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Special article

Expression of MMPs and TIMPs in liver fibrosis – a systematic review with special emphasis on anti-fibrotic strategies

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In liver tissue matrix metalloproteinases (MMPs) and their specific inhibitors (tissue inhibitors of metalloproteinases, TIMPs) play a pivotal role in both, fibrogenesis and fibrolysis. The current knowledge of the pathophysiology of liver fibrogenesis with special emphasis on MMPs and TIMPs is presented. A systematic literature search was conducted. All experimental models of liver fibrosis that evaluated a defined anti-fibrotic intervention in vivo or in vitro considering MMPs and TIMPs were selected. The methodological quality of all these publications has been critically appraised using an objective scoring system and the content has been summarized in a table.

***Keywords*: Liver; Fibrosis; MMP; TIMP; Matrix; Metalloproteinase; Tissue; Inhibitor; Therapy; Expression**

1. Background

The purpose of this review was threefold: first, to present the current knowledge of the pathophysiology of hepatic fibrogenesis and fibrolysis with special emphasis on matrix metalloproteinases (MMP) and their specific inhibitors (tissue inhibitors of metalloproteinases, TIMP); second, to provide a concise table aggregating all experimental approaches targeted towards inhibition of hepatic fibrogenesis or fibrolysis, respectively; and third, to critically evaluate the methodological quality of the reported experimental approaches using an objective scoring system.

2. Introduction

In healthy liver homeostasis of extracellular matrix (ECM) is sustained by a precisely regulated permanent turn-over directed by a group of enzymes called matrix metalloproteinases (MMPs) and their specific inhibitors, TIMPs (tissue inhibitors of metalloproteinases). Upon chronic damage of liver tissue, hepatic stellate cells (HSCs) become activated and differentiate into a fibroblast-like phenotype. In activated HSCs especially the expression of TIMP-1 is upregulated leading to the inhibition of MMP activity and subsequent accumulation of matrix proteins in the extracellular space. A substantial change in ECM composition is the deposition of collagens, mainly fibril-forming types I, III, and IV which increase in fibrotic ECM up to tenfold [1].

The family of MMPs consists of zinc-dependent proteolytic enzymes which comprise 22 different members so far [2]. Although all of them exhibit a broad substrate spectrum, they are divided based on their main substrate into collagenases, gelatinases, stromelysins, matrilysins, metalloelastase, membrane-type MMPs (MT-MMPs), and others. MMPs are secreted as zymogens and become activated by cleavage of their propeptide. Fig. 1 depicts an overview of the domain organization of different MMPs. MMPs have several structural features in common that include a propeptide domain containing the ''cysteine-switch'', the catalytic zinc-binding domain with the sequence HEXGHXXGXXHS, and a hemopexin-like domain. Once secreted, MMP-activity is regulated by the binding of TIMPs. Four TIMPs have been identified

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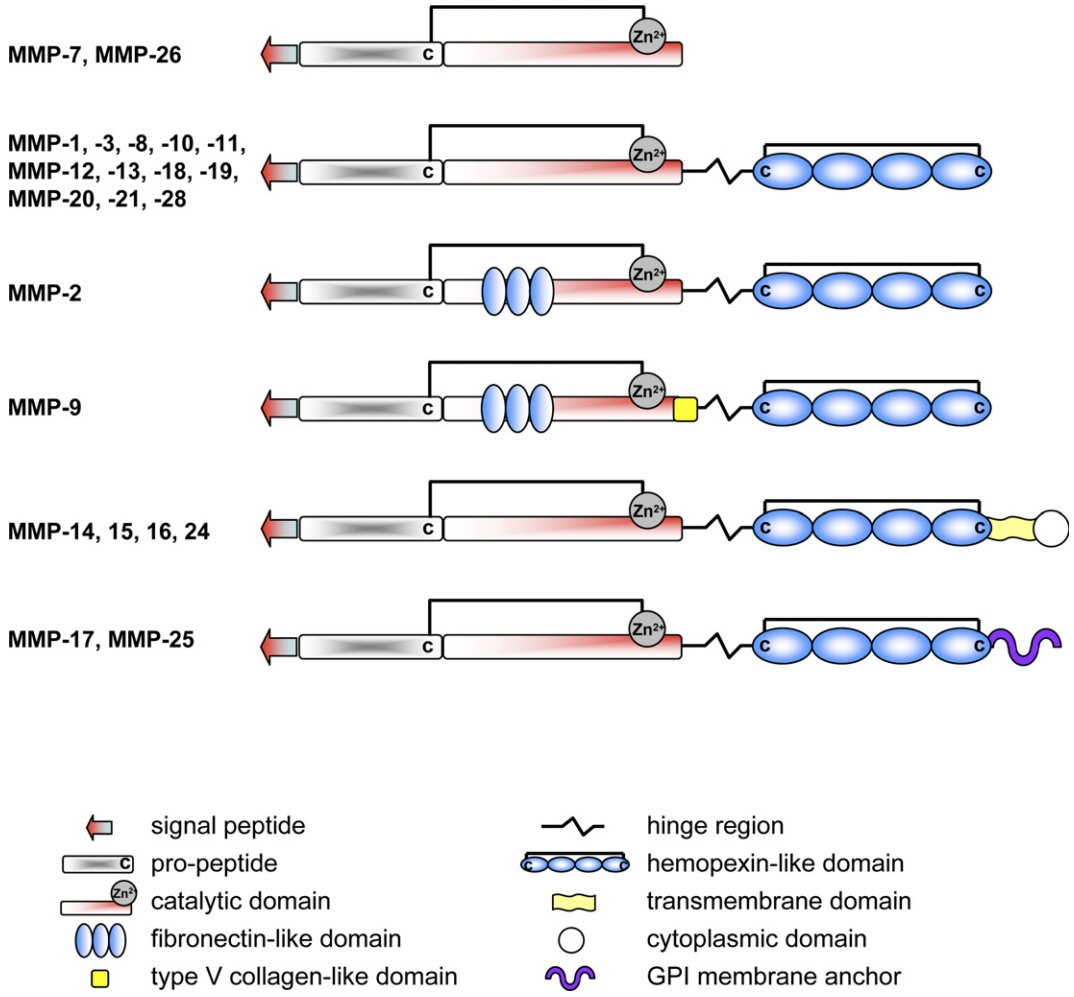

Fig. 1. Domain organization of different mammalian MMPs. GPI (glycosylphosphatidylinositol) membrane anchor.

so far: TIMP-1, TIMP-2, TIMP-3, and TIMP-4. All known MMPs can be inhibited by at least one of the four known TIMPs. Nevertheless, individual differences with regard to bond strength and thus the magnitude of inhibition of a particular MMP do exist. With respect to the phenomenon of fibrosis TIMP-1 plays a pivotal role [3]. Fig. 2 exemplifies the inhibitory mechanism of TIMP-1.

3. MMPs and TIMPs in fibrogenesis

From the known MMPs only a few are expressed in liver tissue and differences between genotype and amino-acid sequence among species have to be considered. In rodents no human MMP-1 (collagenase-1) homologue is known. Nevertheless, sequential and functional similarity exists for rat and mouse MMP-13. MMP-13 is expressed by HSC [4,5], fibroblasts, Kupffer cells, and perisinusoidal cells [6] and its synthesis can be upregulated by cytokines such as IL-1α, IL-1β, TNF-α or EGF [5,7–10]. MMP-1/MMP-13 activation usually occurs in a two-step mechanism via plasmin and MMP-3 [11] or as recently demonstrated, via MMP-14 and MMP-2 in absence of TIMP-2 [12]. Interestingly, TGF-β inhibits MMP-1 expression [13], whereas MMP-13, considered the human MMP-1 homologue in rodents, is not repressed by TGF-β [7,14–16].

Clinically applicable, non-invasive methods allowing a reliable assessment of the presence, magnitude and process of human liver fibrogenesis have not been established yet. Liver biopsy is currently the gold standard for diagnosis and subsequent treatment of liver fibrogenesis. However, liver biopsy is an invasive approach with particular risks and complications and thus studies with human liver tissue are limited. Due to therapeutic implications predominantly patients with hepatitis C virus (HCV)-infection have been investigated. In presence of severe fibrosis or cirrhosis, MMP-1 mRNA was elevated in liver tissue. Nevertheless, MMP-1 was not detectable in the serum of these patients [17]. MMP-1-, as well as MMP-3- and MMP-9-gene-polymorphisms may account for some variability in the progression of HCV-related chronic liver diseases [18]. In animal mod-

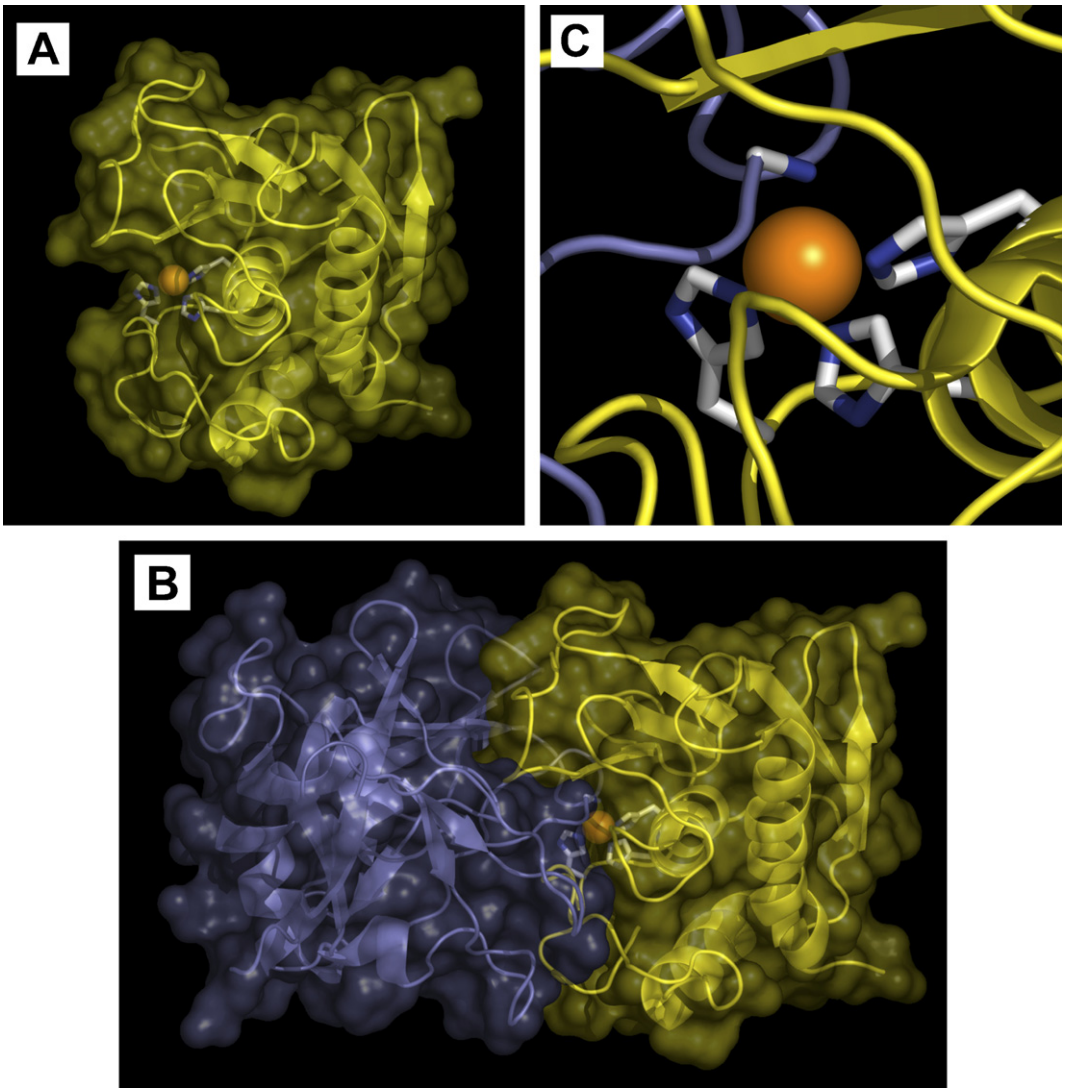

Fig. 2. Mechanism of MMP inhibition by TIMP-1. (A) Structure of MMP-3 (yellow) showing the binding cleft in which the catalytic zinc ion (orange circle) is bound to three His in the active site. One coordination site of the zinc ion is occupied by a water molecule (not shown). Thus, permitting substrate binding. (B) TIMP-1 (blue) bound to active MMP-3 demonstrating the perfect structural fit of the two proteins. (C) Zoom into the active centre of MMP-3. Substrate binding of MMP-3 is inhibited by TIMP-1. N-terminal Cys1 amino group occupies the 4th coordination site of the zinc ion in the active centre. Graphics were generated based on PDB Database Entry No. 1UEA using PyMOL Software (www.pymol.org) [187].

els of acute toxic liver injury MMP-13 mRNA expression peaks within the first hours after CCl_4-injection, and soon returns to control levels [1,19]. In chronic toxic liver injury, namely alcohol- or carbon tetrachloride (CCl_4)-induced rat liver fibrosis, MMP-13 mRNA expression increased during the development of fibrosis but dropped to normal values thereafter [6,20]. Similar results were obtained with cultured HSC, exhibiting a sharp rise in MMP-13 mRNA expression when grown on type I collagen gel in contrast to type I collagen monolayer [21]. Liver cells expressing MMP-13 were identified as quiescent or not fully activated HSC, demonstrating positive staining for desmin, but no staining for α-smooth muscle actin (α-SMA) [6]. From these observations it has been concluded that, preceding fibrogenesis, the early temporary expression of MMP-13 may destroy the surrounding tissue in order to deposit newly synthesized ECM. The degradation of ECM potentially leads to the release of ECM-bound cytokines such as TGF-β and may subsequently induce fibrogenesis. At the same time membrane-anchored MMP-14 activates MMP-13 followed by a disruption of HSC–ECM interactions finally generating an environment for HSC proliferation and migration [22].

MMP-2 (gelatinase A) and MMP-14 (membrane-type matrix metalloproteinase 1, MT1-MMP) are expressed by HSCs during their activation following liver injury [5,23]. The activation of MMP-2 is mediated by MMP-14 and occurs at the cell membrane through the formation of a ternary complex of pro-MMP-2, TIMP-2, and MMP-14 [24]. In HSCs cultured on type I collagen gel [21], and in human fibrotic liver tissue

[25] a transcriptional co-regulation of the involved proteins has been considered the responsible mechanism. In the presence of three-dimensional type I collagen the post-translational activation of MMP-2 increased [7,21,26,27], most likely mediated by the discoidin domain receptor 2 (DDR2) [28]. This could in turn be diminished by an integrin β1 blocking antibody, suggesting the involvement of integrin signaling in pro-MMP-2 activation [21]. In human cirrhotic liver diseases without HCV-infection augmented MMP-2 and MMP-14 expression has been shown in liver homogenates on protein level [29]. Northern blot analyses of liver tissue from HCV-patients with hepato-cellular carcinoma (HCC) demonstrated elevated MMP-2 and MMP-14 levels in advanced fibrosis, but not when cirrhosis was already present [25]. In HCV-patients without HCC competitive RT-PCR revealed a steady increase of MMP-2 and MMP-14 levels associated with the progression from mild fibrosis to cirrhosis [29]. Nevertheless, raised MMP-2 and MMP-14 mRNA has also been observed in liver tissue without any signs of fibrosis suggesting an HCV-driven gene stimulation. A discrimination of HCV-infected patients with or without liver fibrosis utilizing MMP-2 mRNA levels is not feasible [17,30]. Others reported elevated MMP-2 protein in serum only in patients with cirrhosis or at least advanced fibrosis [17,31].

In animal models MMP-2 mRNA and its pro-protein were hardly detectable in healthy liver tissue [32]. During experimental fibrogenesis induced by CCl_4 injections, MMP-2 mRNA expression increased and remained elevated [29,33,34]. About 10% of the total MMP-2 was catalytically active [29], suggesting that MMP-14 remains active during collagen accumulation. This may indicate the inability of TIMP-1 to suppress MMP-14 sufficiently [35]. Yet, high levels of TIMP-1 in fibrotic liver [4,36] were capable of inhibiting active MMP-2 on protein level. TIMP-2, which is able to inhibit the MMP-2 activation itself, was only modestly increased. Therefore in toxic-induced fibrosis enhanced expression of MMP-2 and MMP-14 together with a decline of TIMP-1 may facilitate the rapid resolution of experimental fibrosis [29]. In fibrosis induced via bile duct-ligation (BDL) MMP-2 activity did not differ between injury or repair as assessed by in situ zymography [37], whereas zymography of liver protein extracts revealed an elevated MMP-2 activity [38]. MMP-2 is an autocrine proliferation and migration factor for HSCs [23,39]. Stimulation with both, TGF-β and ROS (reactive oxygen species), often detected in chronic liver diseases, leads to increased MMP-2 expression in cultured human HSCs [40]. Proliferation and invasiveness of ROS-stimulated HSCs can be abrogated by specific MMP-2 inhibitors [41]. The functional relevance of MMP-2 and MMP-14 during fibrogenesis is comparable to that of MMP-1/MMP-13 with respect to local proteinase activity. Continuous MMP-2 overexpression in HSCs with its proliferative and migrative sequels may foster the progression of fibrosis.

MMP-3 (stromelysin-1) is expressed transiently in early stages of HSC activation [5] and has been shown to activate a number of pro-MMPs (e.g. MMP-1, -3, -7, -8, -9, and -13) by cleavage of the pro-peptide [11,42–46]. In the early phase of toxic liver injury in rats in situ hybridization of hepatocytes and non-parenchymal cells demonstrated MMP-3 mRNA expression [47]. This observation was confirmed by Northern blotting of rat liver lysates, with MMP-3 exhibiting approximately the same expression profile as rat MMP-13 [48]. In vitro, however, activated rat HSC exhibited a decreased protein expression of MMP-3 when cultured in 3D collagen, whereas MMP-13 expression was increased [7]. In pancreatic stellate cells TGF-β promotes a downregulation of MMP-3 expression [49]. Data on MMP-3 expression in chronic liver injury or advanced liver fibrosis are scarce.

Compared with healthy controls ELISAs of human serum samples from patients with diverse chronic liver diseases revealed a 50% reduction of serum MMP-3 levels [50]. In contrast, in patients with HCV hepatic MMP-3 mRNA expression was not associated with the magnitude of liver fibrosis [17]. Experimental data lack clarity as well: one group has observed an increased hepatic MMP-3 mRNA expression in mice after 4 weeks of CCl_4 treatment by RT-PCR and microarray analyses [51], whereas MMP-3 transcripts were not detected in Northern blots in bile duct-ligated livers of rats [38]. Moreover, diminished transcript levels of MMP-3 in CCl_4-fibrotic rat livers as assessed by RT-PCR have been reported [48].

Even though MMP-3 exhibits only weak enzymatic activity against matrix proteins, there may well be an indirect contribution to fibrogenesis arising from its pivotal role for the activation of other MMPs. Clearly, the ambiguous role of MMP-3 in fibrogenesis warrants further investigation.

MMP-9 (gelatinase B) is produced by Kupffer cells in primary culture [5], by IL-1β- and TNF-α-stimulated hepatocytes [52], IL-1α-stimulated HSCs [7], and in CCl_4-induced liver injury [53]. MMP-9 is activated by MMP-3, which in turn is activated by plasmin [54]. In liver disease, HSC [7], and inflammatory, mononuclear cells (e.g. lymphocytes and neutrophils) are a source of MMP-9, too [48]. Humans with chronic hepatitis C demonstrate elevated MMP-9 mRNA [17] and MMP-9 activity in liver tissue [55]. However, with respect to MMP-9 serum or plasma levels the results are inconclusive. Further, MMP-9 activity was not associated with the degree of fibrosis but linked to the histologically derived level of tissue inflammation. This is consistent with earlier work where MMP-9 was able to discriminate between the presence or absence of HCV infection,

but unable to detect the level of fibrosis [30]. Human data of MMP-9 expression in chronic liver diseases without HCV-infection are not available.

In animal models of acute and chronic liver injury inconsistent results for the expression of MMP-9 have been reported. In acute liver injury provoked by a single injection of CCl_4 latent and active MMP-9 increased over 3 days after the injection [48], whereas no change in MMP-9 mRNA expression occurred after 24 h [53]. In chronic liver injury induced by an 8-week CCl_4 injection protocol, neither a change in MMP-9 mRNA expression nor appearance of activated MMP-9 was observed [29]. Yet, increased amounts of the latent protein were found during a 7-, 9-, and 14-weeks lasting CCl_4 application protocol [32]. In bile duct-ligated rats increased pro-MMP-9 was detected beginning 2 days after surgery with a peak at day 10 and persistent elevation thereafter [38]. The ability of MMP-9 to activate latent TGF-β [56] may be of utmost importance in earlier stages of fibrogenesis, when collagen production of HSCs is stimulated by TGF-β [57]. Later, a negative feedback mechanism may lead to the subsequent downregulation of the MMP-9 expression via TGF-β-dependent pathways, as demonstrated in pancreatic stellate cells [49]. IL-1α-induced HSC activation was completely prevented by deprivation of MMP-9 [7], clearly identifying MMP-9 a principal performer in the initial phase of HSC activation.

To date four TIMPs are known with differing influence on fibrogenesis in liver tissue. Initial evidence regarding the relatedness of TIMP-1 and TIMP-2, and hepatic fibrosis relies on findings in rat hepatocytes: in this setting treatment with inflammatory cytokines stimulated the transcription of both genes [58–60]. Later, enhanced expression of TIMP-1 and TIMP-2 was shown in two rat models of liver injury, provoked via CCl_4 injection and bile duct ligation [61]. TIMP-1 and TIMP-2 are mainly produced by HSCs [62,63] and upregulated in various human liver diseases [64,65].

In humans suffering from HCV-induced chronic liver disease TIMP-1 protein serum values [31] and TIMP-1 mRNA levels are positively correlated with the grade of liver fibrosis [66]. However, HCV itself also stimulates TIMP-1 mRNA expression [17]. TIMP-1 production could be clearly attributed to activated HSCs [4]. Especially in early stages of liver injury Kupffer cells and hepatocytes may contribute to TIMP-1 production as well [4,38,61,67]. The course of expression was confirmed in animal models of chronic liver injury [4,38,67]. The importance of TIMP-1 for the development of liver fibrosis was highlighted in transgenic mice overexpressing human TIMP-1: CCl_4-treated animals exhibited severely increased symptoms compared to control mice. However, TIMP-1 expression itself did not induce fibrogenesis [68,69]. TIMP-1 is, among others, regulated by TGF-β [13,16,63]. Expression build-up becomes visible within 6–12 h after a single CCl_4-injection in rats returning to control levels after another 3–5 days, thus assigning TIMP-1 to the group of acute phase proteins [19,58].

TIMP-1 protein binds to and inhibits activated collagenases subsequently protecting newly synthesized collagen from immediate degradation by MMPs. But TIMP-1 is also capable of preventing the activation of pro-MMPs demonstrated in stimulated HSCs [7].

In contrast to TIMP-3 which promotes apoptosis [70], TIMP-1 is capable of inhibiting programmed cell-death of HSCs, mediated via inhibition of pro-MMP activation and MMP activity [68,71]. These effects are accompanied by a significantly reduced cleavage of *N*-cadherin in those cells [72]. The inhibitory mechanism of TIMP-1 on the molecular level is shown in Fig. 2. TIMP-1 is also significantly associated with fibrogenesis in the lung [73,74], kidney [75,76], and pancreas [77,78]. Thus, TIMP-1 is obviously a central molecule in tissue fibrosis.

In cultured liver cells TIMP-2 mRNA expression was not detected in native hepatocytes, but in rat liver myofibroblasts, activated HSCs, and Kupffer cells [5]. TIMP-2 has been analyzed only in a minority of studies. In humans data are predominantly based on HCV-positive patients. In patients with chronic HCV-infection an increase in serum TIMP-2 protein [79] and liver TIMP-2 mRNA was noted [17,30]. Without any sign of fibrosis TIMP-2 elevation was presumably inflammation- or virus-related. In animal models of acute toxic liver injury rat TIMP-2 mRNA peaked transiently 2–3 days after a single injection of CCl_4 [48] or LPS [60]. Consistently, in chronic toxic liver injury no significant increase was observed in rats during an 8-week CCl_4-challenge [29]. Yet, in BDL-induced fibrosis TIMP-2 mRNA was elevated starting at day 10, with no further increase until day 30 [38] what was in agreement with earlier data about human biliary atresia, primary biliary cirrhosis, and primary sclerosing cholangitis [64]. In chronic toxic liver injury the relevance of TIMP-2 for fibrogenesis seems restricted to early stages, when a transient TIMP-2 increase activates MMP-2 followed by a pericellular degradation of normal liver matrix. The need for TIMP-2 in order to activate MMP-2 activation has been demonstrated in studies with TIMP-2-null mice: these mice, phenotypically normal, viable and fertile, were unable to activate MMP-2 [80]. Thus, TIMP-2 may be dispensable for a normal development [80], except slight motor dysfunction especially in the early post-natal phase may be noticed [81]. However, for MMP-2 activation in mice TIMP-2 is essential [80].

4. MMPs and TIMPs in fibrolysis

A principal feature of hepatic fibrosis is the disbalance between MMPs and TIMPs. Since either protein family is responsible for both, fibrogenesis and fibrolysis, renders

them ideal targets for anti-fibrotic therapeutic interventions. Along these lines two strategies appear auspicious: upregulation of MMP activity or downregulation of TIMP activity.

Collagenases like MMP-1, -8, -13, and -14, possessing the ability to degrade fibrillar collagens, may well be responsible for key events in the degradation of ECM. Herein, the initial cleavage of collagen type I seems crucial for the overall regression of liver fibrosis because mice expressing a mutated, cleavage-resistant collagen type I were unable to resolve fibrosis [82,83].

Results of MMP-13 expression during fibrolysis were subject to diverse animal models and analysis methods: during the 28-day spontaneous recovery from CCl_4-induced liver fibrosis a constantly elevated expression was noted utilizing ribonuclease protection assays [36]. An expression restricted to certain time points in early stages of recovery after CCl_4-induced fibrosis or after a single CCl_4-injection has been shown via Southern and Northern blots, respectively [6,19]. Mesenchymal cells and in part HSCs were identified a relevant source of MMP-13. A double-transgenic mouse model with inducible, liver-specific expression of TGF-β was utilized for the induction of liver fibrosis. After switching off TGF-β expression a continuous fall of MMP-13 expression during fibrolysis has been observed [16,84]. Most likely Kupffer cells contributed to the prolonged MMP-13 synthesis. It is not yet clarified if collagenase activity of MMP-13 only initiates the process of fibrolysis or actively contributes to it over the whole period. In fibrosis therapy, adenovirus (Ad)-mediated overexpression of MMP-1 in rats with toxic liver injury resulted in fibrolysis and restoration of normal liver structure [85]. Although liver tissue analyses revealed convincing results regarding the efficacy of AdMMP-1 treatment, increasing serum ALT levels indicated hepatocyte cell damage. Unfortunately prolonged MMP-1 expression may increase the susceptibility to tumorigenesis [86]. Adenoviral application may represent a tool to demonstrate the therapeutic potential of an expressed protein only. The transient expression and the resulting requirement of repeated injections render it an artificial system with limited utility for therapeutic interventions in human beings.

Earlier work considered MMP-2 an important factor in fibrogenesis but not in fibrolysis [87]. Elevated expression of MMP-2 and MMP-14 during fibrogenesis permits an immediate metalloproteinase reactivity as soon as TIMP-1 levels declined [29]. In addition, HSC apoptosis induced pro-MMP-2 activation [88]. MMP-2 and MMP-14 exhibit gelatinolytic as well as interstitial collagenolytic properties [89–92], which emphasize their importance for fibrolysis. Accordingly skin fibroblasts from MMP-14 knock-out mice were unable to degrade a reconstituted type I collagen matrix [89]. Elevated MMP-2 mRNA expression in liver only slightly declined in a number of studies during the first week after termination of CCl_4-intoxication but remained significantly increased even after several weeks of spontaneous recovery [16,32,34,48,93]. Zymography of liver samples confirmed these results on protein level [29,32]. During recovery rapidly declining TIMP-1 expression [16,36] may release MMP-2 eventually leading to increased gelatinolytic activity in liver homogenates [29]. The source of MMP-2 mRNA during fibrolysis is still an unresolved issue. Assuming activated HSCs the main source of MMP-2 in fibrotic liver, it remains unclear how MMP-2 is synthesized when these cells undergo apoptosis. Using mirror image sections paired with in situ hybridization, predominantly HSCs and Kupffer cells expressed MMP-2. However, both cell types lacked their characteristic markers (desmin, α-SMA – ED2) [34].

MMP-8 is not considered an MMP of particular importance in fibrogenesis. In chronic liver injury either induced via CCl_4-injection for 8 weeks or by bile duct-ligation for 4 weeks AdMMP-8 was injected once via tail vein at the end of the protocol [94]. Liver biopsies were taken prior to the adenoviral injection, thus establishing a before–after control for each animal. Herein, a single AdMMP-8 injection caused amelioration of cirrhosis in both models. Nevertheless, CCl_4- and BDL-induced liver fibrosis is incommensurable: increased expression of MMP-2 has only been observed in the toxic liver injury model, whereas the BDL-model revealed an increase of TIMP-1.

In different mouse models, upregulation of MMP-9 during the first week of recovery from fibrosis has been demonstrated on mRNA level [84] and protein level by zymograms and immunohistochemistry [34,48]. MMP-9 itself exhibits no activity against collagen type I, the predominant collagen in fibrosis. Nevertheless, since at the beginning of fibrolysis MMP-9 expression sharply increases, an indirect involvement of MMP-9 in fibrolysis has been assumed. Activated HSCs reveal enhanced expression of $\alpha_v\beta_3$ integrin subsequently mediating cell-matrix (type I collagen) interactions thus providing survival signals to the cells. Interruption of type I collagen and $\alpha_v\beta_3$ integrin ligation (by disintegrin echistatin or specific antibodies) induced apoptosis and increased MMP-9 mRNA expression. Concurrently a decline of TIMP-1 expression may be observed. Active recombinant MMP-9 protein promotes apoptosis of these cells [95].

This observation has been reconfirmed in a CCl_4-mouse model with adenoviral-driven overexpression of MMP-9 and MMP-9 mutants [96]. The study demonstrated further that MMP-9 overexpression was able to reduce type I collagen and hydroxyproline content of the liver [96]. Thus, MMP-9 may indirectly contribute to fibrolysis by accelerating HSC apoptosis.

TIMP-1 is dramatically upregulated by several inflammatory cytokines like IL-1β, IL-6, IL-11 [58],

TNF-α [53], and TGF-β [16] and is the most relevant TIMP in toxic liver injury and cholestasis [3,61]. The regulation of TIMP-1 mRNA expression is directly associated with TGF-β protein. After withdrawal of TGF-β overexpression in the liver of transgenic mice, TIMP-1 mRNA levels rapidly declined within 2 days [16]. TIMP-1 antagonization enables the restoration of overall MMP net-activity to finally achieve fibrolysis. For this purpose a mutant MMP-9 gene was designed with retained binding to TIMP-1 but without enzymatic activity [96]. Delivering the gene by adenoviruses via tail vein injection in mice suffering from CCl_4-induced fibrosis resulted in decreased accumulation of collagen, a reduced morphometric stage of fibrosis and less hydroxyproline content of the liver [96]. Additionally MMP-9 mutants suppressed the development of the HSC-myofibroblast phenotype and increased apoptosis of activated HSCs [96]. Alternatively neutralizing antibodies are capable of antagonizing TIMP-1. In a rat model of CCl_4-induced fibrosis anti-TIMP-1 antibodies were injected on day 24 of toxic liver injury and every third day thereafter for a total of 17 days [33]. This led to a significant reduction of hydroxyproline content and α-SMA staining.

Antagonization of inflammatory cytokines (e.g. TGF-β, IL-1β, and TNF-α) also reduces TIMP-1 mRNA expression. Application of IL-1β- and TNF-α-antagonists (anakinra and etanercept, respectively), both approved for the treatment of rheumatoid arthritis, resulted in a significant reduction of TIMP-1 mRNA in experimental toxic liver injury [53]. In addition anakinra augmented MMP-9 mRNA levels. Whether anakinra or etanercept has the potential to inhibit fibrosis or induce fibrolysis remains unknown. Transcriptional or translational inhibition of TIMP-1 has been successfully applied in a rat model of liver fibrosis induced by human serum albumin. Herein, TIMP-1 antisense oligonucleotides were able to block gene and protein expression of TIMP-1 and induce hepatic fibrolysis [97]. Taken together, due to its particular importance for fibrolysis TIMP-1 may represent an attractive therapeutic target. Nevertheless, inhibition of TIMP-1 in rodents alters reproductive cyclicity, uterine morphology and thus prevents pregnancy [98–100] – implications that must not be disregarded.

Besides the resolution of fibrosis through the liberation of bound MMPs, reduced TIMP-1 levels play a pivotal role in the regulation of hepatocyte regeneration: The lack of TIMP-1 led to accelerated hepatocyte proliferation whereas overexpression of TIMP-1 was associated with delayed proliferation in mice after partial hepatectomy [101]. Several MMPs and growth factors have been suggested to contribute to this effect [85,101–104]. Thus, declining TIMP-1 levels may lead to an increase in MMP activity thereby exerting two effects: (i) degradation of ECM to allow hepatocyte expansion and (ii) release of ECM-bound pro-HGF (hepatocyte growth factor). Urokinase plasminogen activator (uPA) conveys pro-HGF to the activated HGF, with HGF binding to its receptor c-met, finally enabling hepatocytes to reenter the cell cycle.

5. Recent experimental anti-fibrotic approaches

A number of interesting experimental studies regarding the role of MMPs and TIMPs in hepatic fibrosis have been conducted since the last reviews have been published [105,106]. Moreover, since then, the methodological quality of both, clinical trials and basic research, has been challenged [107–111]. Therefore, in addition to a structured literature search and a table summarizing all relevant articles in the field, the methodological quality, and thus the strength of the evidence, has been critically appraised applying a self-constructed checklist.

6. Literature retrieval and study selection process

Medline was searched via the internet using the search engine PubMed© (http://www.ncbi.nih.gov/entrez/query.fcgi). Aim of the structured literature search was to identify all models of liver fibrosis that evaluated a defined anti-fibrotic intervention *in vivo* or *in vitro* considering MMPs and TIMPs. The primary search retrieved all studies published between January 2000 and June 2006 using the following search terms: "liver OR hepatic" (*title* & *abstract*) AND "metalloproteinase OR metalloproteinases OR MMP OR tissue inhibitor of OR TIMP" (*title* & *abstract*) AND "fibrosis" (*title* & *abstract*) AND "2000–2006" (*publication date*) AND "journal article" (*publication type*) AND "English OR German" (*language*). A secondary search was conducted to locate reviews, meta-analyses, editorials and monographs. Reference lists of all retrieved papers were searched by hand to detect any additional trial not found by the primary Medline search. All studies had to be published as full papers. Publications solely reporting effects of a substance without considering MMP or TIMP expression were excluded. All selected articles were classified into two categories, (a) in vitro experiments or (b) in vivo experiments.

7. Assessment of the methodological quality

Considering basic principles of good study methodology a checklist comprising four dichotomous variables was accomplished (Table 1). Although not formally validated, robust evidence exists that all individual items of the checklist are indeed associated with methodological quality [108,110,112]. Each item of the checklist scored

Table 1
Checklist for the assessment of methodological study quality

Item		
I.	Experimental question	Did the authors ask an explicit experimental question, i.e. provide a null-hypothesis that has been challenged by their work? Comments such as 'we evaluated the effect of substance xy on fibrosis' were not considered specific and thus scored no point.
II.	Experimental design and method	Were the study design and the applied methods suitable to challenge the proposed question? If no question has been asked, we evaluated whether the reported methods were appropriate to induce and measure hepatic fibrosis, respectively. If, for example, two out of three applied methods were suggested inadequate, no point was given.
III.	Statistical analysis	Were the methods of the statistical analyses reported and were the applied statistical tests adequate? E.g., parametric tests for non-normally distributed data were regarded insufficient and thus scored no point.
IV.	Conclusion supported by results	Were the conclusions supported by the reported results? Exaggerations of the own results and speculations such as 'may be attributed to the modulation of' and thus potential clinical use' without providing clear cut evidence for such statements were awarded zero points.

one point, thus the maximum score was four points and the minimum score zero points. Based on the quality assessment articles scoring two points or less were arbitrarily considered poor methodologic quality. The checklist was applied to all articles in a non-blinded manner by two of the authors (J.G. and E.R.). Discrepancies while judging the methodological quality were resolved by discussion.

8. Results of the literature search and quality assessment process

The primary Medline search retrieved 243 potentially relevant articles, out of which 75 articles met the inclusion criteria and were finally eligible for this overview (Fig. 4 and Table 2). Out of this 44 articles (59%) scored two points or less, thus representing poor methodological quality only. Twenty-four articles scored three and seven articles were awarded four points. An explicit experimental question (*null hypothesis*) has been asked in 45 out of 75 publications (60%). Others just mentioned the evaluation of a given intervention without a statement whether a positive or negative effect was expected. The experimental methods to induce hepatic fibrosis and study the eventual influence of the interventions under investigation were appropriately selected in 53 publications (71%). Some authors failed to provide evidence for liver fibrosis or measured unreliable surrogate parameters, sometimes using test methods not validated for this purpose. Although statistical analyses have been reported in the result sections of all publications, only 18 groups (24%) reported and applied appropriate statistical methods. Most authors used statistical tests suitable for normally distributed data only despite the small sample size and other limitations. Moreover, some authors indicated *p*-values without describing any statistical analysis. The majority of the conclusions were supported by the results (56 out of 75 articles, 75%). However, 19 groups (25%) reported exaggerated conclusions, i.e. the mentioned effects or suggested

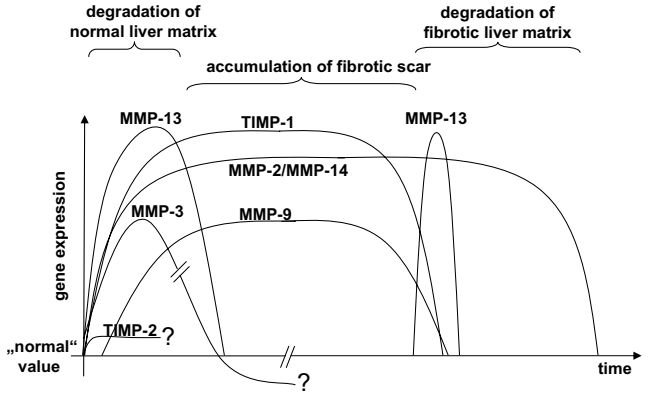

Fig. 3. Possible expression profiles of MMPs and TIMPs incorporating the current knowledge of the pathophysiology of chronic toxic liver injury. Differences in *y*-axis values are arbitrarily set for better understanding.

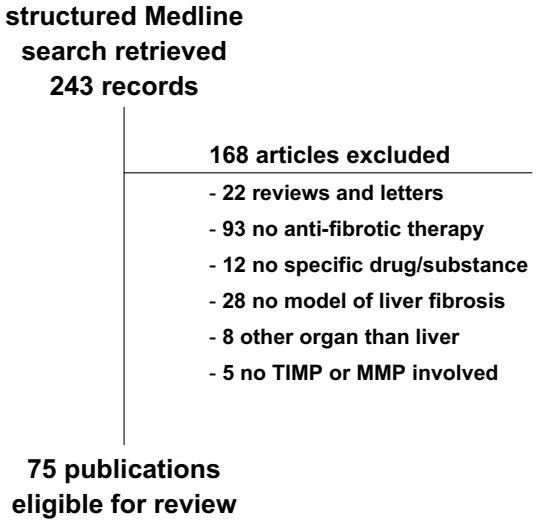

Fig. 4. Results of the systematic literature retrieval process via PubMed (refer to the text for the detailed search strategy). The number and reasons for study exclusion are given.

Table 2
Recent experimental and clinical antifibrotic approaches involving MMPs or TIMPs (index by year, descending)

Author	Agent	Setting/model	MMP and TIMP	Reported effect	Methodological quality
Wang et al. [118]	α-MSH expression plasmid	In vivo TAA-induced fibrosis in mice	MMPs ↑ TIMPs ↓	TGF-β1, collagen α1, cell adhesion molecule mRNA ↓ α-SMA, COX-2 ↓	2 [I, II]
Tasci et al. [119]	PEG-IFN-α2b + UDCA	In vivo CCl_4-induced fibrosis in rats	TIMP-1 ↓ MMP-13 ↑	Fibrosis, hydroxyproline ↓	4 [I, II, III, IV]
Roderfeld et al. [96]	MMP-9 mutant	In vivo CCl_4-induced fibrosis in mice	TIMP-1 mRNA ↓ MMP-2 mRNA ↓	Fibrosis, hydroxyproline ↓ Type I collagen mRNA and protein ↓ α-SMA ↓	3 [I, II, IV]
Popov et al. [120]	Halofuginone (plant alkaloid, inhibitor of collagen synthesis)	In vitro cultured rat HSC	MMP-3 and -13 mRNA and protein ↑ Interstitial collagenase activity ↑	Collagen α1 (I) and (III) ↓ TGF-β1, CTGF mRNA ↔	4 [I, II, III, IV]
		In vivo TAA-induced fibrosis in rats	MMP-2 mRNA ↓ TIMP-1, TIMP-2 mRNA ↔ MMP-3 and -13 mRNA ↑ TIMP-1 mRNA ↓	Fibrosis and hydroxyproline content ↓	
Neef et al. [121]	Imatinib (inhibitor of PDGF receptor tyrosine kinase)	BDL-induced fibrosis in rats	Early phase: MMP-2 activity, TIMP-1 RNA ↓ Late phase ↔	Early phase: ECM formation ↓, activated HSC, collagen I expression ↔ Late phase: ↔	4 [I, II, III, IV]
Migita et al. [122]	FK506 (immunosuppressant)	In vitro LI90 cells + TNF-α (human HSC line)	MMP-9, MMP-3 protein ↓ MMP-9 mRNA ↓ MMP-2 protein ↔	NF-κB-activation, IκB degradation ↓	0 [–]
Li et al. [123]	CTGF siRNA	In vivo CCl_4-induced fibrosis in rats	TIMP-1 mRNA ↓	Fibrosis, CTGF protein, α-SMA ↓ CTGF, type I and III collagen, laminin, TGF-β1 mRNA ↓ Smad2, Smad7 mRNA ↔ Serum procollagen type III ↓	2 [I, IV]
Lee et al. [124]	α-MSH expression plasmid (neuroimmunomodulatory peptide)	In vivo CCl_4-induced fibrosis in mice	MMP-1, MMP-8 mRNA ↑ TIMP-1, TIMP-2 mRNA ↓ MMP-2, MMP-9 activity ↑	Sirius red staining, α-SMA protein ↓ Collagen content and collagen α1 mRNA ↓ TGF-β, TNF-α, ICAM-1, VCAM-1 mRNA ↓	2 [I, II]
Huang et al. [125]	IL-10	In vivo CCl_4-induced fibrosis in rats	MMP-2, TIMP-1 protein ↓	Fibrosis ↓ Collagen I and III, TNF-α protein ↓	2 [I, IV]
Hu and Liu [126]	Bicyclol (synthetic hepatoprotectant)	In vivo DMN-induced fibrosis in mice	TIMP-1 mRNA (prophylactic exp.) ↓ TIMP-1 protein in liver and serum (therapeutic exp.) ↓ Collagenase activity (therapeutic exp.) ↑	ALT, bilirubin, hydroxyproline, prolidase, TNF-α, TGF-β-1, type I collagen, all in serum ↓ TGF-β mRNA (prophylactic exp.) ↓ Body weight, serum albumin and total protein ↑	3 [I, II, IV]
Guido et al. [127]	IFN-α + ribavirin	In vivo chronic HCV in humans	MMP-1 staining ↑	Fibrosis ↓	2 [II, IV]

(*continued on next page*)

Table 2 (*continued*)

Author	Agent	Setting/model	MMP and TIMP	Reported effect	Methodological quality
			Serum MMP-9, MMP-9/TIMP-1 ratio ↑	TGF-β1 staining ↓	
			Serum TIMP-1 ↓	Serum TGF-β, NF-κB staining ↔	
			Serum MMP-2, MMP-2/TIMP-1 ratio ↔	α-SMA staining ↔	
Gianelli et al. [52]	rIFNα-2b	In vitro IL-1β and TNF-α-stimulated human hepatocytes or human LX-2 cells (HSC line)			1 [IV]
			MMP-9 mRNA ↓	n.d.	
			n.d.	α-SMA protein ↓	
Ebrahimkhani et al. [128]	Naltrexone (opioid receptor antagonist)	In vivo BDL-induced fibrosis in rats	MMP-2 activity ↓	Fibrosis development ↓	3 [I, II, IV]
				Activated HSC ↓	
				S-nitrosothiol ↓	
Chou et al. [129]	IL-10 expression plasmid	In vivo CCl_4-induced fibrosis in mice	MMP-2 protein and activity ↓	Fibrosis, COX-2, α-SMA protein ↓	2 [I, II]
			TIMP-1, TIMP-2 mRNA ↓	TGF-β1, TNF-α, collagen α1, fibronectin, ICAM-1, VCAM-1 mRNA ↓	
Cao et al. [130]	DLPC + SAMe	In vitro LX-2 cells (human HSC line)	TIMP-1 protein and mRNA ↓	n.d.	2 [I, II]
Zheng et al. [131]	Intraperitoneal IL-10	In vitro cultured rat HSC from rats with CCl_4-induced fibrosis	MMP-2, TIMP-1 mRNA and protein ↓	n.d.	1 [I]
Yoshiji et al. [132]	Imatinib mesylate (STI-571, Gleevec) (protein tyrosine kinase inhibitor)	In vivo pig serum-induced fibrosis in rats	TIMP-1 mRNA ↓	Fibrosis, hydroxyproline, α-SMA positive cells ↓	2 [II, IV]
				Procollagen α2(I) mRNA, TGF-β mRNA ↓	
Xidakis et al. [133]	Octreotide (synthetic analogue of somatostatin)	In vitro cultured rat Kupffer cells	Early phase: MMP-1 protein ↑	Late phase: TGF-β protein ↓	0 [–]
			MMP-9 protein ↓		
Oakley et al. [134]	Sulfasalazine (inhibitor of κB kinase suppressor)	In vivo CCl_4-induced fibrosis in rats	TIMP-1 RNA ↓	Fibrotic score, α-SMA ↓	2 [I, II]
			MMP-2 activity ↑	Collagen type I RNA ↓	
Nakamuta et al. [135]	Epigallocatechin-3-gallate (polyphenol component of green tea)	In vivo, rat	MMP-1 RNA + activity ↓	Collagen ↓	2 [II, III]
		In vitro, TWNT-4 cells (derived from human HSC)1	TIMP-1 protein ↔	Collagen type I RNA ↑	
				α-SMA RNA ↔	
Nakamuta et al. [136]	Cyclosporine	In vitro human TWNT-4 cells	IMP-1 mRNA ↓	Collagen type I mRNA ↓	3 [II, III, IV]
			MMP-1 mRNA ↑		
Marinosci et al. [137]	PEG-IFN-α2b + ribavirin	In vivo chronic HCV in humans	MMP-9 mRNA and protein ↓	n.d.	1 [IV]
			MMP-2 protein ↔		
Lin et al. [138]	Adenoviral uPA	In vitro HSC-T6 (rat HSC line)	MMP-2 protein ↑	Collagen type I and III protein ↓	2 [I, IV]
Li et al. [139]	ACEI	In vivo CCl_4-induced fibrosis in rats	MMP-2 and MMP-9 activity ↓	Fibrosis, AT1R, TGF-β1, PDGF-BB, serum laminin and hyaluronic acid, NF-κB DNA binding activity ↓	1 [II]
Lebensztejn et al. [140]	IFN-α	In vivo chronic hepatitis B in children	Serum MMP-2 and MMP-9/TIMP-1 complex ↑	Fibrosis ↔	3 [II, III, IV]

				Inflammation (responders only) ↓ Serum laminin-2 and collagen IV ↓	
Jiang et al. [141]	Antisense TIMP-1 expressing plasmid	In vivo pig serum-induced fibrosis in rats	TIMP-1 mRNA and protein ↓	Fibrosis ↓	3 [I, II, IV]
				Interstitial collagenase activity ↑ Hydroxyproline, collagen type I and III ↓	
Hung et al. [142]	IL-10 expression plasmid	In vivo TAA-induced fibrosis in mice	TIMP-1, TIMP-2 mRNA ↓	TGF-β1, TNF-α, ICAM-1, VCAM-1 mRNA ↓ Collagen content and collagen α1 mRNA ↓ Fibronectin mRNA ↔ Fibrosis, COX-2 staining, apoptosis ↓ α-SMA protein ↓	2 [I, IV]
Hsu et al. [143]	*Salvia miltiorrhiza* (Chinese medicine)	In vitro TGF-β1 stimulated HSC-T6 (rat HSC line)	TIMP-1 mRNA ↓	α-SMA protein ↓	4 [I, II, III, IV]
	or Silymarin (milk thistle extract)	In vivo DMN-induced fibrosis in rats	TIMP-1 mRNA ↓	α-SMA, CTGF mRNA ↓ Fibrosis score, hepatic collagen content ↓ α-SMA, TGF-β1, procollagen I mRNA ↓ CTGF mRNA ↔ Plasma AST ↓	
Fiorucci et al. [144]	6-ECDCA (farnesoid X receptor ligand)	In vitro cultured rat HSC	TIMP-1 protein + release↓ MMP-2 protein ↔	SHP protein ↑ Collagen α 1 (I) protein ↓	2 [II, IV]
		In vivo CCl_4-induced fibrosis in rats	MMP-2 activity ↑ TIMP-1 and MMP-2 protein ↓	Fibrosis, α-SMA, hydroxyproline ↓ SXR, SHP protein ↑	
Di Sario et al. [145]	Silybin–phosphatidylcholine–Vitamin E complex	In vivo DMN-induced fibrosis in rats	TIMP-1 mRNA ↓ MMP-2 mRNA ↓	HSC proliferation, α-SMA pos. cells and protein expression, collagen content ↓ TGF-β1, α1(I) procollagen mRNA ↓	2 [I, II]
de Gouville et al. [146]	GW6604 (ALK5 inhibitor)	In vivo DMN-induced fibrosis in rats	TIMP-1 mRNA ↓	Fibrosis, mortality, liver collagen content, α-SMA pos. cells, activated HSC ↓ Serum ALAT, ASAT, PAL, bilirubin, hyaluronic acid ↓ Collagen IA1, IA2, III, TGF-β mRNA ↓ Liver weight, hepatocyte proliferation ↑ Body weight ↔	3 [I, II, IV]
Chen et al. [147]	Adeno-associated virus mediated IFN-γ	In vitro cultured rat HSC	TIMP-1 mRNA ↓ MMP-13 mRNA ↔	α-SMA protein, TGF-β mRNA ↓ Fibrosis, hydroxyproline, serum AST and ALT ↓	2 [II, IV]
		In vivo CCl_4-induced fibrosis in rats	TIMP-1 mRNA ↓ MMP-13 mRNA ↔	TGF-β mRNA ↔	
Zhou et al. [95]	Anti-α_v β_3 integrin antibodies	In vitro cultured rat HSC	TIMP-1 mRNA ↓ MMP-9 protein ↑ MMP-2 protein, MMP-9 mRNA ↔	Apoptosis, caspase-3 activity ↑ Apoptosis, Bax/Bcl-2 ratio, caspase-3 activity ↑ F-actin organization, pFAK ↓	3 [I, II, IV]

(*continued on next page*)

Table 2 (*continued*)

Author	Agent	Setting/model	MMP and TIMP	Reported effect	Methodological quality
	Echistatin (disintegrin)		TIMP-1 mRNA ↓ MMP-14, MMP-2 protein ↔ MMP-9 activity, mRNA and protein ↑	Apoptosis, Bax/Bcl-2 ratio ↑ HSC viability, F-actin organization, pFAK ↓	
	α_v subunit siRNA		TIMP-1 mRNA ↓		
Zhang et al. [148]	IL-10	In vivo CCl_4-induced fibrosis in rats	MMP-2 and TIMP-1 positive cells ↓	n.d.	1 [IV]
		In vitro cultured rat HSC + PGDF	TIMP-1 and MMP-2 mRNA ↔		
Yeh et al. [149]	Thalidomide (α-*N*-phthalimidoglutarimide)	In vivo TAA-induced cirrhosis in rats	TIMP-1, TIMP-2 protein ↓ MMP-13 protein ↓	Cirrhosis, mortality ↓ TNF-α, TGF-β1 protein ↓ α-SMA staining ↓	3 [I, II, IV]
Uchio et al. [150]	TGF-β1 or CTGF antisense oligo	In vivo CCl_4-induced fibrosis in mice	TIMP-1 mRNA ↔	Fibrosis ↔ (preventive and curative) TGF-β1, CTGF mRNA ↓ (preventive and curative) Collagen type I mRNA ↓ (preventive)	0 [–]
Thirunavukkarasu et al. [151]	TAK-044 (endothelin receptor antagonist)	In vivo CCl_4 and phenobarbital-induced fibrosis and cirrhosis in rats	Collagenase activity ↓ TIMP-1 mRNA ↓	Fibrosis, cirrhosis, hydroxyproline ↓ Portal hypertension, systemic hypotension↓ ALT, AST, LDH ↓ Serum albumin ↑ TGF-β1 and collagen type I mRNA ↓ TGF-β1 protein ↓	4 [I, II, III, IV]
Takahara et al. [152]	IFN-α and -γ co-treatment	In vivo CCl_4-induced fibrosis in rats	MMP-2 RNA ↓	Collagen type III and hydroxyproline content ↓	4 [I, II, III, IV]
	IFN-γ	In vivo CCl_4-induced fibrosis in rats	TIMP-1 and -2 RNA ↓	Plasma hyaluronate and transaminase ↓ Collagen type I, desmin and TGF-β1 RNA ↓ Collagen type III ↓	
Reif et al. [153]	Farnesylthiosalicyclic acid (synthetic Ras antagonist)	In vivo TAA-induced cirrhosis in rats	MMP-2 and MMP-9 activity ↑ TIMP-2 mRNA ↑	Fibrosis, hydroxyproline, spleen weight ↓ HSC apoptosis ↔	2 [I, IV]
Refik Mas et al. [154]	Taurine (antioxidant)	In vivo CCl_4-induced fibrosis in rats	MMP-13 ↑ TIMP-1 ↓	Histopathological injury score, activated HSC ↓ Activated HSC apoptosis ↑	2 [II, IV]
Parsons et al. [33]	Anti-TIMP-1 antibody	In vivo CCl_4-induced fibrosis in rats	MMP-2 activity ↓	Collagen accumulation, hydroxyproline content, desmin and α-SMA staining, α-SMA protein ↓	2 [II, IV]
Miranda-Diaz et al. [104]	Adenoviral uPA	In vivo bile duct-ligation in rats	MMP-2, MMP-3, MMP-9 staining ↑	Fibrosis index, α-SMA staining, bilirubin ↓ Hepatocyte regeneration ↑ Acites, gastric varices ↓	2 [II, III]

Matsui et al. [155]	Sulfur-containing amino acids	In vivo TAA-induced cirrhosis in rats	TIMP-1 and TIMP-2 mRNA ↓	Collagen deposition, hydroxyproline, α-SMA expression ↓ PGDFRβ, α-SMA, STAP protein ↓ Collagen type I, TGF-β1 and - β2 mRNA ↓ Serum AST, ALT ↔	1 [IV]
Kotoh et al. [156]	RGD-peptide (adhesive domain of ECM)	TWNT-4 cells (derived from human HSC)	TIMP-1 ↓ MMP-2 ↑	Collagen type I ↓	4 [I, II, III, IV]
Flisiak et al. [157]	Lamivudine (antiviral medication for chronic HBV)	Patients with HBV	Plasma TIMP-1 ↓ Plasma MMP-1 ↑	Fibrosis, inflammation ↔ Plasma TGF-β1 ↓	2 [II, IV]
Fiorucci et al. [158]	6-ECDCA (farnesoid X receptor ligand)	In vivo BDL-induced fibrosis in rats	TIMP-1 and -2 mRNA ↓ MMP-2 mRNA ↔	Fibrosis, hydroxyproline, urinary hydroxyproline ↓ Collagen α 1 (I) and α-SMA mRNA ↓ SHP mRNA ↑	3 [I, II, IV]
Duplantier et al. [159]	SSR182289 (thrombin antagonist)	CCl_4-induced fibrosis in rats	TIMP-1 RNA ↓ TIMP-2 and MMP-2 RNA ↔	Sirius red and α-SMA staining ↓ Collagen α 1 (I) RNA ↔	3 [II, III, IV]
Di Sario et al. [160]	Pirfenidone	In vivo dimethylnitrosamine (DMN)-induced fibrosis in rats	TIMP-1 and MMP-2 RNA ↓	ALT, HSC proliferation, collagen deposition ↓ TGF-β1 and collagen type I RNA ↓	3 [I, II, IV]
Campo et al. [161]	Hyaluronic acid and chrondroitin-4-sulfate (glycosaminoglycans)	In vivo CCl_4-induced fibrosis in rats	TIMP-1, TIMP-2 mRNA and protein ↓	Hydroxyproline, ALT, AST, lipid peroxidation ↓ SOD, GPx activity ↑	3 [I, II, IV]
Bruck et al. [162]	Pyrrolidine dithiocarbamate (antioxidant and inhibitor of NF-κB activation)	In vivo TAA-induced cirrhosis in rats	TIMP-2 staining ↓	Fibrosis score, hydroxyproline, spleen weight ↓ α-SMA, collagen α1(I) staining ↓ Malondialdehyde, protein carbonyls ↓	3 [I, II, IV]
Woo et al. [163]	Butein (antioxidant)	In vitro cultured rat HSC	TIMP-1 mRNA ↓ MMP-13 mRNA ↑	DNA synthesis ↓ α-SMA, type I collagen protein ↓ α1(I) collagen mRNA ↓	3 [I, III, IV]
Sakaida et al. [164]	Gadolinium chloride	In vivo DMN-induced fibrosis in rats	MMP-13, MMP-14 mRNA ↑ TIMP-1 mRNA ↔	Hydroxyproline, ED2-staining ↓ Procollagen type I mRNA, α-SMA staining ↔	2 [I, II]
		In vitro cultured Kupffer cells	MMP-9, MMP-13, MMP-14 mRNA ↑ MMP-14 protein ↑	Type I collagen degrading activity, MAP kinase activity, apoptosis ↑	
Lee et al. [165]	Butein (chalcone)	In vivo CCl_4-induced fibrosis in rats	TIMP-1 mRNA ↓	Hydroxyproline, malondialdehyde, albumin ↓ AST, ALT ↓ α1(I) collagen mRNA ↓	2 [I, IV]
Han et al. [166]	IFN-α2b	Patients with HBV	Serum TIMP-1, TIMP-1 staining ↓	Histological activity index, necrosis and intralobular inflammation ↓ Portal inflammation and necrosis ↔ α-SMA pos. HSC ↓	2 [II, IV]
Bennett et al. [167]	Relaxin	In vitro cultured rat HSC	MMP-13 mRNA and protein ↑	α-SMA protein, total collagen, type I collagen, collagen synthesis ↓	1 [I]

(*continued on next page*)

Table 2 (*continued*)

Author	Agent	Setting/model	MMP and TIMP	Reported effect	Methodological quality
			MMP-2 and MMP-9 activity ↔ TIMP-1 and TIMP-2 protein ↓	HSC proliferation ↔	
Spira et al. [168]	Halofuginone (inhibitor of collagen type I synthesis)	In vivo TAA-induced cirrhosis in rats	TIMP-2 expression ↓	Fibrosis, hydroxyproline ↓	2 [I, II]
				α-SMA staining, collagen content ↓ Collagen type I mRNA ↔	
Raetsch et al. [169]	Pentoxifylline (PTX)	In vivo bile duct-ligation in rats	TIMP-1 mRNA ↑	Hydroxyproline, fibrosis score, ED2 staining, serum PIIINP ↓ α-SMA staining ↔ Procollagen α1(I), CTGF, TGF-β1 mRNA ↓ AST ↑	3 [I, II, III]
Pérez et al. [170]	Dietary nucleotides	In vivo TAA-induced fibrosis in rats	Collagenase activity ↑	Fibrosis, hydroxyproline ↓	2 [I, IV]
			MMP-13 protein ↔	Total collagen, collagen type I, PIIINP, fibronectin. laminin, desmin protein ↔	
			TIMP-1 mRNA and protein ↓	Prolyl 4-hydroxylase activity ↑	
Garcia et al. [171]	Pirfenidone	In vivo CCl_4-induced fibrosis in rats	TIMP-1 RNA ↓	ALT, AST, AP, bilirubin, prothrombin, fibrosis, hydroxyproline, activated HSC ↓ Collagen I, III, IV, TGF-β1, Smad-7, PAI-1 RNA ↓	3 [I, II, IV]
Dubuisson et al. [172]	6-Hydroxydopamine (OHDA) (noradrenergic antagonism)	In vivo CCl_4-induced fibrosis in rats	TIMP-1 RNA ↓	Fibrosis ↓	3 [II, III, IV]
				Type I collagen mRNA ↓	
Cao et al. [57]	DLPC (main phosphatidylcholine species of PPC)	In vitro cultured rat HSC + TGF-β1	TIMP-1 mRNA and protein ↓	Collagen α 1 (I) mRNA ↓	2 [I, II]
			MMP-13 mRNA and protein ↔	Collagen protein ↓	
Williams et al. [173]	Relaxin (reproductive hormone)	In vitro cultured rat HSC	TIMP-1, TIMP-2 secretion ↓	Collagen synthesis and deposition ↓	2 [II, IV]
			MMP-2, MMP-9 secretion ↔ TIMP-1 mRNA ↓ MMP-13 mRNA ↔	Type I collagen, α-SMA, TGF-β mRNA ↔	
Wasser et al. [174]	Ebselen (anti-oxidant)	In vivo CCl_4-induced fibrosis in rats	TIMP-1 mRNA ↓ MMP-13 mRNA ↑	Fibrosis ↓ TGF-β1, procollagen I and III, cytochrome P4502E1, GST mRNA ↓	2 [III, IV]
Ninomiya et al. [175]	IFN-α	Patients with HCV	Serum MMP-1/TIMP-1 ratio ↑ (responders only)	Fibrosis index, serum PIIINP ↓ (responders only)	3 [I, II, IV]
Lee et al. [176]	Tetrandrine (alkaloid)	In vivo bile duct-ligation in rats	TIMP-1 mRNA ↓	Hydroxyproline, AST, ALT, ALP ↓ Collagen α1(I) mRNA ↓	1 [IV]
Jonsson et al. [177]	Captopril (ACE-inhibitor)	In vivo bile duct-ligation in rats	MMP-2, MMP-9 activity ↓	Fibrosis score, hydroxyproline, α-SMA pos. cells ↓ TGF-β1, procollagen α1(I) mRNA ↓ Rel. liver and spleen weight, ALP, AST ↓	2 [III, IV]

				Inflammation ↔	
Jia et al. [178]	Silymarin (standardized extract of milk thistle)	In vivo bile duct-ligation in rats	TIMP-1 mRNA ↓	Collagen content ↓ Procollagen α1(I), TGF-β1 mRNA ↓ Serum PIIINP protein ↓	3 [I, II, IV]
Bruck et al. [179]	Halofuginone (curative and preventive)	In vivo TAA-induced fibrosis in rats	TIMP-2 protein ↓	Collagen α 1 (I) RNA ↓ Hydroxyproline level ↓ α-SMA staining ↓	3 [I, II, IV]
Bruck et al. [180]	Hydroxyl radical scavengers (DMSO, DMTU)	In vivo TAA-induced fibrosis in rats	TIMP-2 staining ↓	Fibrosis, hydroxyproline, spleen weight, collagen content, α-SMA staining ↓ Collagen α1(I) mRNA ↓ Malondialdehyde, lipid peroxides, protein carbonyls ↔ SOD, GSH peroxidase ↑	3 [I, II, IV]
Miyahara et al. [181]	15-D-Prostaglandin J2 (PPAR-γ ligand)	In vitro cultured rat HSC	MMP-3 RNA ↑	Collagen synthesis ↓ Collagen α 1 (I), α-SMA RNA ↓	1 [IV]
Mitsuda et al. [182]	IFN-α	In vivo chronic hepatitis C in human	Serum TIMP-1 ↓ (responders only)	Fibrosis, ALT (responders only) ↓ Serum PIIINP ↓	3 [II, III, IV]
Hironaka et al. [183]	Gadolinium chloride	In vivo pig serum-induced fibrosis in rats	MMP-13 mRNA ↑ TIMP-1 mRNA ↔	Hydroxyproline ↓ Procollagen type I mRNA ↔	1 [II]
		In vitro cultured rat Kupffer cells	Collagenase activity ↑ MMP-13 mRNA ↑	No. of ED2 positive cells ↓ No. of ED1 positive cells ↔ n.d.	
Godichaud et al. [184]	*trans*-Resveratrol (grapevine-derived polyphenol)	In vitro human liver myofibroblasts	MMP-2 secretion ↓	α-SMA staining, collagen type I RNA ↓	2 [II, IV]
Cho et al. [185]	LU 135252 (endothelin-A receptor antagonist)	In vivo bile duct-ligation in rats	TIMP-1 mRNA ↓	Fibrosis, hydroxyproline ↓ Procollagen α1(I) mRNA ↓ Serum pIIINP ↓	3 [I, II, IV]
Bueno et al. [186]	IFNα-2a	In vivo bile duct-ligation in rats	Gelatinase activity ↑ TIMP-1 mRNA ↔	Fibrosis, bile duct mass ↓ ALT, AST, AP ↓	2 [I, IV]
		In vitro non-parenchymal cells	Gelatinase activity ↑	Procollagen αI(III) and αI(IV) mRNA ↓ PAI-1 activity ↓	

The Roman numbers in brackets in the last column refer to the particular item of the checklist that scored a point. Abbreviations used in the table: ↑, increased, ↓, decreased, ↔, not altered, n.d., not determined.

future applications were well beyond the scope of the presented data.

We are aware of the many limitations of any checklist for methodologic study quality, but in the absence of a reference standard for study quality, we ensured content validity of the checklist following a comprehensive review of the literature. We considered construct validity by incorporation of items in the score that were used in previous methodologic scoring systems [113–117], some of which have proven reliability.

9. Summary

The highly controlled interplay between MMPs and TIMPs is responsible for a constant turn-over of liver matrix and the maintenance of homeostasis and a healthy liver architecture. Acute liver injury may significantly disturb the susceptible equilibrium resulting in functional imbalance. In chronic liver injury, differently regulated MMP and TIMP expression leads to a positive feedback loop with subsequent fibrogenesis (an overview of the current knowledge with respect to expression profiles of MMPs and TIMPs in hepatic fibrosis is given in Fig. 3). Rat and mouse models demonstrated that complete reversibility of fibrosis might indeed be possible. Nevertheless, in human beings this seems to be much more complicated since susceptibility with respect to fibrosis is variable, and often toxic liver damage is ongoing. Therefore the induction of fibrolysis often fails and patients thus continue suffering from impaired liver function and eventually develop end-stage cirrhosis.

Gaining more insights into the network of cytokines, MMPs and TIMPs may offer possibilities to interrupt the vicious cycle of fibrogenesis and to induce fibrolysis. The structured literature search retrieved a number of interesting and sometimes promising anti-fibrotic approaches that have been published lately (summarized in Table 2). However, the methodological quality of the majority of articles was disappointing. Besides the investigation of novel experimental approaches challenging hepatic fibrosis there is clearly a need to adhere to basic principles of study methodology to improve the quality of the reports.

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