbreviations:** MMP, matrix metalloproteinase; TIMP, tissue inhibitor of metalloproteinase; ADAM, a disintegrin and metalloproteinase; BDL, bile duct ligation; DDC, 5-diethoxycarbonyl-L-4-dihydrocollidine; MCD diet, a diet deprived of methionine and choline.

membrane proteins. MMP-2 has been reported to be important not only in establishment of CCL<sub>4</sub>-induced fibrosis<sup>52</sup> but also in the resolution phase when fibrotic matrix is degraded and then replaced by the natural matrix components.<sup>53,54</sup> Thus, MMP-2-deficient mice show enhanced fibrosis after CCL<sub>4</sub> treatment and MMP-2 could be therefore critical for inhibiting type I collagen synthesis by activated HSCs, which strongly express this MMP.<sup>55,56</sup> MMP-9 and MMP-2 are elevated in serum and livers of patients with chronic hepatitis type B and C.<sup>57–59</sup> Elevated expression of MMPs in the mentioned fibrotic conditions was shown both in liver parenchyma<sup>60</sup> and portal macrophages.<sup>57,61</sup> Especially, MMP-9 seems to be involved in ongoing liver inflammation,<sup>62</sup> and its level drops after antiviral treatment.<sup>57,63</sup> Induction of MMP-2 is significantly stimulated in the liver of HCV-infected patients.<sup>64</sup>

Although it appears that MMP-9 and MMP-2 induction in fibrosis is the most prominent among MMPs, there are also other MMPs that are able to cleave collagen-IV and other basement membrane components. MMP-19 deficiency causes fibrosis exacerbation in chronic fibrosis models induced by CCL<sub>4</sub> application.<sup>65</sup> MMP-12 is also involved in the regulation of fibrotic matrix processing as the oral



administration of TAA leads not only to accumulation of elastin and increased fibrosis in MMP-12-deficient mice, but also to higher overall fibrosis.<sup>66</sup> Interestingly, these mice did not exhibit difference in overall fibrosis compared to control animals if treated by CCL<sub>4</sub>, although increased perisinusoidal elastin was still detected. This points to a distinct mode of action of toxins used to induce fibrosis. The role of MMP-8 in fibrosis development is still not clearly understood, but it could facilitate HSC migration and invasion, suggesting a potential profibrotic role.<sup>67</sup>

Apart from being matrix cleavage proteases, MMPs also participate directly in certain signaling cascades. Both MMP-9 and MMP-2, with their ability to activate transforming growth factor beta (TGF $\beta$ ), participate in the induction of this main profibrogenic pathway<sup>2</sup> as TGF $\beta$  is crucial for the differentiation of quiescent HSC into collagen-I producing myofibroblast. It also appears that the activation of HSC in three-dimensional culture is dependent on gelatinolytic activity.<sup>68</sup> MMP-19 seems to be involved in TGF $\beta$  signaling as well, as MMP-19 knockout hepatocytes exhibit lower response to the cytokine.<sup>65</sup> Also, MT1-MMP participates in the regulation of fibrosis progression as it cleaves intramolecular RGD motifs of collagen produced by activated HSCs and thus controls  $\alpha$ V $\beta$ 1 activation, providing a survival signal via PI3K/AKT/I $\kappa$ B.<sup>69</sup>

During the entire progress of liver fibrosis, the expression of main inhibitor of MMPs, TIMP-1, is increased to regulate the elevated MMP activity. TIMP-1 has distinct functions in establishment and resolution of fibrosis. This inhibitor may be beneficial at the beginning of the fibrotic tissue formation; yet by inhibiting MMPs during the recovery phase, it may slow down decomposition of collagen-I. Results from animal models are intriguing as both transgenic mice, the overexpressing TIMP-1 mice<sup>70</sup> and TIMP-1 knockout mice, developed more prominent fibrosis after CCL<sub>4</sub> intoxication.<sup>9</sup> Those contradictory observations might be explained by specific engagement of TIMP-1 in different signaling pathways. TIMP-1 is able to inhibit HSC apoptosis,<sup>71</sup> suggesting profibrotic function of TIMP-1. Yet, silencing of TIMP-1 reduces HSC proliferation<sup>72</sup> and, moreover, TIMP-1 inhibits apoptosis of hepatocytes.<sup>9</sup> Probably, loss of this hepatoprotective function in TIMP-1 knockout results in exacerbated fibrosis. Still, expression of TIMP-1 and TIMP-2 is elevated in HCV patients,<sup>57</sup> and some studies examined blockage of TIMP-1 as potential therapeutic treatment for patients with cirrhosis. In one case, TIMP-1 expression was reduced by adenoviral delivery of siRNA.<sup>73</sup> Another approach was inhibiting TIMP-1 activity by MMP-9 proteolytic inactive

mutants.<sup>74</sup> In both studies, the treated animals developed less severe fibrosis, which indicates that inhibition of TIMP-1 can be beneficial in liver fibrosis. By contrast, complete ablation leads to opposite result. TIMP-1 appears to be a key player in liver fibrosis, and an important potential therapeutic target as TIMP-1 might promote liver fibrosis by yet another means than its previously described antiapoptotic effect on HSC.<sup>72</sup>

ADAM10 plays an important role in development and organization of epithelial tissues and is the main alpha-secretase cleaving Notch<sup>75,76</sup> The whole-body ADAM10-deficient mice die prenatally due to limited Notch signaling that results in vasculature failure and numerous other developmental problems.<sup>77</sup> However, specific ablation of ADAM10 in hepatocytes did not exhibit alteration in Notch signal,<sup>78</sup> although some percentage of those animals have problems with liver homeostasis manifested by necrosis of hepatocytes and subsequent fibrosis.<sup>78</sup>

## MMPs in recovery of liver fibrosis

During recovery from fibrosis, removal of the scar tissue formed by collagen-I is needed. In rodents, collagen-I is cleaved predominantly by MMP-13<sup>79</sup> and MMP-8.<sup>80</sup> The expression of MMP-13 peaks during resolution phase.<sup>81</sup> Other MMPs such as MMP-2 and MMP-14 are also upregulated in this phase,<sup>82</sup> by likely participating in restoration of the healthy tissue. MMP-13 is mainly expressed by macrophages that infiltrate the fibrotic lesions.<sup>81</sup> MMP-13-deficient mice suffer from hampered recovery of CCl<sub>4</sub>-induced fibrosis.<sup>81</sup> This finding is in line with adenovirus-mediated MMP-13 expression, which help to resolve the established fibrosis.<sup>83</sup> Apart from direct collagenolytic activity, overexpression of MMP-13 includes healing promoting signals as hepatocyte growth factor (HGF) expression is elevated and activity of MMP-2 and MMP-9 is increased. Similarly, application of adenoviruses carrying MMP-8<sup>84</sup> or MMP-1, the main human collagenase,<sup>85</sup> had beneficial effect in resolution of induced fibrosis.

Another part of recovery from fibrosis is elimination of activated HSCs by apoptosis, and some MMPs are suggested to participate in this process according to several *in vitro* studies.<sup>86,87</sup> In agreement with the mentioned role of TIMP-1 in HSC apoptosis,<sup>71</sup> MMP-2 can promote HSC apoptosis through cleavage of N-cadherin,<sup>86</sup> typical marker of activated myofibroblasts. Recently, the role of degradation products produced by MMPs in regulation of fibrosis has been proposed; cleaved fibronectin peptides induced apoptosis in HSC line and stimulated MMP-9 secretion from human monocytes.<sup>87</sup>

## Inflammation and regulation of TNF $\alpha$ signaling in liver injury

ADAM17 was originally discovered as a protease effectively cleaving TNF $\alpha$  and is therefore also known as TNF $\alpha$ -converting enzymes. TNF $\alpha$  is pivotal in many pathological conditions of the liver and has a distinct effect on hepatocytes depending on the strength and duration of a signal. Experimental approaches against TNF $\alpha$  in models of liver fibrosis have shown reduction of liver injury.<sup>34,35,88</sup> TNF $\alpha$  in liver, mainly produced by Kupffer cells,<sup>89</sup> binds to TNF $\alpha$  receptors (TNFR) on hepatocytes and Kupffer cells after its release. ADAM17 is a key player in this signaling, and mice lacking ADAM17 specifically in myeloid lineage release almost no TNF $\alpha$  into serum after lipopolysaccharide application.<sup>90</sup> Interestingly, ADAM17 ablation in hepatocytes also resulted in reduced shedding of TNF $\alpha$  into serum, suggesting that both cell types cooperate during release of TNF $\alpha$  in the liver. ADAM17 also mediates shedding of TNFR and thus affects activation of the TNF $\alpha$  downstream targets. Subsequently, only coordinated shedding of both, TNF $\alpha$  and TNFR, dictates outcome of the signaling.<sup>91</sup>

In liver diseases, TNF $\alpha$  plays both protective and damaging roles. High levels of TNF $\alpha$  produced in liver after CCl<sub>4</sub> injection contribute to apoptosis of hepatocytes,<sup>92</sup> and neutralization with TNF $\alpha$  antibody showed to be beneficial in this type of injury. Interestingly, with too high doses of neutralizing antibody, beneficial effect disappeared resulting in even higher damage than without antibody.<sup>93</sup> This suggests that a small amount of TNF $\alpha$  has protective effect during liver intoxication. ADAM17 mice deficient in myeloid lineage shed lower amount of TNF $\alpha$  after CCl<sub>4</sub> application compared to WT, leading to lower release of hepatocyte damage markers into serum.<sup>91</sup> However, since this disruption of TNF $\alpha$  signaling could not prevent liver damage as apoptosis and necrosis of hepatocytes were not altered in ADAM17 null mice, CCl<sub>4</sub> causes more complex reaction, which is not controlled by TNF $\alpha$  signaling.

## Regulator of growth factor signaling in the liver

Epidermal growth factor receptor (EGFR) pathway represents an important signaling pathway in the liver as it protects hepatocytes from apoptosis,<sup>94</sup> and it is necessary for hepatocyte division.<sup>95</sup> Epidermal growth factor (EGF), amphiregulin, heparin-binding EGF (HB-EGF), TGF $\alpha$ , epiregulin, and betacellulin are all ligands of EGFR and their bioavailability is provided by ADAM proteases.<sup>96</sup> Individual ligands have different sources; EGF is mainly produced in duodenum,

HB-EGF is produced by Kupffer cells, and amphiregulin and TGF $\alpha$  are made by hepatocytes and HSCs, but generally all have similar effects in liver pathology. They promote proliferation of hepatocytes after liver challenge as reported in many rodent models.<sup>97–102</sup>

EGFR is expressed in both the parenchymal and non-parenchymal liver cells, and several studies have reported that amphiregulin enhances liver fibrosis through its mitogenic effect on HSCs.<sup>102,103</sup> These effects, protecting hepatocytes as well as profibrotic HSCs, could account for the fact that ADAM17 hepatocyte-deficient mice do not develop exaggerated fibrosis after intoxication with CCl<sub>4</sub> (our observation, unpublished). Similarly, despite certain dysregulations, no major liver regeneration phenotypes were observed in mice with ADAM17 deficiency in either hepatocytes or Kupffer cells.<sup>91</sup>

Regulation of the EGFR signaling by ADAMs in liver is complex and is not fully understood so far. One issue is certain redundancy between EGFR ligands, some of which can be cleaved not only by ADAM17 but also by other ADAMs, e.g., ADAM10<sup>104</sup> and ADAM12.<sup>105</sup> Moreover, EGFR ligands are produced and shed by different liver cell types, not just hepatocytes. Thus, deficient models that would distinguish among hepatocytes, cholangiocytes, and HSCs would be useful.

Even though the EGFR signaling plays a significant role in liver homeostasis, it can be quite efficiently compensated by the pathway of HGF and its receptor MET, which is regulated via shedding by both ADAM10 and ADAM17.<sup>106</sup> This regulation does not occur through shedding of ligands as in EGFR but occurs via shedding of the receptor itself. By this action, HGF/MET signaling is disrupted as MET is no longer available on the targeted cells, although the soluble MET can serve as a decoy receptor.<sup>106</sup>

Recent studies have shown that combined disruption of both EGFR and MET signaling but not individual pathways, leads to dramatic effects compromising liver recovery from partial hepatectomy and aggravating necrosis after intoxication.<sup>105,107–109</sup> Even though MET and EGFR pathways both provide mitogenic signals to hepatocytes, they have distinct roles in differentiation of hepatic progenitor cells (HPCs), also called oval cells. Kitade et al<sup>110</sup> showed that MET induced HPC differentiation into hepatocytes, while EGFR signaling led to Notch1-dependent differentiation of cholangiocytes. Roles of ADAMs in this process remain to be established. First hints can be seen in study of ADAM10 knockout targeted to hepatocytes, cholangiocytes, and HPC.<sup>78</sup> These animals developed spontaneous fibrosis, which results

from hepatocyte necrosis caused by high bile acid content in the blood.

## Conclusion

Metalloproteinases are involved in many different processes, playing the central role in tissue damage and remodeling. However, they can function as a double-edged sword. Inhibiting their activity could prevent inflammation and accumulation of the extracellular matrix, but it could also hamper the healing process. Therefore, comprehensive knowledge of the individual functions of metalloproteinases is needed. Nevertheless, as the MMP and ADAM families of metalloproteinases work in the proteolytic network and exhibit number of functional redundancies and substrate promiscuity, it could be beneficial to study several metalloproteinases in parallel to decipher their functional complexity. Moreover, specific attention should be devoted to functional differences in various cell types. All this knowledge could be translated into more feasible therapeutic interventions.

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