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HEPATIC FIBROSIS: Molecular Mechanisms and Drug Targets

Sophie Lotersztajn,[1] Boris Julien,[1] Fatima Teixeira-Clerc,[1] Pascale Grenard,[1] and Ariane Mallat[1,2]

[1] *Unité INSERM 581 Hôpital Henri Mondor, 94010 Créteil, France;*
[2] *Service d'Hépatologie et de Gastroentérologie, Hôpital Henri Mondor, AP-HP, Créteil, France; email: sophie.lotersztajn@im3.inserm.fr, boris.julien@im3.inserm.fr, fatima.clerc@im3.inserm,fr, pascale.grenard@im3.inserm.fr, ariane.mallat@hmn.ap-hop-paris.fr*

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■ **Abstract** Liver fibrosis is the common response to chronic liver injury, ultimately leading to cirrhosis and its complications, portal hypertension, liver failure, and hepatocellular carcinoma. Efficient and well-tolerated antifibrotic drugs are currently lacking, and current treatment of hepatic fibrosis is limited to withdrawal of the noxious agent. Efforts over the past decade have mainly focused on fibrogenic cells generating the scarring response, although promising data on inhibition of parenchymal injury and/or reduction of liver inflammation have also been obtained. A large number of approaches have been validated in culture studies and in animal models, and several clinical trials are underway or anticipated for a growing number of molecules. This review highlights recent advances in the molecular mechanisms of liver fibrosis and discusses mechanistically based strategies that have recently emerged.

INTRODUCTION

Chronic liver injury produces liver fibrosis, and its endstage, cirrhosis, is a major public health problem worldwide owing to life-threatening complications of portal hypertension and liver failure and to the risk of incident hepatocellular carcinoma. A variety of adverse stimuli may trigger fibrogenesis, including viruses, toxins such as alcohol, autoimmune diseases, chronic biliary stasis, metabolic disorders, genetic defects, or hypoxia. In western countries, the prevailing causes of cirrhosis include chronic alcohol consumption, hepatitis C virus, and nonalcoholic steatohepatitis. Current treatment of hepatic fibrosis is limited to withdrawal of the noxious agent, which not only prevents fibrosis progression but may also induce its regression, as discussed below. Major advances have been made in this respect during the past decade, with the advent of efficient antiviral treatments for hepatitis B and C. Nevertheless, suppression of the cause of hepatic injury is not

always feasible, and, therefore, numerous efforts are directed at the development of liver-specific antifibrotic therapies. Although effective antifibrotic treatments are not available as yet, several ongoing clinical trials are evaluating molecules identified from the joint efforts of many researchers. In addition, recent advances in the physiopathology of liver fibrosis are paving the way for the design of new molecules interfering with regulatory pathways in fibrogenic cells. This review highlights recent advances in the molecular mechanisms of liver fibrosis and discusses mechanistically based strategies that have emerged recently.

PROGRESSION AND REGRESSION OF LIVER FIBROSIS

Following acute liver injury, restoration of normal architecture results from an intricate inflammatory reaction and matrix remodeling process that combines matrix synthesis and fibrolysis. In contrast, chronic liver injury is associated with prolonged and dysregulated wound healing, characterized by an imbalance between excessive matrix synthesis and altered matrix degradation. This process leads to a progressive three- to fivefold hepatic accumulation of a large variety of matrix proteins, including collagens, proteoglycans, and glycoproteins. Quantitative changes are associated with qualitative alterations in the composition of matrix, resulting in a predominance of type I and III fibrillar collagens, which accumulate up to tenfold over time and build up a network resistant to fibrolysis following crosslinking of collagen bundles (1). The cirrhotic endstage is characterized by a distorted hepatic architecture associated with fibrotic septa surrounding regenerating hepatocyte nodules, with development of intrahepatic porto-hepatic and arterio-venous shunts within the fibrotic septa.

Although traditionally seen as an irreversible process, advanced fibrosis, even at the cirrhotic stage, may regress following control of the noxious stimulus. Hence, in the rodent model of carbon tetrachloride-induced fibrosis, cessation of dosing is followed by a reversal of fibrosis within four weeks (2). Similarly, fibrosis elicited by bile duct ligation resolves following biliojejunal anastomosis (3). Regression of fibrosis or cirrhosis has also been documented in patients by serial liver biopsies in various settings, including autoimmune hepatitis controlled by immunosuppression (4), chronic hepatitis C responsive to antiviral treatment (5), chronic hepatitis B under long-term treatment with lamivudine (6), or following biliary drainage in patients with chronic pancreatitis or common bile duct stenosis (7). Although older reports raised concerns as to possible false negatives of liver biopsy related to sampling error, recent studies included larger numbers of patients and provided large liver samples, yielding convincing results (7–9).

FIBROGENIC CELLS OF THE LIVER

The cellular source of fibrosis during chronic liver diseases has long been debated. Accumulating data clearly indicate that matrix accumulation originates from different types of smooth muscle α-actin myofibroblastic cells deriving from distinct

cell populations, known as activated hepatic stellate cells and hepatic myofibroblasts (10, 11).

In the normal liver, hepatic stellate cells compose 5% to 10% of cells and are located in the subendothelial space between hepatocytes and sinusoidal endothelial cells. Following acute or chronic liver diseases, they undergo phenotypic changes, switching from a quiescent vitamin A-rich phenotype to a myofibroblastic phenotype (referred as to activated HSC) (12). Activated hepatic stellate cells show de novo fibrogenic properties, including proliferation and accumulation in areas of parenchymal cell necrosis, secretion of proinflammatory cytokines and chemokines, and synthesis of a large panel of matrix proteins and of inhibitors of matrix degradation, leading to progressive scar formation (Figure 1).

Hepatic myofibroblasts are another source of fibrogenic cells that derive from fibroblasts of the portal connective tissue, perivascular fibroblasts of portal and central veins, and periductular fibroblasts in close contact with bile duct epithelial cells. Contribution of these cells to fibrogenesis was initially demonstrated in experimental biliary cirrhosis by showing that myofibroblastic transformation of

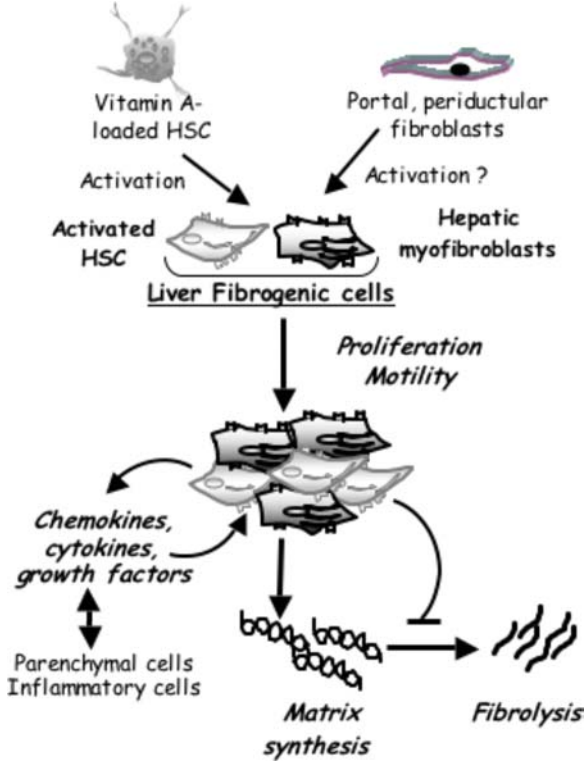

Figure 1 Main properties of liver fibrogenic cells.

portal and periductular fibroblasts precedes activation of hepatic stellate cells in the lobule (13–15).

Phenotypic and functional properties of hepatic myofibroblasts are grossly similar overall to those of activated hepatic stellate cells. However, culture studies have clearly established that several phenotypic markers distinguish both cell types, including selective expression of fibulin-2 and interleukin-6 by hepatic myofibroblasts and protease P100 and reelin by activated hepatic stellate cells (10, 11, 16, 17). Cell-specific expression of these markers has also been described in experimental models (18) and suggests that hepatic myofibroblasts derived from portal (myo) fibroblasts are present within fibrotic septa, whereas activated hepatic stellate cells are found in the subendothelial sinusoidal space close to portal tracts. Regarding biological functions, activated hepatic stellate cells show minor functional differences with hepatic myofibroblasts, such as a short life span owing to rapid apoptosis and low proliferative capacity (10). Further work is needed to fully delineate the precise contribution of each cell type to the fibrogenic process, and characterization of the fibrogenic cell lineage may provide useful information. In this respect, recent studies indicate that as yet undefined bone marrow cells constitute a significant source of hepatic stellate cells (19). In addition, bone marrow myofibroblasts represent a significant proportion of hepatic myofibroblasts in cirrhosis of diverse etiologies (20).

ROLE OF MATRIX-PRODUCING CELLS IN THE PATHOPHYSIOLOGY OF LIVER FIBROSIS

To identify targets for therapeutic intervention, numerous studies have extensively investigated functional properties of fibrogenic cells and mechanisms involved in their phenotypic activation. Selected illustrative examples are provided below.

Acquisition of the Myofibroblastic Phenotype

Mechanisms leading to the acquisition of the myofibroblastic phenotype have been characterized extensively in hepatic stellate cells (for a review, see 21) and remain ill-defined in portal fibroblasts. Briefly, activation of hepatic stellate cells is driven by factors produced by neighboring cells and by remodeling of the surrounding matrix. Thus, parenchymal injury promotes activation of Kupffer cells (resident liver macrophages); endothelial cells and platelets; and an influx of leucocytes, resulting in the generation of lipid peroxides, reactive oxygen species, and a number of cytokines such as TGF-β, interleukin-1, TGF-α, PDGF, and EGF. These factors promote induction of specific sets of transcription factors in hepatic stellate cells within hours, resulting in induction or de novo expression of a variety of cytokines and chemokines and of their receptors, which are involved in fibrogenesis. Transcription factors crucial at this step include ZF9, NFkB, and c-myb (21). Remodeling of matrix also promotes activation of hepatic stellate cells. Thus,

hepatic stellate cells cultured in a three-dimensional matrix of collagen I or matrigel retain a quiescent vitamin-A rich phenotype (22). In contrast, induction of matrix degradation is rapidly associated with acquisition of the myofibroblastic phenotype. Several lines of evidences also indicate that adhesion molecules are important mediators of matrix-induced activation of hepatic stellate cells (23).

Synthesis of Cytokines and Chemokines

Fibrogenic cells produce a variety of proinflammatory chemokines and cytokines with autocrine and paracrine effects (23). Thus, synthesis of TGF-α and TGF-β promotes activation of neighboring quiescent hepatic stellate cells, whereas the release of HGF stimulates regeneration of adjacent hepatocytes. In addition, production of MCP-1 and colony-stimulating factor contributes to the recruitment of mononuclear leucocytes.

Proliferation and Increased Survival

Accumulation of fibrogenic cells during liver injury results from a high mitogenic and an enhanced capacity to escape from apoptosis. Mitogenicity is stimulated by a large variety of growth factors expressed during chronic liver injury, including PDGF, which displays the greater promitogenic effects (23); vasoconstrictors such as thrombin (24); the metalloproteinase MMP-2 (25); or adhesion molecules such as alphaVbeta3 integrins (26). Intracellular pathways governing mitogenicity include the ERK cascade, the PI3 kinase/Akt pathway, STAT 1, production of phosphatidic acid, calcium influx, or acidification via the Na^+/H^+exchanger (23). Mechanisms limiting proliferation of fibrogenic cells have also been the focus of several studies. Typical examples include the vasodilating C-type natriuretic peptide and prostaglandins, which elicit growth inhibitory effects via cGMP and cAMP-dependent pathways, respectively (17, 24, 27, 28).

Survival factors protecting fibrogenic cells from apoptosis and enhancing their accumulation during chronic liver disease have been identified. Tumor-necrosis factor alpha and TGF-β display antiapoptotic effects for activated hepatic stellate cells in culture (29). Other examples include sphingolipid sphingosine-1-phosphate (S1P) accumulation by a pathway involving ERK and PI3 kinase activation (30) and type 1 tissue inhibitor of metalloprotinase (TIMP-1) (26, 31, 32). Finally, interaction with matrix components such as collagen I and fibronectin also plays a crucial role in survival of activated HSC, and interactions with alphaVbeta3 integrins are crucial in this process (26, 33).

Chemotaxis

Migration of fibrogenic cells toward injured areas may contribute to their accumulation at sites of injury. Migration is promoted by growth factors (e.g., PDGF, FGF-2) or chemokines (MCP-1, CCl21) produced by inflammatory cells and involving the PI3 kinase pathway (23, 34).

Fibrogenesis

The profibrogenic potential of activated hepatic stellate cells and hepatic myofibroblasts is due to their capacity to synthesize fibrotic matrix proteins and components that inhibit fibrosis degradation. Among the large number of factors identified as activators of matrix production, TGF-β, CTGF (35), and leptin (36) play a major role.

Hepatic stellate cells express a wide range of metalloproteinases (MMPs) as well as MMP activators that cleave pro-MMP into their active form. In addition, they also produce specific tissue inhibitors of the metalloproteinase family (TIMPs). Production of MMPs and TIMPs is tightly regulated according to the activation state of hepatic stellate cells, and it reflects extracellular matrix remodeling during chronic liver injury. At early stages, hepatic stellate cells express MMP-1, MMP-2, MMP-3, and MMP-9 and their activators, but do not produce TIMPs; this allows degradation of normal matrix in the subendothelial space and its substitution by fibrillar collagens. In contrast, fully activated hepatic stellate cells shut down expression of MMPs and turn on expression of TIMPs, resulting in a dramatic reduction of collagenolytic activity within the liver (37).

Strikingly, a number of cytokines simultaneously govern several functions of fibrogenic cells. Thus, TGF-β, interleukin-1, and leptin promote stellate cell activation, enhance collagen synthesis, and markedly induce TIMP-1. In addition, TGF-β also promotes cell survival (38).

EXPERIMENTAL MODELS AND ASSESSMENT OF HEPATIC FIBROSIS

Development of antifibrotic drugs requires the availability of reliable experimental systems for preclinical studies and the definition of accepted endpoints in clinical trials.

Cell Culture Models

Rodent and human cultures of hepatic stellate cells and of hepatic myofibroblasts are routinely used to define antifibrotic targets and to test potential antifibrotic drugs. Isolation of hepatic stellate cells is based on enzymatic digestion of normal liver (39), and purification of vitamin A-loaded cells through a density gradient or by cell sorting (40). Within a few days, vitamin A-rich hepatic stellate cells spontaneously acquire myofibroblastic features upon culture onto plastic. Hepatic myofibroblasts are obtained from the culture of normal liver explants and do not allow studies of the phenotypic transformation (41). Hepatic stellate cells and liver myofibroblasts culture models display phenotypic properties similar to fibrogenic cells in vivo. However, it should be stressed that several studies have used activated hepatic stellate cells after several passages and these may in fact be largely contaminated by hepatic myofibroblasts, which progressively replace hepatic stellate

cells that spontaneously undergo apoptosis (10). However, this hypothesis needs to be explored by expression profiling of passaged cells. Therefore, in the following sections, we refer to activated hepatic stellate cells or hepatic myofibroblasts, as stated in the publications. Other culture models include rodent or human hepatic stellate cell lines with myofibroblastic features obtained either spontaneously or by transfection of the coding region of SV-40 (12). However, the relevance of these models to the in vivo situation is questionable.

Animal Models

Rodent fibrosis models are widely used because of their convenient time frame. Features of the fibrogenic process depend on the nature of liver injury. Compounds such as carbon tetrachloride, dimethylnitrosamine, or galactosamine generate significant hepatocyte necrosis, associated with marked inflammation. In these models, antifibrotic effects of tested drugs may therefore result either from a direct effect on fibrogenic cells or from nonspecific antiinflammatory effects. Therefore, additional models with low degrees of cell damage and inflammation, such as bile duct ligation or thioacetamide administration, should be used in parallel to validate efficiency of an expected antifibrotic molecule. It should also be stressed that models of fibrosis recovery after cessation of chronic tetrachloride intoxication (2) or following biliodigestive anastomosis in bile duct ligated rats (25) have proved useful recently for the study of curative antifibrotic effects.

Fibrosis Staging in Humans

For years, liver biopsy has remained the gold standard for monitoring fibrosis in clinical studies. Routine staging relies on several semiquantitative scores, such as the widely used Knodell and Metavir scores. However, invasiveness of liver biopsy limits serial repetition of the procedure. Quantification of the area of fibrosis by morphometry shows greater accuracy but carries a significant coefficient of variation (42). Finally, sampling error related to the heterogeneous distribution of fibrosis occurs in 15% to 25% of cases, particularly in advanced stages. These limitations have stimulated the search for noninvasive sensitive and reliable serum markers of fibrosis. Fragments of matrix constituents released in the circulation during remodeling have not proved useful as yet, owing to inadequate diagnostic specificity, particularly for intermediate fibrosis stages. Therefore, recent efforts focused on indexes combining matrix protein markers or based on biochemical and hematological parameters, and more recently, on glycomic serum analysis (43–46). In this expanding field, the Fibrotest combining five biochemical variables currently benefits from the larger experience (45). Finally, measurement of liver elastometry also shows promising results that are currently being assessed for validation in multicenter trials (47). Obviously, validation of noninvasive surrogate markers of fibrosis will be determinant for the rapid assessment of potential antifibrotic therapies in large therapeutic trials.

ANTIFIBROTIC STRATEGIES

An ideal antifibrotic drug should be liver specific to avoid adverse effects on extrahepatic matrix proteins and should selectively attenuate excessive collagen deposition without affecting normal extracellular matrix synthesis. Efforts over the past decade have focused on fibrogenic cells generating the scarring response. Recently, inhibition of parenchymal injury and of liver inflammation has also proved of interest.

Inhibition of Parenchymal Injury

Several studies have shown that during chronic liver injury, hepatocyte and biliary epithelial cells undergo apoptotic cell death. Interestingly, a direct link between hepatocyte apoptosis and liver fibrogenesis has recently been demonstrated in several experimental models. Thus, Fas-deficient lymphoproliferation (lpr) mice show decreased inflammation and fibrosis following bile duct ligation (48). Similarly, immune-mediated liver fibrosis induced by repeated concanavalin A administration is strongly reduced by Fas-specific small interfering RNA (49). These data therefore suggest that inhibiting hepatocyte apoptosis and thereby liver inflammation is an interesting approach for the prevention of liver fibrosis. Proof of concept of this strategy is supported by the demonstration that IDN-6556, a general inhibitor of caspases currently undergoing phase II clinical studies (50), reduces hepatocyte apoptosis and fibrosis in a mouse model of bile duct ligation (51). Although this approach appears promising, administration of molecules interfering with hepatocyte apoptotic pathways may carry a high risk of carcinogenesis on the long term, particularly at the cirrhotic stage, and therefore, this option should be considered at early stages of chronic liver diseases.

Reduction of Liver Inflammation

Inflammation is commonly associated with progression of liver fibrosis during chronic liver diseases. Moreover, leucocytes and Kupffer-derived products stimulate fibrogenic properties of activated hepatic stellate cells and hepatic myofibroblasts. These observations have stimulated studies investigating the effect of antiinflammatory strategies. In this respect, beneficial effects have been observed with inducers of Kupffer cell apoptosis, such as inhibitors of the 5-lipoxygenase pathway, which reduce inflammation and liver fibrosis induced by carbon tetrachloride (52). Interleukin-10 has also been investigated, based on its beneficial effect on the proinflammatory Th1 response. It was shown that IL-10 deficient mice develop greater inflammation and fibrosis than wild-type mice (53, 54). In keeping with these findings, a small pilot trial of interleukin-10 in -24 patients with chronic hepatitis C showed improvement of inflammation and was associated with a decrease in fibrosis (55).

HEPATIC MYOFIBROBLASTS AS TARGETS OF ANTIFIBROTIC DRUGS

Antifibrotic strategies based on inhibition of the scarring response have been extensively studied. Targets include (*a*) inhibition of hepatic stellate cell activation, (*b*) reduction of fibrogenic cell accumulation by growth inhibitory or proapoptotic compounds, and/or (*c*) reduction of extracellular matrix synthesis or enhancement of its degradation. Efficacy of these various strategies has been demonstrated with several molecules in experimental models of liver fibrosis (see Table 1). However, there are currently no molecules with demonstrated antifibrotic activity in humans. The following section depicts selected examples of promising approaches.

Modulation of Cytokine Production and/or Activity

Inhibition of fibrogenic cytokines overproduced within the injured liver has been extensively investigated. The most extensively studied strategy relates to inhibition of TGF-β signaling pathways.

TRANSFORMING GROWTH FACTOR-β TGF-β is markedly overproduced by a variety of cells during chronic liver injury. The cytokine stimulates several steps of the profibrogenic pathway, including phenotypic activation of hepatic stellate cells, enhancement of survival, stimulation of matrix production, and overexpression of TIMP-1 (38). The crucial role of TGF-β is supported by studies showing that overexpression of TGF-β in transgenic animals induces spontaneous liver fibrosis (56).

TGF-β-signaling pathways have been extensively characterized. The cytokine is synthesized as a latent form (LAP) linked to a glycoprotein (latent TGF-β binding protein, LTBP), which anchors the complex to the extracellular matrix (ECM). Proteolytic cleavage of LTBP by plasmin generates active TGF-β, which binds type I and type II receptors associated as heterodimers. Activation of TGF-β RII results in transphosphorylation of TGF-β RI and subsequent phosphorylation of cytoplasmic Smad transducers in cascade, leading to transcription of target genes. Finally, several nuclear oncoproteins such as Smad 7 antagonize the cytoplasmic Smad cascade and limit TGF-β effects (57).

Several anti-TGF-β strategies targeting various signaling steps have proved effective. Thus, inhibition of activation of latent TGF-β by the serine protease inhibitor camostat mesilate prevents and attenuates liver fibrosis induced by porcine serum (58). Prevention of TGF-β binding to type II receptor has also been achieved either by administration of an adenovirus encoding dominant negative truncated form of human TGF-β RII (59) or by treatment with a soluble surrogate type II receptor engineered by the fusion of the Fc portion of immunoglobulin G and the ectodomain of TGF-β RII (60). In both cases, liver fibrosis was strongly attenuated in experimental models. Inhibition of intracellular signaling steps in the TGF-β

TABLE 1 Main potential antifibrogenic compounds

Compound	↓ density of fibrogenic cells in vitro	↓ fibrogenesis and/or fibrolysis in vitro	Antifibrotic effects in animals	Reference(s)
Adiponectin	+	ND	+	(116)
Amiloride	+	+	+	(125)
Antiangiotensin	+	+	+	(86, 88, 91, 92)
Antioxidants (tocopherol, resveratrol, sylimarin, S-adenosylmethionine, Sho-saiko-to. . .)	+	+	+	(67–70)
Anti-TGF-β	+	+	+	(58–61)
Cannabinoid receptor 1 antagonism	ND	ND	+	(121)
Cannabinoid receptor 2 agonism	+	ND	+	(74)
Endothelin A receptor antagonists	ND	ND	+	(84)
Endothelin B receptor agonists	+	ND	ND	(28, 81, 83)
Gliotoxin	+	ND	+	(102, 103)
Halofuginone	+	+	+	(126)
Integrin antagonists	+	+	+	(26, 127)
Interleukin-10	ND	+	+	(53, 54)
Interferon-α	+	+	+	(62)
Interferon-γ	+	+	+	(62)
Noradrenergic antagonists	+	+	+	(128, 129)
Pentoxifylline	+	+	+	(130, 131)
15-D-prostaglandin J2	+	+	+	(73, 104–107)
Prostaglandin E2	+	ND	+	(17, 24, 28, 83, 95)
Sphingosine-1 phosphate	+	+	ND	(17, 30)
Thiazolinediones	+	+	+	(104–106, 108)

signaling pathway may also reduce liver fibrogenesis, as shown by the beneficial effects of an adenovirus carrying Smad 7 cDNA in the bile duct ligation model (61).

Although attractive given their efficiency, systemic anti-TGF-β strategies may be limited by adverse effects, such as the risk of autoimmune disease secondary to its prominent immunoregulatory properties.

OTHER CYTOKINES Among antifibrogenic Th1 cytokines, interferons have been the subject of extensive studies. Interferon-α and interferon-γ inhibit activation, proliferation, and collagen synthesis in cultures of activated hepatic stellate cells and hepatic myofibroblasts (62); in addition, both cytokines directly inhibit collagen gene transcription in vivo and reduce progression of fibrosis, as shown in a model of transgenic mice harboring the α2(I) collagen gene (63). In keeping with these experimental findings, studies in patients with chronic hepatitis C suggest that IFN-α may improve the stage of fibrosis irrespective of virological response, suggesting a direct inhibitory effect of the cytokine on fibrosis progression (5, 64). This hypothesis is being further evaluated in several ongoing clinical trials. Beneficial effects of hepatocyte growth factor (HGF) delivered as a recombinant protein or by gene therapy have also been reported following dimethylnitrosamine administration (65). However, HGF being a promitogenic factor for parenchymal cells, long-term administration raises concern as to the risk of epithelial tumors.

Reduction of Oxidative Stress

Oxidative stress has been detected in the vast majority of experimental and clinical chronic liver diseases (66). Several lines of evidence suggest that oxidative stress modulates fibrogenic properties of activated hepatic stellate cells and hepatic myofibroblasts. Thus, activation of hepatic stellate cells is associated to oxidative stress and may be prevented by antioxidants, such as α-tocopherol or resveratrol. In addition, extracellular reactive oxygen species originating from Kupffer cells, mononuclear cells, and polymorphonuclear cells stimulate transcription of collagen genes (66). In keeping with these observations, antioxidant compounds such as α–tocopherol (67), the flavonoid sylimarin (68), the Japanese herbal medicine Sho-saiko-to (69), and resveratrol (70) display antifibrogenic properties in cell cultures and in experimental animal models (Table 1). However, data from clinical trials are often conflicting or disappointing compared with results in experimental models (67, 71, 72). Discrepancies are probably related to several factors, including the use of inadequate low dosages in clinical trials, the short time frame of treatment, and the possible inefficiency of antioxidants at late stages of fibrosis. Finally, the role of reactive oxidative stress may be more subtle than merely profibrogenic. Indeed, we recently showed that intracellular oxidative stress mediates antifibrogenic properties of 15-D-PGJ2 and cannabinoids in hepatic myofibroblasts (73, 74; see below). Therefore, future studies should further clarify the properties of specific reactive intermediates.

Modulation of Vasoactive Peptides

A number of vasoregulatory peptides are overproduced during liver fibrogenesis and show pro- or antifibrogenic properties. These observations have stimulated assessment of pharmacological activator or inhibitors of these compounds. Endothelin-1, the angiotensin system, and prostaglandins have provided the most convincing data.

ENDOTHELIN-1 Endothelin-1 is a potent vasoconstrictor that binds at least two G protein–coupled receptors, ETA and ETB (75–77). Investigation of the role of endothelins in liver fibrogenesis was stimulated by the finding that both endothelin-1 and its receptors are markedly induced in fibrogenic cells during chronic liver diseases (78, 79) and by the previous demonstration of a profibrogenic role of the peptide in kidney fibrogenesis (80). Culture studies have shown that endothelin-1 displays dual pro- and antifibrogenic effects in the liver according to receptor subtype: thus, binding of ETA receptors stimulates activation of hepatic stellate cells and induces a weak mitogenic effect. In contrast, binding of ETB receptors promotes marked growth inhibition (28, 81) by a mechanism involving the sequential generation of sphingosine-1-phosphate (S1P), cyclooxygenase-2 (COX-2)-derived prostaglandins, and elevation of cAMP (28, 82, 83). Therefore, these results suggested that antifibrotic effects may be achieved by selectively inhibiting ETA receptors, whereas beneficial antifibrogenic effects of ETB receptors should be protected, or even better enhanced. In keeping with these in vitro studies, administration of a selective ETA receptor antagonist prevents the development of liver fibrosis in bile duct–ligated rats (84), whereas treatment with a nonselective ETA/ETB receptor antagonist accelerates liver fibrosis in carbon tetrachloride-treated rats (85).

THE ANGIOTENSIN SYSTEM Angiotensin II is involved in cardiac and kidney fibrogenesis, and several recent studies support a significant role in liver fibrosis. AT1 receptors are upregulated in fibrotic areas during experimental liver fibrosis (86). Accordingly, cultured activated stellate cells express AT1 receptors and produce angiotensin II in response to growth factors via the renin angiotensin system (87). Furthermore, activation of AT1 receptors stimulates secretion of TGF-β and proliferation of cultured activated stellate cells (88, 89). Finally, the relationship between angiotensin II and liver fibrogenesis is supported by experimental and clinical studies. Thus, mice invalidated for AT1 receptors show reduced liver fibrosis following administration of carbon tetrachloride (90). These observations are corroborated by the beneficial effect of angiotensin antagonism in experimental models of liver fibrosis, whether using angiotensin inhibitors or antagonists of AT1 receptors (88, 91, 92). In patients with chronic hepatitis C, there is a statistically significant relationship between inheritance of a high angiotensinogen-producing genotype and progression of hepatic fibrosis (93). Finally, a controlled pilot study in hepatitis C recently showed that losartan reduces liver fibrosis as compared to

untreated controls (94). Multicenter prospective trials assessing angiotensin antagonism in liver fibrosis are currently under way.

PROSTAGLANDINS A number of studies have demonstrated antifibrogenic potential of prostaglandins. Thus, PGE2 reduces fibrosis progression in bile duct–ligated rats (95). Beneficial effects are related to inhibition of proliferation and collagen synthesis in hepatic myofibroblasts and activated hepatic stellate cells, as shown in culture studies (95, 96). Interestingly, we have shown that growth inhibitory effects of several factors, such as endothelin-1, TNF-α, and S1P, involve induction of COX-2 and subsequent generation of PGE2 (17, 83, 96). Finally, we also demonstrated that the mitogenic effects of PDGF-BB and thrombin result from a balance between a promitogenic pathway and a parallel COX-2-dependent growth inhibitory pathway (24). Together, these data point to COX-2 as a source of antifibrogenic prostaglandins in the liver.

Enhancement of Apoptosis

It has been demonstrated conclusively in experimental models that apoptosis of hepatic fibrogenic cells is a key mandatory step in the recovery process following fibrosis induction. Thus, available data indicate that during liver fibrogenesis, proliferation of fibrogenic cells predominates over spontaneous apoptosis, whereas cessation of liver injury is associated with a reduction of proliferation and a marked increase in apoptosis. Importantly, apoptosis of fibrogenic cells is accompanied by a restoration of the collagenolytic capacities of MMP-1 and MMP-2 in the liver, subsequent to a decrease in TIMP-1 and TIMP-2 expression, which allows progressive matrix degradation (2, 97).

These observations have been strong incentives to characterize pathways regulating apoptosis and survival of fibrogenic cells. Available studies have been performed mainly in cultures and have identified a number of apoptotic stimuli. Classical apoptotic factors such as Fas-L, TRAIL 2, and TRAIL 5, and their receptors Fas and TRAIL, are upregulated during transition of hepatic stellate cells to their activated myofibroblastic phenotype (98–100). Other receptor-mediated stimuli include nerve growth factor and benzodiazepines (25, 101); however, expression of the benzodiazepine receptor is transient and declines in activated hepatic stellate cells. Nonreceptor-mediated apoptosis of hepatic myofibroblasts also occurs in response to a COX-2-derived prostaglandin, 15-deoxy $\Delta^{12,14}$ prostaglandin J2 (15-D-PGJ2) (73). Furthermore, we have also recently shown that hepatic myofibroblasts undergo apoptosis following exposure to sphingomyelinase metabolites, including ceramide, sphingosine, and sphingosine-1-phosphate (S1P) (30). Investigation of the role of S1P arose from the findings that hepatic myofibroblasts express Edg receptors for the molecule (17, 30) and that sphingosine kinase activity is increased in carbon tetrachloride–treated rats (P. Grenard, T. Levade, A. Mallat & S. Lotersztajn, unpublished results). We found that S1P stimulates two parallel pro- and antiapoptotic pathways in human hepatic myofibroblasts, probably

via distinct receptors. The apoptotic signal is mediated by caspase-3, whereas the survival signal is conveyed by activation of ERK and PI3K (30).

Two experimental studies using the fungal toxin gliotoxin have documented the potential efficiency of a proapoptotic strategy in vivo. It was shown that the compound kills activated hepatic stellate cells in culture (102), and that in both carbon tetrachloride- and thioacetamide-treated rats, treatment with gliotoxin reduces the number of fibrogenic cells and decreases fibrosis (102, 103). A major issue of a proapoptotic strategy is that of cell specificity because nonselective effects may result in life-threatening side effects, such as severe or fulminant hepatitis.

Emerging Therapeutic Targets

Potential new antifibrotic targets have been recently described. Selected examples are described below.

LESSONS FROM ADIPOCYTES Recent studies point to similar regulatory mechanisms in liver fibrogenic cells and in adipocytes.

PPAR γ Agonists Peroxisome proliferator activated receptor gamma (PPAR γ), a member of the nuclear receptor superfamily of ligand-dependent transcription factors, is predominantly expressed in adipocytes and plays a key role in the regulation of adipogenesis. PPAR γ binds antidiabetic thioazelinediones compounds, as well as eicosanoids (namely, 15-D-PGJ2), that display antiinflammatory, growth inhibitory, and apoptotic properties. Expression of PPAR γ decreases during activation of hepatic stellate cells to almost undetectable levels (73, 104–106), but is reexpressed upon exposure to PPAR γ agonists. Moreover, thioazelinediones and 15-D-PGJ2 inhibit the main fibrogenic properties of activated hepatic stellate cells and hepatic myofibroblasts via PPAR γ-dependent and independent mechanisms (73, 105–107). Finally, thiazolininediones decrease fibrosis progression in several experimental models (108), suggesting that these compounds may represent a promising approach for the treatment of liver fibrosis.

Leptin Leptin, an obese gene product, is a potent adipocyte-derived hormone that controls energy balance and food intake through widely expressed receptors (OB-R). Leptin serum levels are increased in patients with alcoholic cirrhosis (109), and in patients with chronic hepatitis C (110). In addition, leptin is an independent predictor of the severity of fibrosis in alcoholic cirrhosis (110). Liver fibrogenesis is reduced in mice with leptin deficiency (ob/ob) or bearing mutations in leptin receptor (db/db and fa/fa), supporting a profibrogenic role of leptin. Accordingly, the peptide is undetectable in the normal liver and is produced by activated hepatic myofibroblasts in vitro and in vivo during fibrogenesis elicited by thioacetamide (111, 112). The precise mechanism of action of leptin during liver fibrogenesis is not clearly defined but may involve direct effect on matrix synthesis by myofibroblasts and upregulation of TGF-β synthesis by liver cells (111, 112). These observations suggest that antagonists of leptin receptors should be investigated as antifibrotic agents.

Adiponectin Adiponectin is also produced by adipocytes and acts as a major insulin-sensitizing hormone by increasing glucose uptake and fat oxidation in muscle and reducing fatty acid uptake and hepatic glucose production (113). Decreased circulating levels of adiponectin are found in patients with obesity, insulin resistance, type 2 diabetes, and NASH, and administration of adiponectin causes glucose-lowering effects, ameliorates insulin resistance in mice, and alleviates nonalcoholic steatohepatitis (113, 114). The peptide binds two receptors, R1, with an ubiquitous distribution, and R2, which predominates in the liver (115). Several recent lines of evidence support an antifibrogenic role of adiponectin during chronic liver diseases. Thus, mice knocked-out for adiponectin show enhanced liver fibrosis following chronic administration of carbon tetrachloride, whereas treatment with an adenovirus encoding adiponectin reduces liver fibrogenesis in wild-type mice (116). Recent studies have partially elucidated targets of adiponectin in fibrogenic cells and show that the peptide reduces proliferation and migration of activated hepatic stellate cells as well as TGF-β1-induced collagen synthesis. Unexpectedly, serum adiponectin levels are elevated in patients with cirrhosis, suggesting that the peptide may counteract progression of fibrosis at advanced stages (117). Although promising, these results await confirmation when pharmacological agonists of adiponectin receptors are available.

CANNABINOIDS The cannabinoid Δ9-tetra-hydrocannabinol (THC) is the main psychotropic constituent of *Cannabis sativa* and exerts a wide array of effects via two G protein–coupled receptors, CB1 and CB2. Recently, THC has been FDA-approved for the treatment of nausea following chemotherapy and the treatment of anorexia and weight loss in immunocompromised patients (118). There is also growing interest in the use of pharmacological antagonists of cannabinoid receptors, and the CB1 antagonist SR141716A (Rimonabant) is currently being evaluated in phase III trials for the treatment of obesity and tobacco withdrawal (119). Several studies also indicate that cannabinoids may also be potential antineoplastic agents owing to their ability to induce regression of various types of tumors. These antineoplastic effects are mainly attributed to antiproliferative and apoptotic properties of CB2 receptors (120).

We have recently demonstrated that the cannabinoid system may be a crucial regulator of liver fibrogenesis. Thus, CB1 and CB2 receptors are marginally expressed in the normal liver and undergo marked upregulation in the cirrhotic liver, predominating in smooth muscle α-actin expressing cells within fibrotic septa (74). Strikingly, functional studies show that CB1 and CB2 receptors display opposite effects on liver fibrogenesis. Thus, in human hepatic myofibroblasts, selective activation of CB2 receptors triggers two antifibrogenic properties, growth inhibition and apoptosis (74). Moreover, CB2 knock-out mice develop enhanced liver fibrosis following chronic carbon tetrachloride treatment, demonstrating an antifibrogenic role of CB2 receptors. In contrast, CB1 knock-out mice show reduced fibrosis following carbon tetrachloride administration, indicating a profibrogenic role of CB1 receptors (121). In keeping with these results, we have shown that daily cannabis smoking is an independent predictor of fibrosis progression in patients with chronic

hepatitis C (122). These promising results obviously warrant investigation of the effects of pharmacological antagonists of CB1 receptors and of selective agonists of CB2 receptors.

DRUG TARGETING As outlined in this review, a number of antifibrotic approaches are limited by the lack of cell and or tissue specificity, with a high risk of potentially severe adverse side effects. Recently, drug carriers have been designed that specifically target liver fibrogenic cells. According to this approach, selected antifibrotic compounds are covalently linked to a cyclic peptide that selectively binds receptors specifically expressed and upregulated in liver fibrogenic cells. Examples of carriers showing the desired cell specificity include the sugar mannose 6-phosphate/insulin-like growth factor II (M6P/IGF II), which binds the M6P/IGFII receptor, and a peptide selective for the PDGF-BB receptor and collagen VI receptor (123, 124). Such carriers appear promising for targeted delivery of antifibrotic agents.

CONCLUSION

During the past decade, characterization of molecular mechanisms of liver fibrogenesis and resolution has revealed novel approaches for therapeutic intervention based on interference with major pro- or antifibrogenic pathways in liver fibrogenic cells. A large number of approaches have been validated in culture studies and in animal models. Clinical trials are underway or anticipated for a growing number of molecules, and will obviously be facilitated by the availability of noninvasive methods for staging fibrosis. However, proof of effectiveness is still lacking in humans. Combination of drugs with distinct antifibrogenic actions may result in therapeutic benefits at low dosages and reduce the risk of unwanted side effects.

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