Expert Review

Matrix Metalloproteinase Gene Delivery for Liver Fibrosis

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Abstract. The resolution of advanced liver fibrosis has been recently recognized to be possible, if the causative stimuli are successfully removed. However, whether complete resolution from cirrhosis, the end stage of liver fibrosis, can be achieved is still questionable. Delivery of interstitial collagenases, such as matrix metalloproteinase (MMP)-1, in the liver could be an attractive strategy to treat advanced hepatic fibrosis from the view point that the imbalance between too few interstitial collagenases and too many of their inhibitors is the main obstacle to the resolution from fibrosis. Remodeling of hepatic extracellular matrix by delivered interstitial collagenases also facilitates the disappearance of activated hepatic stellate cells, the main matrix-producing cells in the liver, and promotes the proliferation of hepatocytes. This review will focus on the impact of the gene delivery of MMPs for the treatment of advanced liver fibrosis while discussing other current therapeutic strategies for liver fibrosis, and on the need for the development of a safe and effective delivery system of MMPs.

KEY WORDS: gene delivery; hepatic stellate cells; liver fibrosis; MMP; TIMP.

INTRODUCTION

Liver fibrosis results from continuous damage to the liver, including viral hepatitis, alcohol abuse, drugs, metabolic diseases involving an overload of iron or copper, autoimmune diseases, or congenital abnormalities (1,2). The end stage of liver fibrosis, cirrhosis, is characterized by increased deposition and altered composition of the ECM and the appearance of regenerative nodules (3,4), accompanied by hepatic failure and portal hypertension (5). This represents an enormous worldwide healthcare problem.

From a treatment viewpoint, several approaches have successfully prevented the progression of liver fibrosis in animal models. However, because fibrotic liver disease may not present clinically until an advanced or cirrhotic stage, the possibility of reversing established fibrosis is an essential issue. Advanced hepatic fibrosis had been believed to be a passive and irreversible process due to the collapse of the hepatic parenchyma and its substitution with collagen-rich tissue (6). Recently, however, it is thought that hepatic fibrosis may be reversible if the underlying injurious stimuli are removed. Indeed, some investigators have reported improvement in liver fibrosis and cirrhosis in selective patients with effective antiviral therapies in chronic viral hepatitis (7,8) and with surgical decompression of secondary biliary fibrosis (9), suggesting a capacity for recovery even

from established cirrhosis. Transplantation is another effective treatment for advanced cirrhosis (10,11) but is limited by the number of available donor organs. Therefore, effective antifibrotic treatments for established liver fibrosis are earnestly required, and biotechnology and pharmaceutical companies are increasingly interested in developing new antifibrotic therapies.

PATHOGENESIS OF LIVER FIBROSIS

Liver fibrosis is associated with major alterations in both the quantity and composition of the extracellular matrix (ECM) (3,4). Accumulation of ECM, including fibrillar collagens, fibronectin, laminin, hyaluronan, and proteoglycans, results from both increased synthesis and decreased degradation (12).

Hepatic stellate cells (HSC) are believed to be the main ECM-producing cells in the injured liver (1), while portal fibroblasts also play important roles in several kinds of liver fibrosis (13,14). In the normal liver, HSC reside in the space of Disse and are the major storage sites of vitamin A. In injured areas of the liver, HSC activate or transdifferentiate into myofibroblast-like cells, expressing contractile proteins and secreting large amounts of ECM, especially fibrillar collagens (type I and type III) (15,16). Many researchers are now investigating the mechanisms mediating the activation of HSC, and activated nonparenchymal cells (NPC), such as Kupffer cells, are believed to play important roles in the mechanism (17,18).

The decreased activity of ECM-removing MMPs is mainly due to an increased expression of their specific inhibitors, tissue inhibitors of metalloproteinase (TIMPs). The key enzymes in the degradation of fibrillar collagens are matrix metalloproteinase (MMP)-1 in humans and MMP-13

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in rodents (19,20). However, during fibrogenesis, the expression of MMP-1 or MMP-13 is very limited, whereas that of MMP-2 increases (21,22). On the other hand, fibrotic livers have high expression of the TIMPs, including TIMP-1 and TIMP-2 (23–25). Thus, there is a combination of low expression of interstitial collagenases and high TIMPs that prevents the degradation of the fibrillar collagens. Moreover, it has been recently well documented that fibrillar collagens act as a survival factor for activated HSC. An *in vitro* study has shown that HSC are activated on collagen type I, whereas they are quiescent on Matrigel (26), implying that signals from the ECM through some types of adhesion molecules, such as integrins, play critical roles in HSC activation. TIMPs are also reported to block HSC apoptosis in fibrotic livers (27,28). Thus, the imbalance between too few MMPs, too much ECM and too many TIMPs possibly account for the advanced liver fibrosis and the failure to resolve the fibrous scar (Fig. 1).

RESOLUTION OF LIVER FIBROSIS

Recently, many researchers have focused on the reversal of hepatic fibrosis (29,30). As mentioned above, recent evidence indicates that even advanced fibrosis is reversible, in contrast with the traditional view that cirrhosis is an irreversible disease (6). Increased collagenolytic activity is a major mechanism of fibrosis resolution (31), and fibrillar collagens (type I and III) are degraded by interstitial MMPs. According to several animal models of liver fibrosis resolution, the expression of TIMPs rapidly decreases while interstitial MMPs continue to be expressed during the resolution, resulting in increased MMP activity and consequent matrix degradation within the liver (27,29,30).

Together with these changes in MMP activity, the apoptosis of activated HSC has been observed in the resolution process of hepatic fibrosis (29,32,33). In a progressive fibrogenic process, activated HSC secrete large amounts of ECM. When the injurious stimulus is withdrawn and the survival factors for activated HSC, including soluble factors and ECM, are partially removed, the loss of activated HSC by apoptosis is thought to occur. HSC apoptosis, in turn, facilitates the remodeling process by removing the major cellular source of collagen and TIMPs.

However, it is still questionable whether the complete resolution of advanced liver fibrosis is possible. Data from animal experiments indicate that resolution from an extensive micronodular to an attenuated macronodular cirrhosis is possible when injurious stimuli are removed, while complete resolution is limited by ECM cross-linking and a failure of activated HSC to undergo apoptosis (30). Therefore, it seems that treatments possessing MMP activity against matrix-cross linking are necessary for complete, or nearly complete, histologic resolution from advanced cirrhosis.
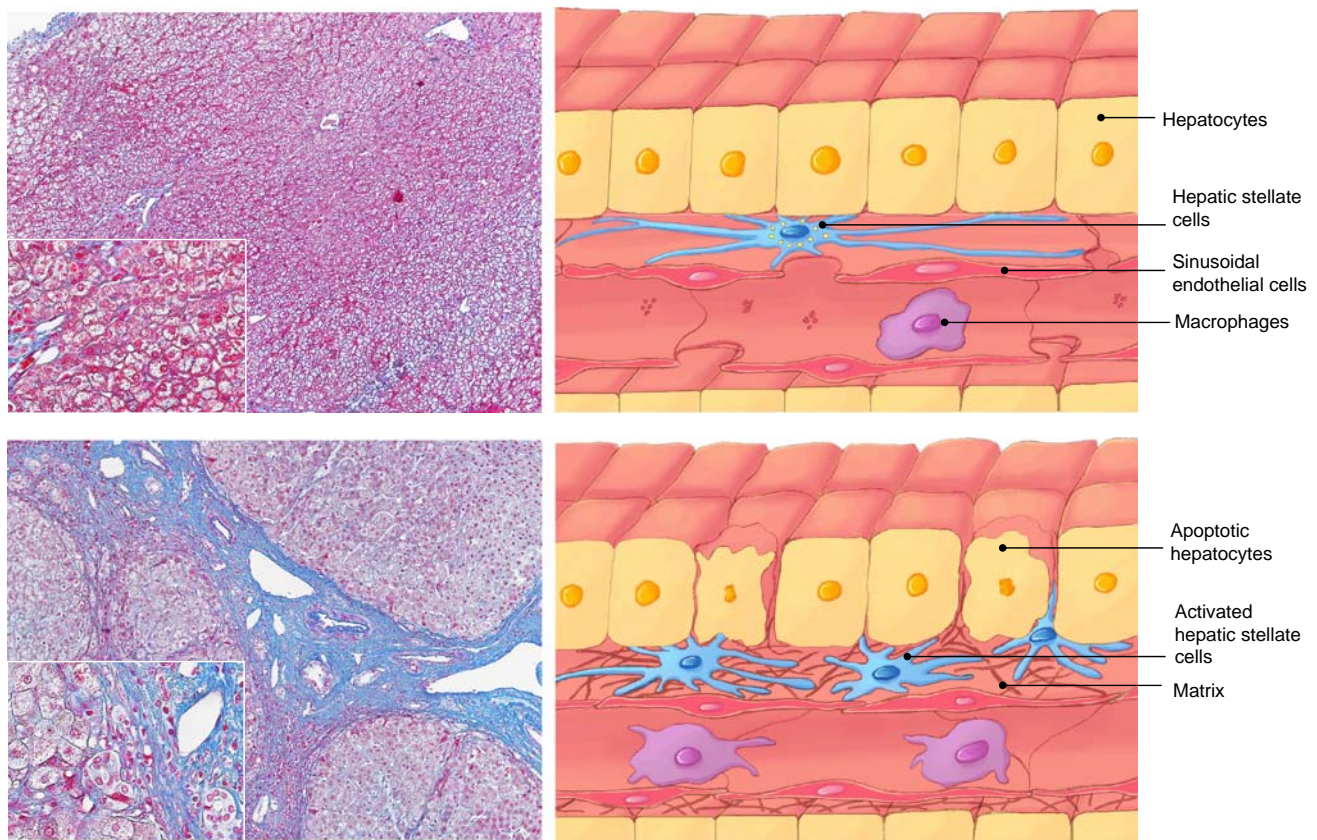
Fig. 1. Azan-Mallory staining of human samples from normal (*top*) and cirrhotic (*bottom*) livers. *Blue-stained area* represents collagen and reticular fibers. Regenerative nodules surrounded by abundant fibrous band are observed in the cirrhotic liver (*bottom*). Schematic changes in the hepatic architecture between normal (*right top*) and cirrhotic (*right bottom*) livers are also shown.

RECENT ADVANCES AND CHALLENGES IN TREATMENT OF LIVER FIBROSIS

Removal of the underlying stimulus is believed to be the most effective strategy for the treatment of hepatic fibrosis. Meanwhile, alternative approaches to treat this disease at earlier stages are currently in progress in consideration of the mechanism of liver fibrosis and its resolution (Table I).

Anti-inflammatory Approaches

Hepatic inflammation invariably precedes fibrosis as mentioned above. Consequently, a number of anti-inflammatory agents have been evaluated intensively. Corticosteroids have been used to successfully treat autoimmune hepatitis, and also shown to improve survival and reduced progression to cirrhosis in selected patients with severe alcoholic hepatitis (34) with no benefit in the treatment of primary biliary cirrhosis (PBC) (35). Colchicine, a well-known anti-inflammatory plant extract, has been used as an antifibrotic agent on the basis of a beneficial effect in experimental fibrosis models and cirrhotic patients (36), although there is some discrepancy between the subsequently reported study (37). Other anti-inflammatory agents such as malotilate (38) and octreotide (39) have been reported to be effective for the treatment of liver fibrosis in animal models, but promising results with these agents in cirrhotic patients remains obscure. Antagonism of pro-inflammatory cytokines is also an attractive approach. An antibody against TNFα (40) or a soluble TNFα receptor (41) has shown potential for reducing liver injury in experimental models. However, randomized controlled trial concerning the effect of the humanized monoclonal anti-TNFα antibody, infliximab, in acute alcoholic hepatitis was stopped early due to an excess of deaths through sepsis (42). Thalidomide has also shown its antagonistic effect against TNFα, and been shown to be beneficial in experimental models of liver injury (43,44). Blockade of IL-1, another important pro-inflammatory cytokine, using IL-1 receptor antagonist reduced liver fibrosis in an experimental model (45). IL-10 also has potent anti-inflammatory and antifibrotic effects. Recombinant IL-10 reduced inflammation and fibrosis in patients with chronic hepatitis C, but increased HCV viral burden (46).

Inhibition of HSC Activation and Proliferation

Hepatic stellate cells (HSC) are believed to be the main ECM-producing cells in the injured liver. Therefore, inhibition of HSC activation or proliferation is critically important in the treatment of liver fibrosis.

Many kinds of antioxidants have been investigated because oxidative stress can induce HSC activation (47). Vitamin E inhibited HSC activation in several experimental models (48,49), and also in a pilot study of humans with chronic hepatitis C infection (50). Polyenylphosphatidylcholine, an extract from soybean, has shown beneficial effect in ethanol- (51) or carbon tetrachloride (CCl_4)-induced (52) experimental hepatic fibrosis. Other antioxidants, such as silymarin (53), n-acetylcysteine (54,55), and S-adenosyl-L-methionine (56) can inhibit HSC activation in experimental models of liver fibrosis, but silymarin had no effect in another controlled trial in human (57).

Table I. Therapeutic Strategies for the Treatment of Liver Fibrosis

Therapeutical Target	Agent
Supress inflammation	corticosteroids
	colchicine
	malotilate
	octreotide
	TNFα antagonist (e.g. anti- TNFα antibody, soluble TNFα receptor, thalidomide)
	IL- 1 receptor antagonist
	recombinant IL- 10
Inhibit HSC activation and proliferation	antioxidants (e.g. vitamin E, phosphatidylcholine, silymarin, n- acetylcysteine, S- adenosyl- L-methionine)
	tyrosine kinase inhibitors (e.g. genistein, imatinib mesylate)
	soluble dominant- negative form of the PDGF receptor
	PI3K inhibitors (e.g. wortmanin, canrenone, LY294002)
	Ras inhibitor S- farnesylthiosalicylate
	ROCK inhibitor Y- 27632
	TGF- β antagonist (e.g. camostat, soluble or dominant- negative TGF- β type II receptors, TGFβ1 antisense mRNA, smad7, HGF)
	AT receptor inhibitors (e.g. losartan, candesartan, olmesartan)
	ACE inhibitors (e.g., peridinopril, captopril)
	Trichostatin A
Stimulate HSC apoptosis	Fas ligand
	NGF
	gliotoxin
	sulfasalazine
Enhance matrix degradation	MMPs
	TIMP antagonist
	uPA
	bone marrow transplantation

Numerous mitogens and cytokines have been implicated in hepatic fibrogenesis, promoting HSC proliferation or stimulating ECM production by HSC. Platelet-derived growth factor (PDGF) is a powerful mitogen for HSC (58), and its signaling pathway can be targeted for fibrosis therapy. Inhibition of the PDGF receptor using tyrosine kinase inhibitors such as genistein and imatinib mesylate blocked HSC activation and proliferation *in vitro* or *in vivo* (59,60). A soluble dominant-negative form of the PDGF receptor showed similar results in bile duct-ligated rats (61). PI3 K, a downstream effecter of PDGF signaling, has also been studied as therapeutic target. Inhibitors of PI3 K such as wortmanin, canrenone, and LY294002 suppressed HSC migration and proliferation (62–64). The small GTPase Ras can be activated by PDGF to lead to cellular proliferation and migration, and the Ras inhibitor S-farnesylthiosalicylate suppressed HSC proliferation and migration and attenuated thioacetamide-induced liver fibrosis in rats (65). A Ras-related GTPase, RhoA, is also implicated in HSC activation and treatment with an inhibitor of Rho's associated kinase ROCK, Y-27632, blocked HSC activation and proliferation *in vivo* and *in vitro* (66,67).

TGF-β1 is one of the most profibrogenic cytokines and is a potent activator of HSC. Several inhibitors of the TGF-β pathway have been effective in experimental models of liver fibrosis. These include camostat, a serine protease inhibitor that prevents release of latent TGF-β (68), soluble or dominant-negative TGF-β type II receptors (69,70), adeno-viral expression of TGF-β1 antisense mRNA (71), and gene transfer of smad7 that blocks TGF-β intracellular signaling (72). However, no anti-TGF-β strategy has been studied in human. Hepatocyte growth factor (HGF) has also shown its anti-fibrogenic effect in experimental models. Recombinant HGF and gene therapy with HGF cDNA promoted regression of fibrosis *in vivo* (73,74), suppressing TGF-β expression and up-regulating MMP activity.

Angiotensin-II (AT-II) is a vasoconstrictive cytokine in the rennin-angiotensin system, which has been shown to be associated with HSC activation and fibrosis (75). A variety of angiotensin-I (AT-I) and -II receptor inhibitors such as losartan, candesartan, and olmesartan (76–78), and inhibitors of angiotensin-converting enzyme (57) such as peridinopril and captopril (77,79) have also shown beneficial effects in animal models of liver fibrosis.

Meanwhile, histone deacetylation is a key gene regulatory process during HSC activation. Trichostatin A, a deacetylase inhibitor, reduced experimentally HSC activation *in vitro* (80).

Stimulation of HSC Apoptosis

Spontaneous resolution of liver fibrosis is associated with apoptosis of HSC as described above, so induction of HSC apoptosis could be make a useful anti-fibrotic therapy. HSC express a number of cell-surface death receptors, including Fas, TNFα receptor, and nerve growth factor (NFG) receptor. Activation of such death receptors following exposure to Fas ligand (81) or NGF (82) stimulated HSC apoptosis *in vitro*. Nuclear factor (NF)-κ B is a transcription factor which can induce transcription of anti-apoptotic genes,

and NF-κB protects HSC from apoptosis. Thus inhibition of NF-κB in HSC can promote HSC apoptosis. A fungal metabolite gliotoxin has been shown to promote HSC apoptosis, and it accelerated recovery from experimental liver fibrosis (33). Another potent NF-κB inhibitor, sulfasalazine, also increased HSC apoptosis *in vitro* and *in vivo* (83). Ras inhibitor, S-farnesylthiosalicylate, also induced HSC apoptosis in TAA-induced established liver fibrosis (84).

Promotion of Matrix Degradation

Animal experiments suggest that total degradation of fibrous scar observed in advanced liver fibrosis, cirrhosis, could be impossible even if causative stimuli are successfully removed (30). Therefore, some anti-fibrotic therapy targeting the existing fibrous scar is required. Such strategies will be discussed in detail later in this review.

Bone Marrow Transplantation as an Anti-fibrotic Therapy

Recruitment of bone marrow-derived cells (BMC) into a variety of injured organs and differentiation of these cells into organ specific cells has been reported in the last few years. As for liver fibrosis, BMC has been reported to participate in the remodeling process during fibrosis resolution, and bone marrow transplantation has proved to be an attractive anti-fibrotic therapy (85). The molecular mechanism underlying such BMC-related matrix degradation possibly includes MMP activities (85,86). Meanwhile, there is another aspect of BMC in liver fibrosis. Several studies have shown that BMC also participate in fibrogenesis in the liver (87–89), so more detailed analysis of the role of BMC in liver fibrosis is required.

REMODELING OF EXTRACELLULAR MATRIX BY DELIVERING INTERSTITIAL MMPS

In human studies, partial resolution of cirrhosis, such as resolution from an extensive micronodular to an attenuated macronodular cirrhosis, has been reported, if the fibrogenic stimuli are successfully removed (7,90), suggesting the current best treatment for hepatic fibrosis is the removal of the underlying injurious causes. However, many clinicians may feel that advanced cirrhosis cannot return to mild fibrosis even if the ongoing fibrogenic stimuli have appeared to resolve or diminish during their follow-up observation. Accordingly, a more promising strategy might require other approaches in addition to the simple removal of the underlying causes for the treatment of advanced liver fibrosis or cirrhosis, especially in humans.

Molecular studies of the expression of mRNA for MMPs have shown that they are expressed in the liver even in cirrhosis, but their activity is suppressed by powerful specific inhibitors, TIMPs (91). Therefore, it is speculated that the potential for matrix degradation is present even in advanced cirrhosis. Manipulation of the imbalance between too few interstitial collagenases and too many TIMPs may possibly facilitate matrix degradation in fibrotic or cirrhotic livers, resulting in the resolution of the fibrosis.

There are logically two kinds of strategy to manipulate the imbalance between too few interstitial collagenases and

too many TIMPs, leading to up-regulation of collagenase activity in fibrotic livers. One possible approach is the overexpression of interstitial MMPs in the livers, while another possibility is the inhibition of the excessive amount of TIMPs. In this review, the authors have focused on the former approach.

Several types of MMPs can digest fibrillar collagens; however, the most potent MMPs against these collagens are collagenases including MMP-1, MMP-8, and MMP-13 (92). Delivery of collagenases into advanced liver fibrosis has been reported by us and others using experimental animal models (93,94). In our experiment, a complementary DNA encoding human pro-MMP-1 was delivered into established liver fibrosis using an adenoviral vector in a rat model (Fig. 2a). In that experiment, we tested our hypothesis that the transient manipulation of the imbalance between collagenases and TIMPs might attenuate established liver fibrosis induced by long-term treatment with thioacetamide (TAA). Long term administration of TAA has been reported to result in a characteristic lesion in rat liver, which corresponds to cirrhosis-like patterns of micronodular cirrhosis type. During its development a reproducible temporal course of biochemical and morphological changes can be recognized. After withdrawal of the toxic agent this lesion persists for at least 2 months (95). Western blot analysis with liver tissue homogenates showed that the delivery of human pro-MMP-1 complementary DNA by Ad5MMP-1 (5×10^9 pfu per rat intravenously) successfully expressed pro-MMP-1 in all livers infected with Ad5MMP-1. Moreover, a significant amount of the active form of MMP-1 (41 kilodaltons) was also detected in this assay, indicating that the expressed pro-MMP-1 protein was gradually converted to the active form *in vivo* (Fig. 2b). One week after their last TAA treatment, the rats
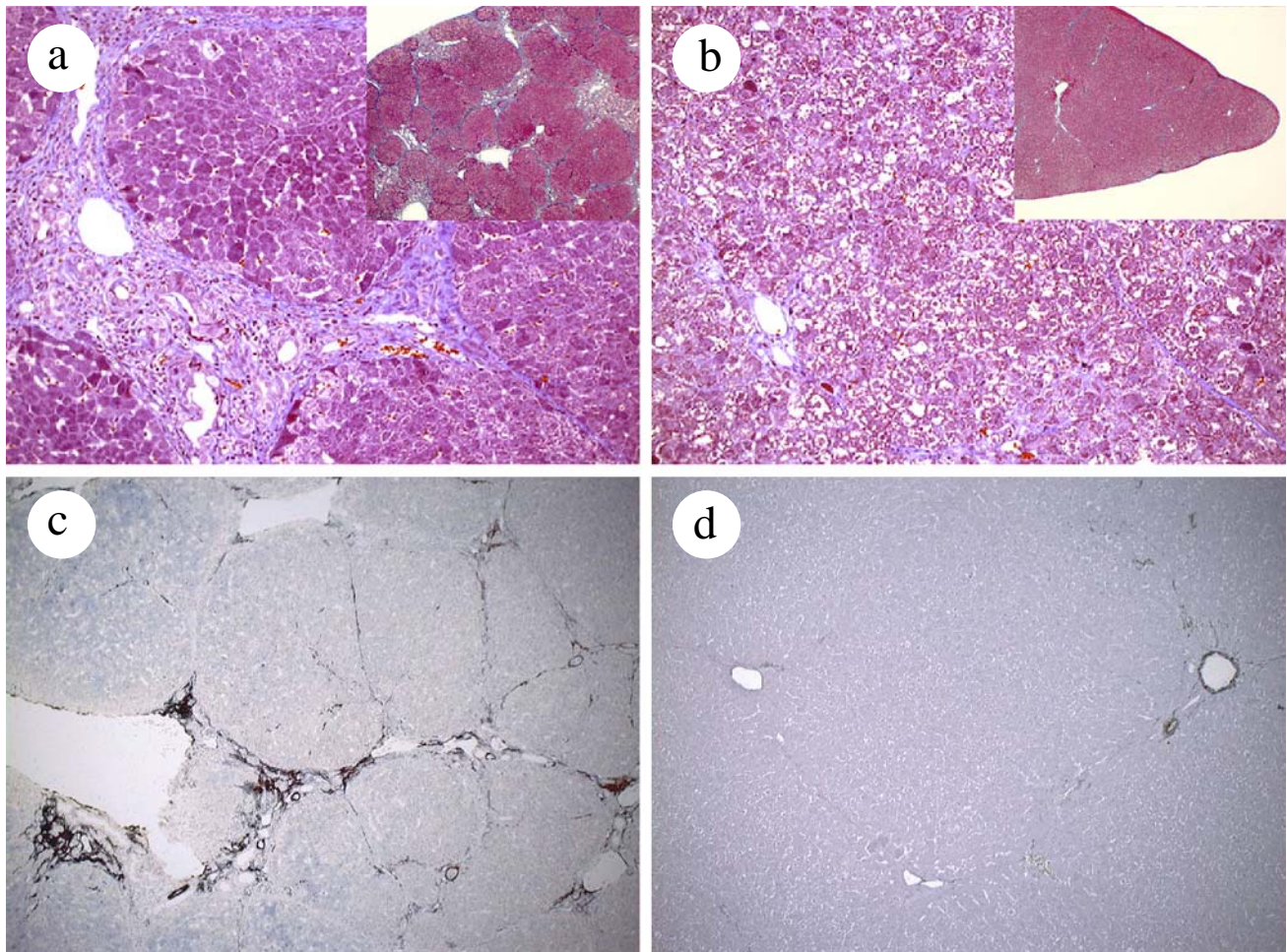
were infected with Ad5MMP-1 or Ad5LacZ, a control adenovirus (5×10^9 pfu per rat). Histological changes in the liver were examined at 2 and 4 weeks after the infection. The control Ad5LacZ had no obvious effect on the established liver fibrosis, which developed after 7 weeks of TAA treatment (Fig. 3a). After the Ad5MMP-1 infection, the fibrosis was significantly attenuated by 2 weeks and remained attenuated by 4 weeks, as indicated by Masson's trichrome staining (Fig. 3b). Preferential target cells in the liver following adenovirus-mediated gene transfer are generally hepatocytes as well known. Indeed, main target cells in our experiment were also hepatocytes (96), while human MMP-1 protein expression was observed rather diffusely because the expressed MMP-1 was secreted out of the infected cells.

Notably, in Ad5MMP-1-infected rats, the area of α-SMA-positive cells (a marker of activated HSC) dramatically decreased at 2 weeks, whereas infection with Ad5LacZ had no effect (Fig. 3c, d). A significant decrease in the α-SMA-positive area was observed only in the Ad5MMP-1-infected animals, indicating that degradation of fibrillar collagens because of MMP-1 overexpression in the liver can lead to a decrease in the number of activated HSC. This phenomenon is consistent with the hypothesis that the loss of survival factor, such as fibrillar ECM, facilitates the disappearance, or apoptosis, of activated HSC, which in turn leads to lower expression of TIMPs and ECM, resulting in the resolution of liver fibrosis.

Another key observation in our study was that the proliferation of hepatocytes was induced by MMP-1 expression in the liver. Degradation of fibrillar collagens due to MMP-1 overexpression possibly affects the interaction between ECM and hepatocytes. Because hepatocytes are known to be responsive to the biophysical state of ECM (97,98), modification of their interaction possibly stimulates
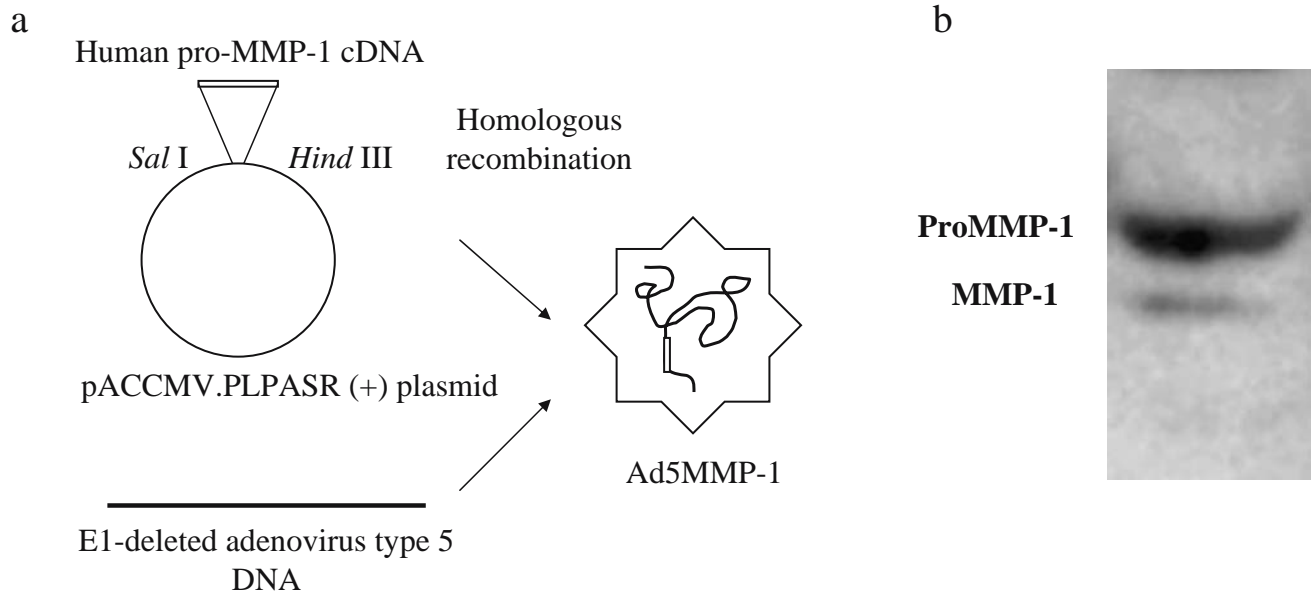
Fig. 2. a Schema of construction of adenovirus carrying human pro-MMP-1 cDNA (Ad5MMP-1). Plasmid pRc/CMV-pro-MMP-1, which contains a human pro-MMP-1 complementary DNA, was subcloned into the *Sal*I/*Hin*dIII site of the pACCMV.PLPASR (+) plasmid to construct the plasmid pACCMV/MMP-1, in which pro-MMP-1 is driven by the cytomegalovirus promoter/enhancer. The recombinant adenovirus was then constructed by cotransfection of the 293 embryonic human kidney cell line with the pACCMV/MMP-1 plasmid plus the purified fragment of *Cla*I-digested DNA from E1-deleted adenovirus type 5 (Ad5). **b** Western blotting for human pro-MMP-1 and MMP-1 using liver homogenate obtained from rats infected with Ad5MMP-1.

Fig. 3. Liver tissues treated with thioacetamide (200 mg/kg) for 7 weeks were stained with Masson's trichrome at 2 weeks after the adenoviral infections (**a**, **b**). Liver tissues were collected from control Ad5LacZ-infected (**a**) or Ad5MMP-1 infected (**b**) rats. Fibrous tissues were markedly attenuated with Ad5MMP-1 infection (**b**). Immunostaining against α-smooth muscle actin (α-SMA) was also performed in the liver from rats treated with TAA (200 mg/kg) for 7 weeks (**c** and **d**). Ad5LacZ-infected (**c**) and Ad5MMP-1-infected (**d**) livers at 2 weeks after the infection are shown. α-SMA-positive area was remarkably reduced with Ad5MMP-1 infection (**d**). Figures were modified from ref. 46 (Gastroenterology **124**: 445–58, 2003).

hepatocyte proliferation directly. Alternatively, it is possible in our model that several growth factors bound to hepatic ECM, such as hepatocyte growth factor (99), are freed from ECM by MMP-1 overexpression and that the activated growth factors facilitate hepatocyte proliferation. Indeed, this proliferative response of hepatocytes is not specific to the fibrotic liver, because we have observed the same proliferative effect of MMP-1 on hepatocytes when MMP-1 was expressed in normal rat livers (96).

ECM-cross linking has been implicated in the mechanism for the limited resolution of advanced liver fibrosis (30). Interestingly, it has been reported that the degradation of this form of ECM-cross linking can be achieved by MMP-1 (30). Thus, gene delivery of MMP-1, an interstitial collagenase, seems to offer several advantages for the treatment of advanced liver fibrosis, or cirrhosis.

A similar approach using gene delivery of MMP-8, human neutrophil collagenase, has been reported in animal models (94). In that experiment, liver fibrosis induced by CCl₄ or bile duct ligation was ameliorated by the adenoviral gene transfer of MMP-8, and was accompanied by improved ascites and gastric varices. Meanwhile, adenoviral gene transfer with human urokinase-type plasminogen activator (uPA) also reversed experimental rat liver fibrosis inducing collagenase expression (100). Considering these experimental data by us and others, gene delivery of interstitial collagenases seems promising for the treatment of advanced cirrhosis in humans. However, persistent overexpression of interstitial collagenases in the liver possibly digests normal architectures in addition to pathologically deposited ECM. Therefore, precisely controlled delivery of active interstitial MMPs may be necessary for developing a treatment for clinical use.

STRATEGIES TO ANTAGONIZE TIMP

For the purpose of antagonizing the excess amounts of TIMPs in liver fibrosis, some strategies, employing an anti-TIMP antibody or mutant MMP, have been reported. In the former report, an anti-TIMP antibody successfully decreased HSC activation and attenuated fibrosis in a CCl₄-induced rat liver fibrosis (101). In the latter report, mutant MMP was delivered into a CCl₄-induced mouse liver fibrosis (102).

From the viewpoint that the balance between MMP and TIMPs is a pivotal parameter of the deposition and breakdown of the ECM, a catalytically inactive MMP-9 which still binds TIMP-1 was delivered as a specific antagonist of TIMP-1 in this study. MMP-9 mutants delivered into CCl_4-induced experimental fibrotic livers using an adenoviral vector effectively suppressed liver fibrosis and inhibited mRNA expression of type I collagen. Reduced expression of α-SMA, a marker of activated HSC, was also observed in the livers expressing MMP-9 mutants. This report has raised the possibility for the development of a new therapy without adverse side effects derived from MMP activity exogenously delivered.

APPROACHES TO EFFICIENT AND SAFE DELIVERY OF MMP GENE

Vectors that have been used for gene therapy are divided into two broad categories: viral and nonviral vectors. Viral vectors are derived from viruses with either RNA or DNA genomes and are represented as both integrating and nonintegrating vectors (103). RNA virus vectors are derived from retroviruses, and can be classified into oncoretroviruses, lentiviruses, and spumaviruses. Among the vectors derived from DNA viruses, the most prominent are those based on adenovirus (Ad) and the adeno-associated virus (AAV). As with any viral vector there are distinct advantages and disadvantages to its use. Retroviral vectors have shown to transduce and stably express transgenes for a long period, although the transduction efficiency is relatively low. Attractive properties of Ad vectors include their very efficient transduction of target cells *in vitro* and *in vivo*, but immunogenicity and toxicity of the vectors could make an obstacle against clinical use. Among the viral vector systems, AAV vector, which is derived from nonpathogenic human parvovirus, has been suspected as a promising one in treatment of many kinds of hereditary diseases.

The efficacy of MMP gene delivery using adenoviral vectors for the treatment of liver fibrosis has been reported as mentioned above (93,94). However, the clinical use of adenoviral vectors is limited by the adverse effects of the virus vector itself, such as immunogenicity and toxicity as well as the possible mutagenesis of the cells transfected. Moreover, expression of MMP genes in the liver for a long period using viral vectors such as retroviral vectors or AAV vectors may possibly induce adverse side effect by over-degradation of ECM in normal architectures. Thus, it is important to create safe non-viral vectors with significant transduction efficiency and limited expression for clinical gene therapy of liver fibrosis.

Nonviral vectors should circumvent some of the problems occurring with viral vectors such as endogeneous virus recombination, oncogenic effects and unexpected immune response, and are categorized into two general groups: 1) naked DNA delivery by a physical method, such as electroporation, gene gun, ultrasound and hydrodynamic pressure and 2) delivery mediated by a chemical carrier such as cationic polymer and lipid (104). In consideration of selective MMP gene transfer into the liver, selective injection of naked DNA into the liver via the portal vein or the hepatic artery, or gene delivery using a chemical carrier possibly possess therapeutic potential. From such a view point, an interesting experiment where plasmid DNA encoding MMP-1 was delivered to streptozotocin-induced fibrotic kidneys using cationized gelatin hydrogels has been reported (105). In that study, in order to facilitate the sustained release of plasmid DNA, biodegradable hydrogels and microspheres were formulated from cationized gelatin prepared through aminization. Plasmid DNA was released from the cationized gelatin hydrogels as a result of hydrogel degradation. The hydroxylproline content in the murine fibrotic kidneys was effectively reduced when the gelatin microspheres incorporating plasmid MMP was administered. A combination of this kind of strategy and plasmid DNA of catalytically inactive MMPs may prove useful in treating advanced liver fibrosis in humans in the future.

SUMMARY

The removal of the causative factors is currently the most effective intervention in the treatment of liver fibrosis. Although experimental studies have revealed several targets to prevent fibrosis progression in rodents (17), whether complete regression from advanced cirrhosis is possible is still controversial, especially in humans. Therefore, the development of more effective and safe therapies against established liver fibrosis is critically important. In this review, the authors focused on MMP gene delivery that attempts to reset the imbalance between too little MMPs and too much TIMPs in advanced liver fibrosis. The delivery of interstitial MMPs into advanced liver fibrosis seems rational and is directed against the excess amount of deposited fibllilar collagens. More importantly, the data imply that even transient shifts in the imbalance between MMPs and TIMPs are sufficient for the resolution of advanced fibrosis, if the underlying causative stimuli have been successfully removed. The development of a safe and efficient MMP-delivering system may prove to be an attractive pharmaceutical target for the treatment of advanced liver fibrosis and cirrhosis.

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