Pathogenesis of Liver Fibrosis

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Abstract

Liver fibrosis is a major cause of morbidity and mortality worldwide due to chronic viral hepatitis and, more recently, from fatty liver disease associated with obesity. Hepatic stellate cell activation represents a critical event in fibrosis because these cells become the primary source of extracellular matrix in liver upon injury. Use of cell-culture and animal models has expanded our understanding of the mechanisms underlying stellate cell activation and has shed new light on genetic regulation, the contribution of immune signaling, and the potential reversibility of the disease. As pathways of fibrogenesis are increasingly clarified, the key challenge will be translating new advances into the development of antifibrotic therapies for patients with chronic liver disease.

INTRODUCTION

Hepatic fibrosis: a wound-healing response to either acute or chronic liver injury; characterized by the accumulation of interstitial or fibrillar ECM

Extracellular matrix (ECM): tightly organized molecular network that provides functional and structural integrity for liver parenchyma

Cirrhosis: end-stage liver disease characterized morphologically by the presence of diffuse fibrosis, regenerative nodules, and distortion in both liver parenchyma and vascular architecture

HSC: hepatic stellate cell

KC: Kupffer cell

MF: myofibroblast(s)

Hepatic fibrosis is a reversible wound-healing response characterized by the accumulation of extracellular matrix (ECM) following liver injury. If the insult is acute or self-limited, these changes are transient, and liver architecture is restored to its normal composition. However if the injury is sustained, chronic inflammation and accumulation of ECM persist, leading to a progressive substitution of liver parenchyma by scar tissue. This process results in cirrhosis, the end consequence of progressive fibrosis, which can have a poor outcome and high mortality. Progression to this end stage is typically variable but slow, developing over 20 to 40 years in patients with chronic liver injury; the pace is influenced by both genetic and environmental factors.

The hepatic parenchyma is composed of epithelial cells (hepatocytes), endothelial cells, and resident nonparenchymal cells, including hepatic stellate cells (HSCs) and Kupffer cells (KCs). The sinusoid is the hepatic microvascular unit. It has an endothelial lining distinguished by fenestration of pores and is separated from the hepatocytes by the subendothelial space of Disse, where HSCs reside. This space contains a low-density basal membrane–like matrix that is essential for maintaining the differentiated function of parenchymal cells yet is sufficiently porous to enable metabolic exchange between the bloodstream and hepatocytes.

CLINICAL AND PATHOLOGIC CONTEXTS

Deposition of ECM in the space of Disse leads to loss of the normal fenestrations that are characteristic of the endothelial lining, which provokes the impairment of the normal bidirectional metabolic exchange between portal venous flow and hepatocytes. This process is termed capillarization of the sinusoids (1).

Different patterns of fibrosis progression have been described on the basis of their etiology, the region of injury (e.g., portal or central), the source of fibrogenic cells involved, and the predominant fibrogenic mechanism(s) (**Figure 1**) (2). Chronic viral hepatitis B and C are the major causes of bridging fibrosis, which is characterized by the presence of interface hepatitis and portal-central vein bridging necrosis, resulting in the formation of portal-central fibrotic septa. Perisinusoidal or pericellular fibrosis are typically found in alcohol-related disorders and nonalcoholic fatty liver disease. Alcohol-related fibrosis is characterized by deposition of ECM in the space of Disse around sinusoids or hepatocytes (chicken-wire pattern). Biliary fibrosis incorporates the proliferation of bile ductules and periductular myofibroblasts (MF), which leads to the formation of portal-portal fibrotic septa surrounding liver nodules. Conditions that alter venous outflow are the main cause of centrolobular fibrosis, which is characterized by central-central fibrotic septa.

Progression of disease with sustained fibrogenesis leads to cirrhosis, which is not merely the end-stage accumulation of scar, but rather is characterized by a distortion of the liver parenchyma and vascular architecture. The main pathological feature of cirrhosis is the formation of nodules of regenerative parenchyma surrounded by fibrotic septae, which may incorporate terminal hepatic venules and portal tracts when the nodules are especially large (i.e., macronodular cirrhosis). Porto-systemic shunts and venous occlusion often occur, leading to impairment in liver function and the development of portal hypertension. The formation of vascularized fibrous septa that link portal tracts and central veins is stimulated by angiogenesis and contributes to porto-systemic shunting that bypasses the liver parenchyma (**Figure 2**) (3).

Whereas hepatic fibrosis is largely asymptomatic, progression to cirrhosis confers a risk of significant morbidity and mortality. Among digestive diseases, cirrhosis is the most common nonneoplastic cause of mortality in the United

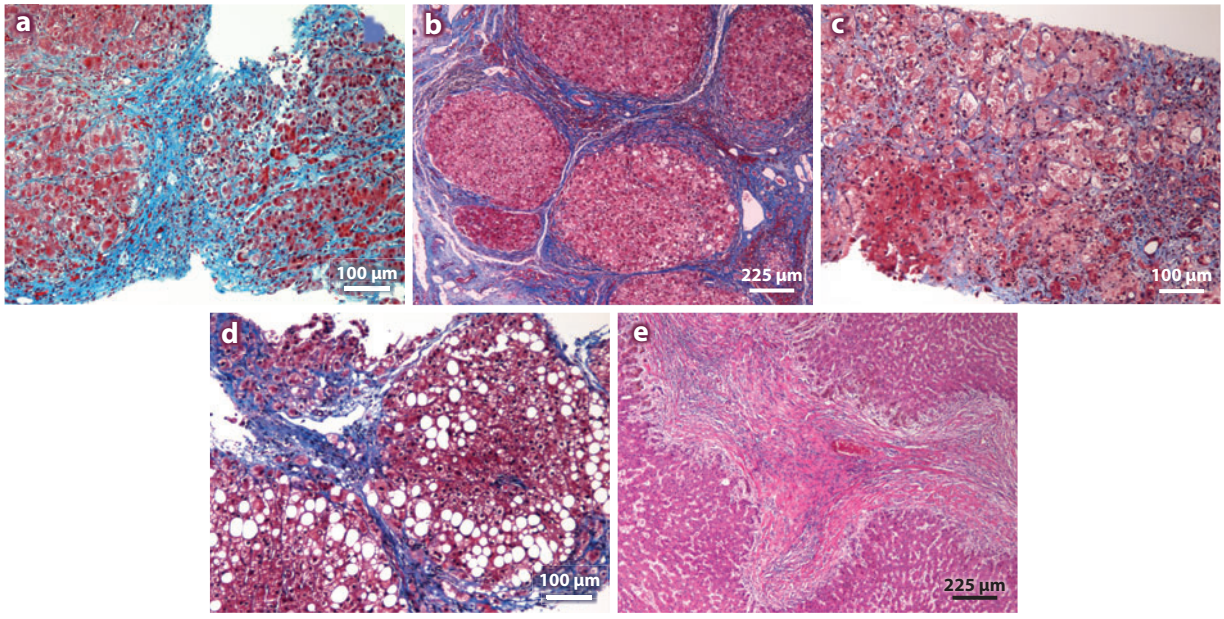


Figure 1

Photomicrographs of fibrosis patterns in different etiologies of liver disease. (*a*) Autoimmune hepatitis. Portal-central vein bridging necrosis. (*b*) Chronic viral hepatitis C. Trichrome staining showing portal-central fibrotic septa and nodule formation. (*c*) Acute alcoholic hepatitis. Deposition of extracellular matrix around hepatocytes (so-called chicken-wire pattern) and ballooning degeneration of hepatocytes. (*d*) Nonalcoholic steatohepatitis. Trichrome staining showing macrovesicular steatosis and pericellular fibrosis. (*e*) Biliary cirrhosis. Portal-portal fibrotic septa and proliferation of bile ductules. Images reproduced courtesy of Dr. M. Isabel Fiel, Mount Sinai School of Medicine.

States, causing 30,000 deaths per year. An additional 10,000 deaths occur due to liver cancer, which usually arises in the setting of cirrhosis (4). Once cirrhosis has developed, its natural history typically includes progression from a compensated phase to a decompensated phase; the latter is defined by the development of portal hypertension and liver failure. Portal hypertension is therefore a major complication of cirrhosis that leads either to death or to the need for liver transplantation.

According to the principle of Ohm's law ($P = Q \times R$, where P is the change in pressure along a vessel, Q is the flow, and R is the resistance to that flow), portal hypertension may arise from increased hepatic resistance to blood flow and/or from increased flow. The initial event in the pathophysiology of portal hypertension is increased vascular resistance that occurs mainly in the sinusoids.

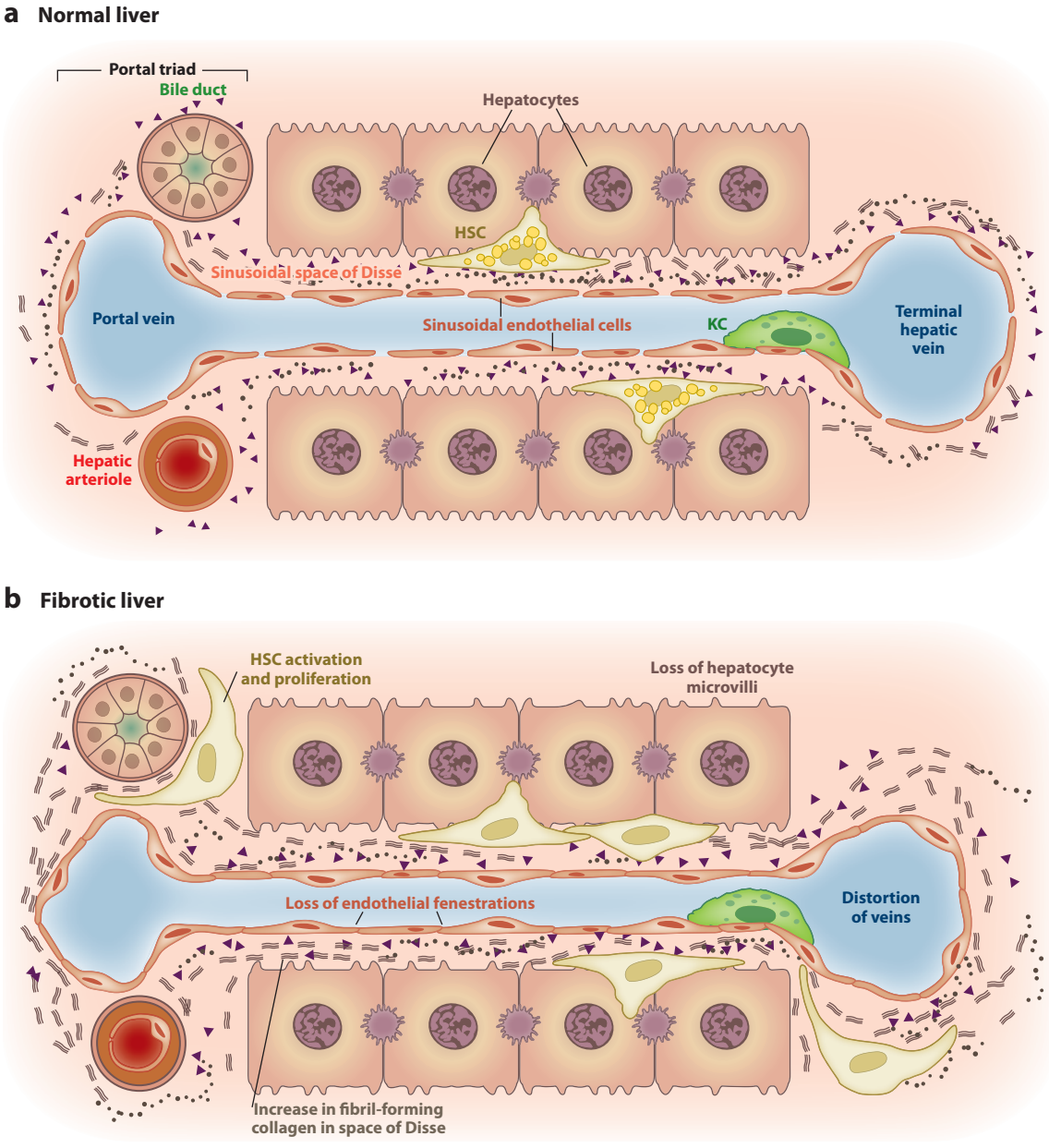
COMPOSITION OF THE HEPATIC SCAR, OR EXTRACELLULAR MATRIX, OF LIVER

In normal liver, ECM is a highly dynamic substratum with a precisely regulated balance between synthesis and degradation. During chronic liver injury, however, ECM production exceeds ECM degradation, and hepatic fibrosis develops as a result of the progressive thickening of fibrotic septae and chemical cross-linking of collagen. Moreover, these changes in ECM composition directly stimulate fibrogenesis (5).

Hepatic fibrosis affects both the quality and quantity of hepatic ECM, which is a tightly organized molecular network that provides functional and structural integrity for liver parenchyma (5). Normally, the hepatic ECM comprises less than 3% of the relative area on a liver tissue section, and approximately 0.5%

of the wet weight (6). It is also a component of Glisson's capsule, portal tracts, central veins, and the subendothelial space of Disse. The most important structural ECM components in liver are collagen, proteoglycans, laminin, fibronectin, and matricellular proteins.

The low-density basement membrane–like matrix of the space of Disse in normal liver



is composed mainly of collagens IV and VI. After liver injury, disruption of this matrix and replacement by fibrillar collagens occur; this matrix is composed of collagens I and III and fibronectin (7, 8). These quantitative and qualitative changes in ECM composition (termed capillarization; see above) alter the matrix microenvironment and create a functional and physical impediment to the bidirectional flow of plasma between sinusoidal lumen and hepatocytes, which leads to altered hepatic function.

In addition to incorporating structural molecules, ECM also incorporates a range of growth factors and matrix metalloproteinases (MMPs) that are specifically bound and preserved in latent forms (5). ECM may thereby regulate cellular activity and the availability of growth factors. For instance, decorin and biglycan, two ECM components, bind transforming growth factor β (TGF-β); fibronectin and laminin bind tumor necrosis factor α (TNF-α); and collagen binds platelet-derived growth factor (PDGF), hepatocyte growth factor (HGF), and interleukin (IL)-2. The binding of survival factors to the ECM may prevent apoptosis in the damaged liver and also prevent growth factor proteolysis (9).

Interactions between ECM and its surrounding cells are bidirectional. After injury, ECM can modulate the activation and proliferation of HSC, angiogenesis, and the availability and activity of growth factors and MMPs. ECM also provides cells with signals for polarization, adhesion, migration, proliferation, survival, and differentiation. ECM-cell interactions are determined largely by specific membrane adhesion receptors. Among these receptors, the integrin family, ADAM (a disintegrin and metalloproteinase domain) molecules, and discoidin domain receptors have been the most extensively studied, as detailed below.

Integrins are heterodimeric transmembrane receptors composed of α- and β-subunits; they have a globular head domain that can bind components of the ECM and cell adhesion molecules (10). Classic integrin ligands contain an arginine–glycine–aspartic acid sequence, which is necessary but not sufficient for signal transduction. Integrins can also modulate signal transduction pathways downstream of other receptors following cell adhesion (11). Cultured HSCs express α1β1, α2β1, α5β1, and α6β4 (12). Upregulation of α2β1, α6β4, αVβ8, and α5β1 has been identified in experimental hepatic fibrosis models; however, cholestatic human diseases are also associated with αVβ6 induction (13). Integrins can also interfere with TGF-β1, PDGF, and hedgehog signaling pathways, and they play a role in cancer biology, including hepatocarcinogenesis (14–16).

ADAM molecules are a family of ECM membrane receptors implicated in liver fibrosis. In liver, two molecules have been identified: ADAMSTS-13 and ADAMSTS-1, which are expressed by HSCs and endothelial cells, respectively (17, 18).

Discoidin domain receptor 2 (DDR2) is a tyrosine kinase receptor activated primarily by collagen type I and secondarily by collagens II, III, and V. DDR2 contributes to HSC activation and epithelial-to-mesenchymal transition (EMT) (19–21). Increased production of collagen type I during HSC activation induces DDR2 via its phosphorylation, leading to an increase in MMP-2 production and growth stimulation. Higher expression of DDR2 has also

MMP: matrix metalloproteinase

HSC activation: transition following liver injury of a resident nonparenchymal cell type from a quiescent vitamin A–rich cell to a proliferative, contractile, and highly fibrogenic MF cell type

EMT: epithelial-to-mesenchymal transition

Figure 2

Matrix and cellular alteration in hepatic fibrosis. Normal liver parenchyma contains epithelial cells (hepatocytes) and nonparenchymal cells: fenestrated sinusoidal endothelium, hepatic stellate cells (HSCs), and Kupffer cells (KCs). (*a*) Sinusoids are separated from hepatocytes by a low-density basement membrane–like matrix confined to the space of Disse, which ensures metabolic exchange. Upon injury, the HSCs become activated and secrete large amounts of extracellular matrix (ECM), resulting in progressive thickening of the septa. (*b*) Deposition of ECM in the space of Disse leads to the loss of both endothelial fenestrations and hepatocyte microvilli, which results in both the impairment of normal bidirectional metabolic exchange between portal venous flow and hepatocytes and the development of portal hypertension. Adapted from Reference 216 with permission.

been demonstrated in the small bile ducts of patients with primary biliary cirrhosis (22).

qHSC: quiescent hepatic stellate cell

CELLULAR SOURCES OF EXTRACELLULAR MATRIX IN LIVER

The scarring response of liver is a common pathway that results from a range of injuries, including, toxic, metabolic, and viral insults. Underlying this response is the activation of resident mesenchymal cells into contractile MF, primarily derived from HSCs, that generate scar, which encapsulates injury. HSCs are a resident mesenchymal cell type located in the subendothelial space of Disse, interposed between sinusoidal endothelium and hepatocytes (12). Following liver injury, HSCs become activated, which leads to the conversion of a resting vitamin A–rich cell [a quiescent HSC (qHSC)] to one that has lost vitamin A droplets, leading to increased proliferation and contraction and the release of proinflammatory, profibrogenic, and promitogenic cytokines. These activated cells are capable of enhanced migration and deposition of ECM components (23, 24).

HSC activation can be conceptually divided into two phases: initiation and perpetuation (25). Initiation, also known as the preinflammatory stage, refers to early changes in gene expression and phenotype. It is the result of primarily paracrine stimulation from damaged parenchymal cells. Maintenance of these stimuli leads to a perpetuation phase regulated by autocrine and paracrine stimuli. Perpetuation involves at least six distinct changes in HSC behavior, including proliferation, chemotaxis, fibrogenesis, contractility, matrix degradation, and retinoid loss (23).

MF are the prototypical mesenchymal cell type regulating repair following injury in a range of tissues, including liver, kidney, skin, lung, and bone marrow, as well as the central nervous system (26). MF are defined primarily by their ability to produce ECM and exhibit contractile activity. Although HSCs are the primary source of this fibrogenic population in the liver (23), contributions from other cells, listed below, are increasingly being appreciated (**Figure 3**).

1. Portal fibroblasts. Because of their location within the connective tissue of portal areas, the recruitment and activation of resident fibroblasts into MF are especially relevant in diseases associated with ischemia and cholestasis (27, 28). Increasing attention is being focused on the identification, purification, and analysis of this fibrogenic population, whose contribution to fibrosis is especially important in biliary diseases (29).
2. Bone marrow–derived cells and circulating fibrocytes. Several studies have demonstrated that, following liver injury, the bone marrow supplies MF-like cells that may participate in the progression of liver fibrosis (30–33). However, the contribution of these bone marrow–derived cells to collagen production during liver injury may be limited (34).
3. EMT. Epithelial cells can contribute to the replacement of dead or damaged hepatic cells through a biological process known as EMT. This process allows a closely attached epithelial cell with apical-basal polarity to migrate and accumulate in the interstitium of the tissue and acquire a mesenchymal cell phenotype (e.g., migratory capacity, invasiveness, resistance to apoptosis, and production of ECM) (35–37). EMT has been associated not only with tissue regeneration and fibrosis but also with embryonic development and cancer progression (38).

During chronic liver inflammation, cells with characteristic markers of epithelial cells (cytokeratin, E-cadherin) and mesenchymal cells [α smooth muscle actin (ASMA), fibroblast-specific protein 1 (FSP1)] appear to represent an intermediate stage of EMT (39, 40). Signals that induce and regulate EMT after injury have been extensively studied in carcinogenesis and in lung and kidney fibrosis. After liver injury, the most important trigger to EMT is the release of chemokines, MMPs,

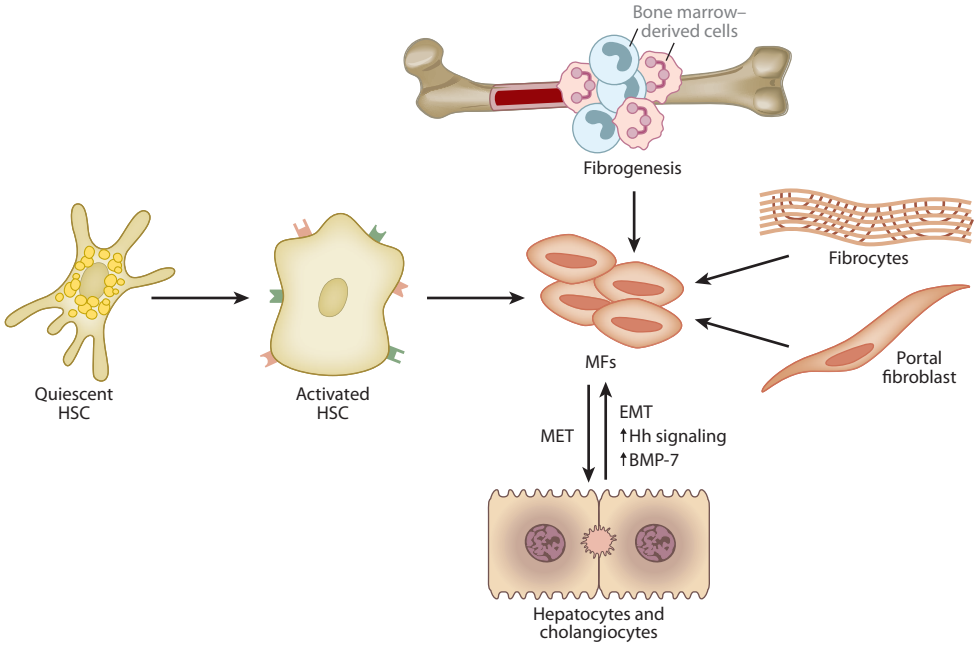


Figure 3

Sources of extracellular matrix. Liver fibrosis is characterized by the proliferation of contractile and fibrogenic myofibroblasts (MFs). The primary and best-characterized source of MFs is activated hepatic stellate cells (HSCs); other cells may also transdifferentiate into MFs, although their exact contribution to human disease remains unclear. These cells include bone marrow–derived cells, portal fibroblasts, and epithelial-to-mesenchymal transition (EMT) from hepatocytes and cholangiocytes. Abbreviations: BMP-7, bone morphogenetic protein 7; Hh, hedgehog; MET, mesenchymal-to-epithelial transition.

and growth factors such as PDGF and TGF-β [via both the Smad2/3 and mitogen-activated protein kinase (MAPK)-dependent pathways] (41). More recently, hedgehog signaling has been implicated in this process as well (15, 42). EMT is dynamic and bidirectional, given that fibrogenic cells can undergo mesenchymal-to-epithelial transition and revert back to an epithelial phenotype (35).

Biliary epithelial cells coexpressing epithelial and MF markers have also been identified in animal models following bile duct ligation (43) and in human fibrotic livers, particularly in primary biliary cirrhosis and biliary atresia (44, 45). TGF-β-mediated stimulation of primary human cholangiocytes upregulates p-Smad2/3, S100A4, and ASMA, which confer a motile phenotype (45)

Hepatocyte EMT may also contribute to hepatic fibrogenesis. In vitro studies show that TGF-β induces both EMT in mature hepatocytes and expression of collagen α1(I) (46). Increased FSP1 (S100A4) expression in hepatocytes following carbon tetrachloride (CCl_4)-induced fibrosis (47) has also been reported. Despite these data, type I collagen production by EMT hepatocytes has not yet been conclusively proven in in vivo models (48), and a recent study refutes the participation of EMT as a significant source of fibrosis (48, 49). Recently, endothelial cells have also been implicated in the transformation of mesenchymal cells during kidney (50) and cardiac (51) fibrosis via an analogous process (endothelial-to-mesenchymal transition); however, their role in liver fibrosis is unknown.

CYTOKINES AND SIGNALING PATHWAYS

Cytokines

Inflammatory cytokines play a key role in fibrosis, given that persistent inflammation almost always precedes fibrosis. Following liver injury, several cell types can secrete inflammatory cytokines; these cell types include KCs, hepatocytes, HSCs, natural killer (NK) cells, lymphocytes, and dendritic cells.

Cytokines are a family of proteins that include chemokines [monocyte chemotactic protein 1 (MCP-1), RANTES, IL-8], interferons (IFN-α, IFN-γ), interleukins (IL-1, IL-6, IL-10), growth factors, adipokines, and soluble neurohumoral ligands (endocannabinoids) (**Table 1**). Adipokines (adipose tissue cytokines) are polypeptides secreted mainly by adipocytes and, to a lesser extent, by stromal cells including macrophages, fibroblasts, and infiltrating monocytes (52). Leptin and adiponectin are the main adipokines implicated in liver injury. Translation of the *obese* (*ob*) gene results in the expression of leptin. Leptin can mediate its biological effects through one of several leptin receptors (ObRa to ObRf) via activation of the Janus kinase 2 (JAK2) and signal transducer and activator of transcription 3 (STAT3) pathways (**Figure 4**) (52). Leptin has a profibrogenic effect; it directly modulates the HSC phenotype through ObRb and activates KCs, macrophages, and endothelial cells to produce TGF-β (53, 54). Leptin also plays a role in promoting the proliferation, migration, and metastasis of hepatocellular carcinoma and cholangiocarcinoma cells (55, 56).

In addition to leptin, the JAK-STAT signaling pathway is activated by a large variety of cytokines, including IFN-γ (112). Binding of the cytokines to their receptors activates receptor-associated tyrosine kinases (JAK1, JAK2, JAK3, Tyk2), which interact with the STAT proteins. Phosphorylation of the STAT proteins (STAT1–6) at their phosphotyrosine-binding SH2 domain allows the complexes to translocate to the nucleus and regulate target gene transcription (59, 113).

STAT1 and STAT3 play a key role in liver fibrosis. STAT1 can be activated by IFN-α/-β and IFN-γ, and STAT3 can be activated mainly by IL-6 and IL-22. STAT1 and STAT3 regulate the transcription of many target genes involved in antiviral defense, liver inflammation, and liver regeneration.

STAT1 has been proposed to negatively regulate liver fibrosis through several mechanisms, including inhibition of HSC proliferation, suppression of β-PDGF receptor (β-PDGFR) expression, inhibition of TGF-β/Smad3 signaling, and stimulation of NK cell cytotoxicity (114). In vivo, mice with a selective knockout of STAT1 in HSCs develop accelerated liver fibrosis in response to liver injury due to CCl_4 (114).

Adiponectin inhibits hepatic fibrogenesis both in vitro and in vivo (54, 57). In HSCs, adiponectin binds its specific receptors, AdipoR1 and AdipoR2, whose downstream effects are mediated by adenosine monophosphate (AMP)–activated protein kinase (AMPK) and peroxisome proliferator–activated receptor α (PPAR-α) (58, 115). AMPK activation generates ATP and inhibits processes that consume ATP, apart from those crucial for short-time survival.

A recent study has implicated an additional adipokine, ghrelin, in attenuating hepatocellular injury and fibrosis (59). Ghrelin-deficient mice have enhanced injury and fibrosis following toxic injury, whereas recombinant ghrelin attenuates injury in wild-type animals. Moreover, human polymorphisms of the *ghrelin* gene may influence fibrosis progression in patients with chronic hepatitis.

Peptide growth factors are also members of the cytokine family. The most important growth factors implicated in HSC activation and collagen synthesis are PDGF and TGF-β. PDGF is a dimeric protein composed of varying combinations of four polypeptide chains (A, B, C, and D) that signal via the tyrosine kinase receptors PDGFR-α and PDGFR-β. All PDGF isoforms are upregulated during HSC activation and correlate with the degree of fibrosis and inflammation (59–63).

Table 1 Repertoire of cytokines and membrane receptors associated with hepatic stellate cells[a]

Cytokine family	Cytokines	Receptors	Effect(s)
TGFs	TGF-β1/TGF-α, BMP4, BMP6	TGF-β receptor types I, II, III; mannose-6-phosphate receptor	Proliferative fibrogenic
PDGFs	PDGF-B	β-PDGFR, α-PDGFR	Proliferative fibrogenic
EGF	Unknown	EGF receptor	Proliferative fibrogenic
Stem cell factor	Stem cell factor	Unknown	Proliferative fibrogenic
HGF	HGF	c-Met	Proliferative fibrogenic, regenerative, antifibrogenic
CTGF	CTGF (CCN2)	αvβ3-integrin, low-density lipoprotein receptor-related protein	Proliferative fibrogenic
FGFs	aFGF and bFGF	FGF receptor 2	Proliferative fibrogenic
ET-1	ET-1, ECE	ET-A and ET-B receptors	Proliferative fibrogenic, chemotactic/inflammatory
Leptin	Leptin	OB-Ra and OB-Rb	Proliferative fibrogenic
Plasminogen	uPA/PAI-1	uPA receptor	Proliferative fibrogenic
VEGFs	VEGF	VEGF receptors 1 and 2	Proliferative fibrogenic
IGFs	IGF-I, IGF-II	IGF-IR	Proliferative fibrogenic
Thrombin	Unknown	Thrombin receptor	Proliferative fibrogenic
RGD-containing and integrin ligands	—	Integrins α1β1, α2β1, α6β4, α5β1,α8β1, αvβ1, and αvβ3; integrin-linked kinase	Proliferative fibrogenic
Fibrillar collagens	Collagens I, II	Discoidin domain receptors 1 and 2	Proliferative fibrogenic
Cannabinoids	Unknown	CB1 receptor	Proliferative fibrogenic
Purines	Ubiquitous	P2Y receptors	Proliferative fibrogenic
Adenosine	Ubiquitous	A(2a)adenosine receptor	Proliferative fibrogenic
Renin-angiotensin	Angiotensin II, renin, ACE	Angiotensin II types 1 and 2 receptors	Proliferative fibrogenic
Serotonin	Unknown	SSR2, SSR3, and SSR5 receptors	Proliferative fibrogenic
Hedgehog	Indian hedgehog and sonic hedgegog	Patched	Proliferative fibrogenic
Galectins	Galectin-3	Unknown	Proliferative fibrogenic
AGE	Unknown	Receptor for AGE	Chemotactic/inflammatory
M-CSF	M-CSF	Unknown	Chemotactic/inflammatory
PAF	PAF	PAF receptor	Chemotactic/inflammatory
CD40	CD40 ligand	Unknown	Chemotactic/inflammatory
TNF-α	TNF-α	TNF receptor 1, p75NTR	Chemotactic/inflammatory
Chemokines	CXCL1, MCP-1, RANTES, MIP-1, eotaxin, IL-8	CXCR3	Chemotactic/inflammatory
Opioids	Unknown	δ1 and δ2 opioid receptors	Chemotactic/inflammatory
Oxidized LDl	Unknown	CD36	Chemotactic/inflammatory
TLR ligands	Unknown	TLR4, CD14	Chemotactic/inflammatory
IL-6	IL-6	Unknown	Regenerative
NTs	NGF, BDNF, NT-4, NT-4/5	p75-NTR, Trk-B, Trk-C	Regenerative
IL-10	IL-10	IL-10 receptor	Antifibrogenic
Adiponectin	Adiponectin	CB2 receptor	Antifibrogenic
Follistatin	Follostatin	Unknown	Antifibrogenic

(*Continued*)

Table 1 (*Continued*)

Cytokine family	Cytokines	Receptors	Effect(s)
Fas signaling	Unknown	Fas	Unknown
Cystatin	Cystatin	Unknown	Miscellaneous
Catecholamines	Norepinephrine	α1A- and β-adrenergic receptors	Miscellaneous
5-hydroxytamine	Unknown	5-hydroxytamine receptor subtypes 1A, 2A, and 2B	Miscellaneous
Adrenomedullin	Adrenomedullin	Unknown	Miscellaneous
Complement cascade	Unknown	C5a receptor	Miscellaneous
Natriuretic peptides	Unknown	Natriuretic peptide receptor B	Miscellaneous

[a]Adapted from Reference 12. Abbreviations: ACE, angiotensin-converting enzyme; AGE, advanced glycation end products; BDNF, brain-derived neurotrophic factor; BMP, bone morphogenetic protein; CTGF, connective tissue growth factor; ET, endothelin; FGF, fibroblast growth factor; HGF, hepatocyte growth factor; IGF, insulin-like growth factor; IL, interleukin; M-CSF, macrophage colony–stimulating factor; MCP, monocyte chemotactic protein; MIP, macrophage inflammatory protein; NGF, nerve growth factor; NT, neurotrophin; PAF, platelet-activating factor; PAI, plasminogen activator inhibitor; PDGF, platelet-derived growth factor; TGF, transforming growth factor; TLR, Toll-like receptor; TNF, tumor necrosis factor; uPA, urokinase-type plasiminogen activator; VEGF, vascular endothelial growth factor.

PDGFRs transmit their activity in part through phosphoinositol 3-kinase (PI3K)/Akt, which also transduces signals for other tyrosine kinases [e.g., vascular endothelial growth factor (VEGF)], cytokine receptors (e.g., MCP-1), integrins, adipokines (leptin), and G protein–coupled receptor stimulators [e.g., angiotensin II (AII), thrombin] (96–98). When a receptor tyrosine kinase binds to its cognate receptor, its tyrosine residues become autophosphorylated, which leads to the recruitment of PI3K to the membrane. Once activated and localized to the membrane, PI3K phosphorylates phosphoinositol lipids, which translocate Akt to the plasma membrane. After its recruitment, Akt is phosphorylated by phosphoinositide-dependent kinase and thereby activated. Activated Akt regulates several cell functions through phosphorylation of many different targets, including mammalian target of rapamycin (**Figure 4**) (99).

Growth factor receptors may also utilize MAPK signaling pathways. The MAPK family includes extracellular signal–regulated kinase, c-Jun N-terminal kinase (JNK), and p38 MAPK. These molecules are activated by proliferative peptides (PDGF, thrombin, AII, VEGF, leptin) and chemokines. Once activated, they recruit the signaling molecule Ras, which leads to the transcription of cell-proliferative and profibrogenic factors (98, 100, 101).

TGF-β is secreted by a variety of cell types; it has three major isoforms (TGF-β1, TGF-β2, and TGF-β3). TGF-β1 is produced mainly by monocytes and macrophages and is the principal isoform implicated in liver fibrosis. TGF-β1 is stored as an inactivated protein bound to a latency-associated protein. Once activated, TGF-β1 signals via its cognate receptors to Smad proteins, which enhance the transcription of target genes, including *procollagen I* and *procollagen III* (64). TGF-β1 signaling is initiated by binding to the type II receptor. Subsequently, this receptor dimerizes with its type I receptor and binds Smad2 and Smad3; this complex becomes phosphorylated and is released into the cytosol, where it associates with Smad4. The resulting heterodimer can then translocate into the nucleus and regulate transcription. The pathway can be endogenously inhibited, which prevents the binding of Smad2/3 to the receptor by Smad6/7 (**Figure 4**) (102, 103).

VEGF, a well-characterized angiogenesis modulator, is upregulated during HSC activation and stimulates cell proliferation, migration, and collagen production (65, 66). Other

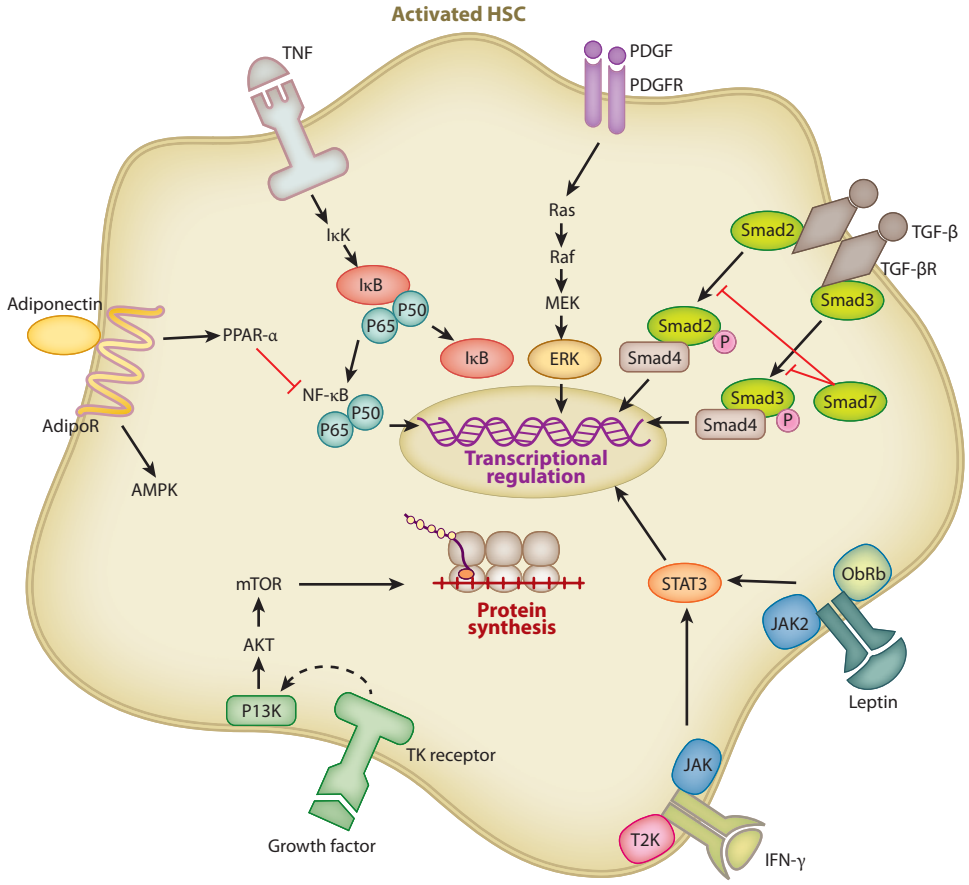


Figure 4

Main cytokine pathways regulating liver fibrosis. Liver injury is followed by the secretion of cytokines that mediate the activation of several intracellular signaling pathways, primarily through their binding to specific receptors. Leptin and interferon (IFN)-γ can activate STAT3 and regulate the transcription of many target genes involved in liver fibrosis. Adiponectin, through its binding to its receptor, AdipoR, can inhibit hepatic fibrogenesis via peroxisome proliferator–activated receptor (PPAR)-α signaling. The dimerization of the transforming growth factor β receptor (TGF-βR), following binding by TGF-β, recruits Smad2 and Smad3 proteins, which are then phosphorylated and released into the cytosol, where they can associate with Smad4. These heterodimers can then translocate into the nucleus and regulate fibrogenic gene transcription. Platelet-derived growth factor (PDGF) mediates its transcriptional regulation in part through the activation of the extracellular signal–regulated kinase (ERK) pathway following binding to the PDGF receptor (PDGFR). In addition to PDGF, several other growth factors can activate tyrosine receptors, which lead to the recruitment of phosphoinositol 3-kinase (PI3K) and the phosphorylation of AKT; activated AKT regulates synthesis of fibrogenic proteins via the mammalian target of rapamycin (mTOR) pathway. Abbreviations: JAK, Janus kinase; NF-κB, nuclear factor κB; TK, tyrosine kinase; TNF, tumor necrosis factor.

peptide growth factors related to liver fibrosis are HGF, fibroblast growth factor, and insulin-like growth factor 1 (43, 67–69)

Endogenous cannabinoids are a family of molecules derived from arachidonic acid that signal through CB1 and CB2 receptors. Major endogenous ligands are anandamide (arachidonylethanolamide), 2-arachidonylglycerol, noladin ether, and virodhamine (70). Chronic liver disease is associated with the upregulation

of endocannabinoids and their receptors; however, the two CB receptors have completely divergent activities. Whereas CB1 is fibrogenic, CB2 has the opposite effect; thus, CB1 antagonism and CB2 agonism are two opposing strategies that represent new therapeutic options to reduce fibrosis (70–73).

Vasoactive Mediators

HSCs can regulate intrahepatic blood flow during injury according to several lines of evidence:

1. In their perisinusoidal orientation, HSCs, which contain extensive long cytoplasmic foot processes, embrace the sinusoids and resemble tissue pericytes, a cell population thought to regulate blood flow by modulating pericapillary resistance (74).
2. Activation of HSCs includes the adoption of a contractile phenotype.
3. In vivo microscopy has demonstrated direct sinusoidal constriction by HSCs (75).

Moreover, contraction of HSCs in response to vasoactive substances has been demonstrated in vitro and in vivo, and the actual force generated by HSC contraction is sufficient to contract sinusoids [the average force contraction generated by endothelin 1 (ET-1) stimulation of a single HSC exceeds sinusoidal pressure] (76).

Several vascular mediators provoke hepatic stellate cell contractility. The endothelin (ET) family consists of three members (ET-1, ET-2, and ET-3) that are produced by endothelial cells, which bind to two G protein–coupled receptors, ETA and ETB, and exert paracrine and autocrine effects. ETA receptors are found mainly on vascular smooth muscle cells, and ETB receptors are found on endothelial cells (77, 78).

After liver injury, ET-1 is secreted by HSCs, whereas its synthesis by sinusoidal endothelial cells is reduced (79). Liver injury is associated with both an increase in local ET production and enhanced ET receptor expression, which lead to HSC contractility that increases sinusoidal constriction and intrahepatic blood flow resistance (78, 80).

Nitric oxide (NO) is a tightly regulated, unique messenger molecule that is produced from L-arginine by three isoforms of NO synthase. NO modulates intrahepatic resistance in an autocrine manner by stimulating a soluble guanylate cyclase that decreases Ca^{2+} levels, provoking vasodilation and HSC relaxation (78, 81). In the cirrhotic liver, sinusoidal endothelial cells have decreased NO secretion, thereby contributing to the imbalance between vasoconstrictor and vasodilator substances that is typical of advanced liver disease (82).

The renin-angiotensin system (RAS) is an endocrine system that can regulate intrahepatic vascular resistance. The main mediator is AII, which is either produced through endothelial cleavage of angiotensin I (AI; synthesized from angiotensinogen by hepatocytes) or generated de novo in damaged tissues. AII binds AI receptor in MF and promotes fibrogenesis and inflammation. In the fibrotic liver, HSCs highly express AI receptors and secrete AII, thereby inducing cell proliferation and contraction (83, 84). Moreover, RAS activity correlates with the degree of portal hypertension (85).

AII mediates its effects (*a*) by directly stimulating Smad signaling and (*b*) through an increase in intracellular calcium and ROS production, which stimulate the PI3K/Akt, Rho kinase, nuclear factor κB (NF-κB), and MAPK pathways (83, 86). Like many systems, RAS contains an endogenous antagonistic pathway in which a truncated form of AII, Ang1–7, exerts effects on fibrosis that are opposite to those of AII (87). Ang1–7 is generated by a homolog of angiotensin-converting enzyme 1 (ACE1) known as ACE2 (88). Thus, whereas ACE1 and AII are profibrotic, ACE2 and Ang1–7 are antifibrotic.

Other vasoactive factors. Several vascular agents are also implicated in hepatic vascular homeostasis. Carbon monoxide is produced in the hepatic sinusoidal cells and mediates relaxation in sinusoids and HSCs (89). The

serine protease thrombin regulates platelet aggregation and endothelial cell activation. It can act as a hormone or a cytokine, depending on which receptor isoform is engaged (protease-activated receptors 1 through 4). Expression of protease-activated receptor 1 by HSCs increases during activation and induces the contraction, proliferation, and secretion of several chemokines, as well as platelet-activating factor. The binding of atrial natriuretic peptide to its receptor in HSCs antagonizes the effects of ET on Ca^{2+} and contraction (90). Prostaglandins are also implicated in HSC contraction; some of them (PGI_2, PGE_2) induce relaxation, whereas others (tromboxane, $PGF_{2\alpha}$) induce contraction (91).

Vasopressin and thrombin elicit contraction of HSCs by releasing Ca^{2+} from intracellular stores (92). Adenosine, substance P, and lysophosphatidic acid also induce HSC contraction in vitro (93–95).

REGULATION OF GENE EXPRESSION

Transcriptional Regulation

Regulation of gene expression in eukaryote cells is a complex, precise, and cell-specific process. There has been tremendous progress in revealing the regulatory mechanisms that control gene expression in HSCs during fibrosis, and research has focused primarily on transcriptional control pathways (104, 105, 116). Recent advances have also highlighted the impact of posttranslational modifications, including phosphorylation, SUMOylation, prenylation, acetylation, and glucosylation, which can regulate a range of effects in transcriptional activity; binding affinity to DNA; oligomerization; and/or targeting for degradation of transcription factors, corepressors, and coactivators (117). Rather than comprehensively review all facets of transcriptional biology in HSCs, we describe here a few examples of key transcription factors implicated in the regulation of HSC activation (**Table 2**).

Basic helix-loop-helix (bHLH) transcriptional factors constitute a two-class family involved in HSC contractility. Class A bHLH factors are ubiquitously expressed, whereas class B bHLH factors are tissue specific; both can be inhibited by related bHLH proteins known as inhibitors of DNA binding/differentiation (Id proteins). The best-characterized HLH members in HSCs are MyoD, sterol regulatory element–binding protein 1c, c-Myc, and c-Myb. MyoD is a myogenic transcriptional factor expressed in rat and human HSCs that is implicated in the acquisition of a contractile phenotype (118). The role of Id proteins in liver fibrosis remains poorly defined, as there are some contradictory findings. Id1 is implicated in the maintenance of the in vitro quiescent phenotype of HSCs (118), and forced overexpression of Id1 enhances HSC activation (119). In contrast, Id2 plays an important role in perpetuating fibrosis and is upregulated during early HSC activation (120); however, it later leads to the suppression of fibrogenic markers (ASMA, collagen I, MMP-2) (121). These divergent data may reflect the effects of different modes of cross talk between Id proteins and the major profibrogenic factor TGF-β (105).

Three members of the CCAAT/enhancer binding protein (C/EBP) family, C/EBPα, C/EBPβ, and C/EBPδ, are expressed in HSCs (122). C/EBPα, a key factor in adipocyte differentiation (123), is also a negative regulator of HSC activation and liver fibrosis (124). Its expression declines with activation, and its forced overexpression inhibits HSC proliferation, decreases production of ECM and expression of ASMA, and enlarges cytoplasmic lipid droplets (122). C/EBPβ modulates collagen I expression in HSCs in response to oxidant stress induced by acetaldehyde (125, 126). It also plays an important role in preventing apoptosis of activated HSCs and promoting progression of fibrosis. During HSC activation in an experimental model of injury, phosphorylation of Thr217 C/EBPβ by ribosomal S-6 kinase (RSK) induces HSC proliferation and prevents apoptosis. In contrast, HSCs in *C/EBPβ*$^{-/-}$ mice or in wild-type animals treated with a selective inhibition in RSK undergo apoptosis following liver injury with CCl_4 (127).

Table 2 Transcription factors expressed by hepatic stellate cells[a]

Factor	Function of target gene(s)
Miscellaneous	
NF-κβ	Inflammation and apoptosis survival regulation
AP-1 (c-Jun, JunB, JunD, c-Fos, Fra1, Fra2, Fos-B)	*TGF-β1*, *TIMP-1*, and *IL-6* gene regulation
AP-2	Collagen gene regulation
Ets-1	Activation
NF-1	Collagen gene regulation
Smads	Collagen gene regulation, gene arrest
C/EBP	Collagen gene regulation
Mef2	Activation
E-box factors	Mannose-6-phosphate/IGF-II regulation
c-Myb	α smooth muscle actin
CREB	Activation
CRP2	Quiescence
SREBP	Quiescence
Lhx2	Quiescence
KLFs	
KLF6	Varied
Sp1, Sp3	Collagen gene regulation
BTEB	Collagen gene regulation
ZNF267	MMP-10
Egr-1	Activation
Nuclear hormone receptors	
FXR	Quiescence
PPAR-γ	Quiescence
LXR	Quiescence
PXR	Quiescence
Vitamin D receptor	Activation
RAR-α,-β; RXR	Varied
Forkhead factors	
Foxf1	Activation
FoxO1	Activation

[a]Adapted from Reference 12. Abbreviations: AP, activator protein; C/EBP, CCAAT/enhancer-binding protein; CREB, cyclin AMP response element–binding; CRP2, cysteine- and glycine-rich LIM (Lin11, Isl1, Mec3) domain protein 2; FXR, farnesoid X receptor; IGF, insulin-like growth factor; IL, interleukin; KLF, Krüppel-like transcription factor; LXR, liver X receptor; MMP, matrix metalloproteinase; NF, nuclear factor; PPAR, peroxisome proliferator–activated receptor; PXR, pregnane X receptor; SREBP, sterol regulatory element–binding protein; TGF, transforming growth factor; TIMP, tissue inhibitor of matrix metalloproteinase, ZNF, zinc finger protein.

Mef2, a member of the MADS (MCM1, AG, DEFA, SRF)-box family implicated in differentiation and organogenesis (128), also drives HSC proliferation and contractility (129). Although Mef2 is not detectable in qHSCs, during activation its messenger RNA increases, which in turn increases expression of ASMA and collagen I and stimulates HSC proliferation.

Cysteine- and glycine-rich LIM (Lin11, Isl1, Mec3) domain protein 2 is expressed solely by HSCs among liver cells and induces their activation. It expression is regulated by TGF-β (130). The LIM homeobox gene, *Lhx2*, preserves the quiescent phenotype of HSCs (105), and its overexpression in human HSCs reduces ECM production and ASMA expression

(131). Activator protein (AP)-1 expression becomes evident at early stages of HSC activation, when it enhances the transcription of tissue inhibitor of metalloproteinase (TIMP)-1, IL-6, and TGF-β1 (132). AP-2 (133), NF-1 (134), and SOX9 (135) also regulate collagen αI expression in activated HSCs.

Nuclear receptors [pregnane X receptor (PXR), PPAR] regulate the expression of target genes following ligand activation in the cytoplasm and translocation to the nucleus. Although retinoid storage is the most prominent and characteristic feature of HSCs in normal liver, the relative importance of the nuclear receptors, RXR and RAR, in liver homeostasis and injury responses is surprisingly obscure. Nonetheless, expression of RAR and RXR is downregulated during HSC activation (136).

The farnesoid X receptor (FXR) is activated by bile acids and diminishes collagen and TIMP-1 expression in HSCs without affecting either ASMA expression or cell proliferation (137, 138). PXR is implicated in the response to several xenobiotica and endogenous metabolites through upregulation of the CYP3A family of cytochrome p450 enzymes, particularly CYP3A4. Activation of PXR by pregnelone-16-α-carbonitrile prevents rat HSC activation and fibrosis following liver injury with CCl_4 (139).

The PPARs regulate lipid and glucose metabolism and are expressed in HSCs. Although there are several isoforms (i.e., PPAR-α, -β, -γ, and -δ) the most extensively investigated in liver fibrosis is PPAR-γ, which plays an important role in the maintenance of qHSCs. Forced expression of PPAR-γ in activated HSCs inhibits collagen I expression, blocks TGF-β1 signaling, reduces proliferation, and increases cytoplasmic lipid droplets (140–142). PPAR-γ exerts these effects though physical interaction with JunD (an AP-1 protein) and the blockade of TGF-β signaling by Smad3 inhibition, which leads to reduced collagen and connective tissue growth factor expression, respectively (140). Stimulation of PPAR-γ, with either a natural ligand [15-d-PJ(2)] or a synthetic ligand (GW7845, pioglitazone), inhibits HSC proliferation and induces apoptosis in vitro and in vivo (140, 143). PPAR-γ requires formation of a heterodimer, primarily with RXR, to become transcriptionally active, but it also dimerizes with RAR and FXR (144).

Epigenetic Regulation

Changes in gene expression can also arise without modifications in DNA sequences through epigenetic regulation. Epigenetic modifications of gene expression are typically stable and are retained through mitosis, without leading to DNA mutations. They are highly responsive to environmental and developmental cues (see Reference 145 and references therein). Epigenetic modifications consist of three main processes: histone modification, DNA methylation, and silencing by noncoding RNAs (**Figure 5**) (146).

Several studies have identified a potential role for histone modification in HSC activation. Trichostatin A, a histone deacetylase inhibitor, blocks the morphological features of HSC activation in vitro and reduces both cellular proliferation and transcription of the *ASMA* and *collagen I* genes (147, 148). Interestingly, ethanol, the chronic ingestion of which is linked to hepatic fibrosis, can induce a posttranslational histone modification through acetylation of histone H3 lysine (149).

Gene silencing, which results from the addition of a methyl group to the 5′ position of cytosine residues (i.e., methylation) in the cytosine phosphoguanine dinucleotide, is also implicated in the regulation of HSC activation. The DNA methylation inhibitor 5-aza-2′-deoxycytidine blocks HSC activation through sustained repression of IκBα in qHSCs and PPAR-γ in activated HSCs, respectively (108).

Chromatin silencing can also be accomplished by small noncoding RNA genes that bind to their target messenger RNA and downregulate their stability and/or translation (150). These RNA modulators can act at either the transcriptional (antisense RNA) or posttranscriptional level [e.g., via small interfering RNA or microRNA (miRNA)] and are involved in

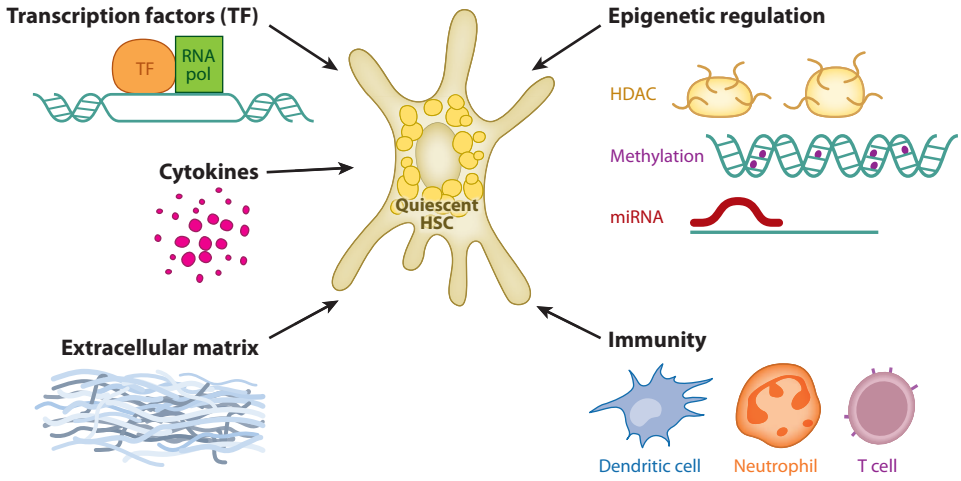


Figure 5

Regulation of gene expression in hepatic stellate cells (HSCs) during fibrosis. Transcription factors (TFs) can promote or block the recruitment of RNA polymerase (RNA pol) binding to a specific DNA sequence and thereby control the rate of gene expression. Changes in genes expression can also occur without modification in DNA sequences through at least three distinct epigenetic processes: histone deacetylation (HDAC), DNA methylation, and silencing by noncoding microRNAs (miRNAs). Activation of immune cells following liver injury is also an important stimulus to HSC activation that occurs mainly through the secretion of proinflammatory and fibrogenic molecules. Cytokines and extracellular matrix components also play an important role in initiating fibrosis and perpetuating HSC activation.

many biological pathways such as cell differentiation, proliferation, and death (151, 152).

miRNA-mediated RNA silencing is associated with HSC activation through the upregulation of 13 miRNAs and the regulation of 22 signaling pathways (153). Specifically, the overexpression of miR-27a and miR-27b is involved in the reversal of the activated phenotype of rat HSCs in vitro (154, 155).

Intriguingly, cross talk between transcription factors and epigenetics occurs during HSC activation. Specifically, HSC activation is associated with a persistent suppression of IκBα caused by the methylation of a CpG island upstream of the IκBα promoter by the repressors centromere-binding factor 1 (CBF1) and methyl CpG–binding protein 2 (MeCP2) (107, 108). In the context of hepatocellular carcinoma, activated HSCs overexpress the transcription factor Mef2, which interacts with class II histone deacetylase. This interaction leads to the hyperacetylation of histones H3 and H4 (156).

IMMUNE REGULATION AND HOST GENETICS IN LIVER FIBROSIS

As the first solid organ beyond the gut to process ingested antigens, the liver is constantly exposed to antigen-rich blood and therefore is a major line of defense against such antigens, especially microorganisms. Both the adaptive and innate immune systems of the liver are highly evolved to serve this function. In doing so, however, innate immune pathways may also drive fibrogenesis, given that their primary activity is to protect against acute insult; however, fibrosis is a late-stage response that does not pose an immediate threat to an organism's survival. Both the innate and adaptive immune systems play an important role in hepatic fibrosis modulation (**Figure 6**).

Innate Immune Response

Innate immune mechanisms are critical to defense from microorganisms through a range

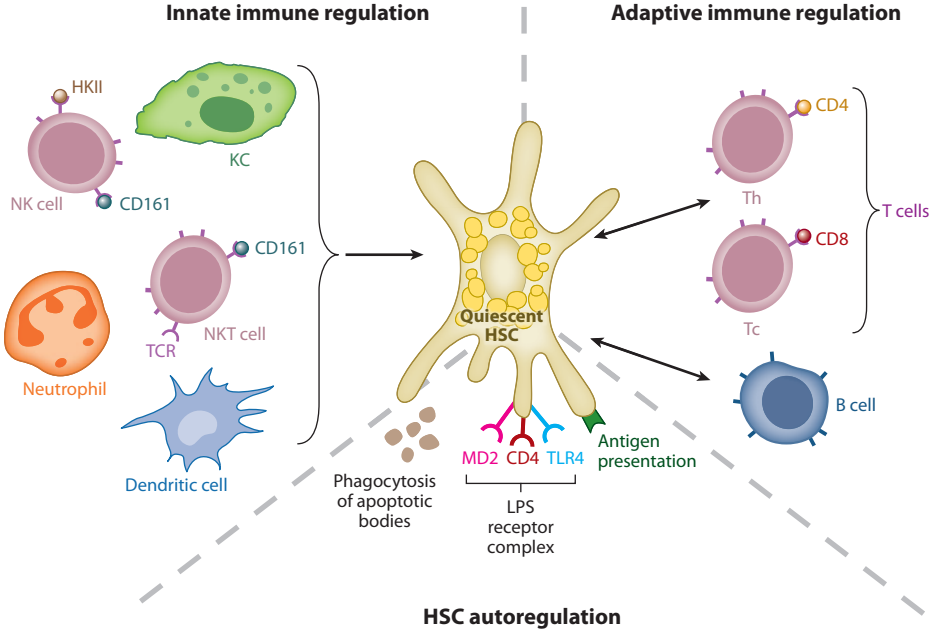


Figure 6

Both the adaptive and innate immune systems play important roles in hepatic fibrosis modulation. Liver Kupffer cells (KCs) can secrete soluble mediators and function as antigen presenting cells, thereby modulating hepatic stellate cell (HSC) activation. Neutrophils are implicated in the early response following liver injury. Natural killer (NK) cells confer a protective role by inducing HSC apoptosis and secreting antifibrotic mediators, whereas NKT cells have a profibrogenic profile. HSCs also function as professional antigen presenting cells and can phagocytose apoptotic debris, especially from hepatocytes. HSCs express the innate immune receptor Toll-like receptor 4 (TLR4), whose main ligand is lipopolysaccharide (LPS). Abbreviation: TCR, T cell receptor.

of pathways. A secondary consequence of this response, however, may include effects on fibrogenesis.

Hepatic macrophages and monocytes. KCs are tissue macrophages derived from circulating monocytes; they constitute 15% of the total liver cell population and reside predominantly in the periportal areas. They have a wide repertoire of functions in liver pathophysiology, including phagocytosis, antigen presentation, and secretion of soluble mediators that can modulate innate immune and inflammatory responses. Once activated, KCs secrete a large number of proinflammatory and fibrogenic mediators, which can drive HSC activation (157, 158).

KCs are also the first point of contact for bacterial products, including endotoxin, that are derived from the gastrointestinal tract. KCs are therefore the main target of lipopolysaccharide (LPS), and they strongly express Toll-like receptor 4 (TLR4), the innate immune receptor whose main ligand is LPS. TLRs are a family of mammalian transmembrane pattern-recognition receptors that distinguish pathogen-associated motifs. TLR4-mediated signals are transduced through two major pathways, which are either MyD88 dependent or MyD88 independent, and they signal through NF-κB, MAPK, and PI3K/Akt (157, 158). Most resident liver cells, including HSCs, express TLR4. Activated human HSCs that express TLR4 respond to LPS by secreting cytokines and activating IκB kinase/NF-κB and JNK

(159). Moreover, LPS downregulates the TGF-β pseudoreceptor BAMBI in qHSCs, thereby promoting TGF-β signaling and enhancing hepatic fibrogenesis (160). Interestingly, two single-nucleotide polymorphisms of the *TLR* gene (D299G and T399I), which are associated with reduced LPS responsiveness, confer a significantly reduced risk for fibrosis progression in patients with chronic hepatitis C virus infection (161, 162).

TLR9 is also expressed by HSCs and can be activated by apoptotic hepatocyte DNA, which leads to cellular activation and collagen production (163). Accordingly, TLR9-deficient mice display decreased hepatic fibrosis in experimental liver injury (164).

Neutrophils. Neutrophils are implicated in the early response of the innate immune system following liver injury, and they are especially prominent in both alcoholic and nonalcoholic steatohepatitis. Moreover, they may be stimulated to directionally migrate into injured liver by numerous chemokines, including IL-8 (165). However, their role in directly driving fibrogenesis, rather than through their amplification of tissue damage per se, is not clear, as few pathways that directly link neutrophils to fibrogenic pathways have been identified.

Natural killer and natural killer T cells. NK cells confer a protective role in fibrosis development (*a*) by inducing HSC apoptosis and (*b*) through the production of antifibrotic mediators (166–170). The inhibitory effect of NK cells on HSCs may be mediated by STAT1, given that induction of HSC killing by NK cells is attenuated in *STAT1*$^{-/-}$ mice (114).

NKT cells are a heterogeneous group of cells that express both T cell markers (αβ TCR) and NK cell markers (NK1.1, CD161) (171). A profibrogenic role in fibrogenesis has been suggested on the basis of the finding that depletion of NKT cells in mice treated with CCl_4 has a protective effect against liver damage and fibrosis (172).

Dendritic cells, which are classical antigen presenting cells, are abundant in liver, and their contribution to hepatic fibrosis is an area of active interest. Recent evidence suggests that they may modulate the inflammatory milieu via TNF-α (173).

Adaptive Immune Response

Growing interest has also uncovered a vital role for the adaptive immune system in hepatic fibrosis.

T lymphocytes. T cells can be classified as $CD8^+$ and $CD4^+$ cells, as well as cells defined by the cytokine secreted. For example, classical T helper 1 (Th1) cells secrete IL-2 and IFN-γ, whereas Th2 cells secrete IL-4, -5, -10, and -13 (174). In general, Th2 cytokines from $CD8^+$ cells play a profibrotic role in liver fibrosis, whereas Th1 cytokines play a protective role (157, 175).

B lymphocytes. B cells may play a profibrogenic role in liver fibrosis because B cell–deficient mice demonstrate reduced collagen deposition after CCl_4 injury compared with wild-type animals. The impact of B cell deficiency is probably antibody independent (176).

Influence of Hepatic Stellate Cells on Immune System Cells

Both immune cells and HSCs are important mediators of hepatic fibrosis through bidirectional interactions. HSCs can be regulated by immune cells and can modulate inflammatory cell behavior (175, 177). They can produce macrophage colony–stimulating factor, which is an important KC regulator (178); the lipid chemoattractant platelet-activating factor (179); and several chemokines (165, 180). HSCs are also immunoregulatory because they both secrete soluble mediators and upregulate leukocyte adhesion molecule receptors, including intracellular adhesion molecule type 1 (181, 182), vascular cell adhesion molecule type 1 (183), and neural cell adhesion molecule type 1 (184, 185).

RESOLUTION OF FIBROSIS

Fibrosis Reversibility

Recent clinical evidence contradicts the long-standing belief that cirrhosis is always irreversible (186, 187). In experimental animal models of fibrosis, cessation of the causative agent results in fibrosis regression (188, 189). Even in humans, successful treatment of the underlying disease may reverse liver fibrosis. Regression of liver fibrosis has been observed in patients with iron and copper overload; alcohol-induced liver injury; chronic hepatitis B, C, and D; hemochromatosis; secondary biliary cirrhosis; nonalcoholic steatohepatitis; and autoimmune hepatitis (reviewed in References 186 and 187).

Fibrosis regression: any reduction in ECM content of any degree, without necessarily a return to normal histology

Extracellular Matrix Degradation

Liver fibrosis following chronic liver injury entails both qualitative and quantitative changes in ECM composition as a result of an imbalance between the rates of matrix synthesis and degradation. The ECM becomes progressively insoluble and resistant to protease digestion because of the thickening of fibrotic septae and the increase in cross-linking (189, 190).

MMPs, also known as matrixins, are the major family of calcium-dependent enzymes that degrade collagenous and noncollagenous ECM substrates. They are a tightly regulated 25-member family traditionally classified into five categories on the basis of their substrate specificity: interstitial collagenases, gelatinases, stromelysins, membrane types, and metalloelastases (**Table 3**). To tightly regulate the turnover and constant remodeling of ECM, MMPs are regulated at several levels. They are secreted as inactive proenzymes, have complex transcriptional control, and are modulated by a family of endogenous proteinase inhibitors known as TIMPs (**Figure 7**) (189, 191, 192). Four TIMP members bind reversibly to the active site of all MMPs and have different affinities for specific MMPs. Thus, TIMPs play an important role in preventing degradation of the accumulating matrix during liver injury by antagonizing the activity of metalloproteinases. TIMP-1 has also an antiapoptotic effect on HSCs: It prevents clearance of activated HSCs during injury and promotes their survival through induction of B cell lymphoma 2 (Bcl-2) (193). HSCs are a key source of MMPs, especially MMP-2, -3, -9, and -13. Hepatic macrophages also regulate matrix remodeling and play a decisive role in matrix degradation by increasing MMP-13 production during resolution of liver fibrosis (194, 195).

In acute liver injury in rats, expression of both MMPs and TIMPs increases within hours. After a single dose of CCl_4, increased MMP-13, MMP-2, MMP-9, MMP-3, MMP-10, TIMP-1, and TIMP-2 can be detected. During the recovery phase, levels of all of them—apart from MMP-2 and the TIMPs—are rapidly reduced (196). In chronic human liver disease and animal models of fibrosis, however, levels of MMP-1/-13 do not change, but there is a progressive increase in TIMP-1 and -2 as fibrosis advances. TIMP expression can be detected soon (6 h) after liver injury and may precede the induction of procollagen I (197).

Mechanisms of Fibrosis Reversibility

Animal models remain essential to the study of the basic mechanism of fibrosis progression, reversibility, and the development of new antifibrotic therapies. The most standardized models are rodents (mouse and rat). Mouse models are more cost-effective because they usually require lower amounts of therapeutic agents (198). Bile duct ligation and CCl_4 are well-validated models of fibrosis progression and resolution. Spontaneous resolution may be observed four to six weeks after establishment of a bilio-jejunal anastomosis to reverse biliary obstruction or after cessation of CCl_4 administration. Analysis of mechanisms underlying fibrosis regression requires the use of more than one model to ensure that the findings are not model-specific artifacts.

Experimental evidence for the reversal of cirrhosis was rigorously characterized in a 12-week CCl_4 injury model (190). Following

Table 3 Main metalloproteinases involved in liver fibrosis[a]

Name	Family	Substrate	Sources
Collagenases			
Collagenase-1	MMP-1	III, I, II, VII, VIII, X, gelatin	HSCs
Neutrophil collagenase	MMP-8	I, III, II, V, VII, X, gelatin	Neutrophils
Collagenase-3	MMP-13	II, III, I, VII, X, gelatin	HSCs, MFs, KCs
Stromelysins			
Stromelysin-1	MMP-3	III, IV, V, IX, X, XI, gelatin, laminin, fibronectin, proteoglycans, glycoproteins, elastin, pro-MMP-1, pro-MMP-13	HSCs
Stromelysin-2	MMP-10	III, IV, V, gelatin, elastin, aggrecan	HSCs
Stromelysin-3	MMP-11	PAI-1; weak activity against matrix proteins	Hepatocytes
Gelatinases			
Gelatinase A	MMP-2	Gelatin, V, IV, VII, X, XI, elastin, laminin, III, II, I	HSCs, MFs
Gelatinase B	MMP-9	Gelatin, V, IV, VII, X, XI, elastin, laminin, III, II, I	KCs, HSCs, hepatocytes
Matrilysin	MMP-7	Entacin, gelatin, elastin, fibronectin, vitronectin, laminin, fibrinogen	HSCs
Metalloelastases			
MT-MMPs	MMP-12	Elastin, gelatins, IV, laminin, fibronectin, entactin, vitronectin, proteoglycan, myelin basic protein, a1-antitrypsin	Macrophages
MT-MMP-1	MMP-14	I, II, III, gelatin, fibronectin, vitronectin, laminin, fibrogen, pro-MMP-2, pro-MMP-13	HSCs, MFs, KCs
MT-MMP-2	MMP-15	Pro-MMP2, fibronectin, tenascin, laminin, aggrecan, perlecan	Hepatocytes, bile duct epithelial cells
TIMPs			
TIMP-1	TIMP-1	Pro-MMP-9, MMP-1, MMP-2, MMP-3, MMP-13	HSCs, MFs, KCs, hepatocytes
TIMP-2	TIMP-2	MT-MMPs, pro-MMP-2, MMP-3, MMP-13, MMP-7	KCs, HSCs, MFs
TIMP-3	TIMP-3	MT-MMPs, TACE, MMP-13	—
Others			
α2-Macroglobulin	—	Nonspecific proteinase scavenging	—

[a]Adapted from Reference 217. Abbreviations: HSC, hepatic stellate cells; KC, Kupffer cell; MF, myofibroblast; MMP, matrix metalloproteinase; PAI, plasminogen activator inhibitor; TIMP, tissue inhibitor of metalloproteinase.

cessation of toxin administration, liver pathology revealed the coexistence of macro- and micronodular cirrhosis. Micronodular cirrhosis was progressively replaced by macronodular cirrhosis, which persisted up to one year postinjury. Similar results were obtained in the bile duct occlusion model.

The best-validated explanation for spontaneous liver fibrosis regression is provided by apoptosis of hepatic MF. Although this response has been well characterized in rodent models (CCl_4- and bile duct ligation–induced fibrosis in rats), evidence in human disease is limited. Moreover, human hepatic MF have relatively higher levels of Bcl-2 than do rodent cells, and they are therefore less susceptible to apoptosis (199).

Regulation of Myofibroblast Apoptosis

Because the switch from survival to apoptotic mode appears critical to the clearance of fibrogenic MF during fibrosis resolution, extensive studies have explored its underlying mechanisms. For example, NF-κB prevents

apoptosis by maintaining expression of anti-apoptotic Bcl-2 and suppressing JNK activation of p53, thereby inhibiting Bax and PUMA expression (104, 200). NF-κB signaling refers to a family of dimeric transcription factors that regulate inflammation, innate and adaptive immunity, wound-healing responses, and cell survival. The molecules p50, p52, p65, ReIB, and cReI are the five subunits of NF-κB that usually form dimers that operate as transcriptional activators (104, 105).

Given its central role in preventing MF apoptosis, a more detailed summary of NF-κB signaling is relevant. Activation of NF-κB can occur via at least two signal transduction routes known as the canonical and noncanonical pathways. In the canonical pathway, the transcriptionally active NF-κB is the heterodimer p65:p50, although it remains inactivated in the cytoplasm bound to its inhibitory protein, IκBα. Various activators (TNF-α, LPS) can phosphorylate IκBα and allow its removal, permitting NF-κB to translocate to the nucleus and initiate transcription. When NF-κB binds to its cognate promoters, it also induces the expression of IκBα as an autoregulatory mechanism to prevent persistent NF-κB activation. NF-κB proteins are expressed in qHSCs; however, during cellular activation, the level of transcriptionally active NF-κB increases dramatically, which is crucial for two reasons: (*a*) It induces the expression of proinflammatory and profibrotic genes (*IL-6*, *IL-8*, *MCP-1*, and *ICAM1*), and (*b*) it confers resistance to apoptosis (106). Despite the autoregulatory ability of NF-κB to induce the expression of the *IκBα* gene, HSC activation is associated with a paradoxically persistent suppression of IκBα that is mediated by the transcriptional repressors CBF1 and MeCP2 (107–109).

Other molecules are implicated in MF apoptosis and survival. For example, FXR stimulates apoptosis via the induction of short heterodimer partner, a nuclear receptor that interacts with JunD, thereby preventing FXR from binding to the TIMP-1 promoter (138, 201). C/EBPβ induces apoptosis through caspase-8 activation when it is phosphorylated at threonine 127 by its regulatory molecule, RSK (127).

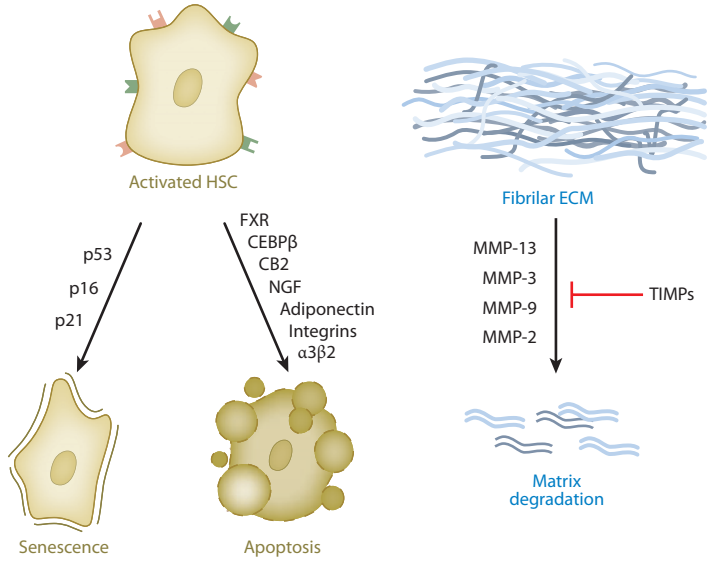


Figure 7

Fibrosis resolution pathways. Two key events in fibrosis resolution are the degradation of the fibrillar extracellular matrix (ECM) and reduction in myofibroblast survival. Tissue inhibitors of metalloproteinase (TIMPs) play an important role in preventing degradation of the accumulating matrix during liver injury by antagonizing the activity of metalloproteinases (MMPs) and promoting survival of activated hepatic stellate cells (HSCs). In contrast, several mediators have been implicated in inducing apoptosis and clearance of HSCs. Similarly, p21 and p16 proteins can limit the fibrogenic response by promoting senescence of HSCs. Abbreviations: CEBP, CCAAT-enhancer–binding protein; FXR, farnesoid X receptor; NGF, nerve growth factor.

Endogenous cannabinoids can either stimulate or inhibit liver fibrosis, depending on which receptor (CB1 or CB2) is engaged. As mentioned above, *CB1*$^{-/-}$ mice are resistant to fibrosis induced by CCl_4, thioacetamide, or bile duct ligation, and they display high rates of MF apoptosis (72, 202). Additionally, stimulation of CB2 induces MF apoptosis via induction of intracellular oxidative stress (73).

Nerve growth factor, a member of the mammalian neurotrophin family, can stimulate human melanocyte apoptosis via the inhibition of NF-κB (203–205). HGF promotes human melanocyte apoptosis in vitro and in vivo and suppresses PDGF-stimulated proliferation. However, other antifibrogenic effects have been attributed to the inhibition of TGF-β and to the reduced recruitment of

bone marrow–derived cells expressing MMP (206–208).

Adiponectin can suppress MF proliferation and stimulate their apoptosis (209, 210). This antifibrotic effect has been partially attributed to the downstream effect of adiponectin in AMPK activation (see above), which inhibits NF-κB activation and RSK phosphorylation. In contrast to adiponectin, leptin promotes survival of HSCs and is thus antiapoptotic (211), as well as a profibrogenic signal (212).

NK cells expressing NKG2D and TRAIL can directly induce MF apoptosis, although they are unable to kill qHSCs (167). Interestingly, alcohol may abrogate the antifibrotic effects of NK cells by blocking their apoptotic activity (167).

Components of the ECM may also be involved in the regression process and human melanocyte apoptosis. For example, disruption of the integrin α3β2 increases the ratio of Bax/Bcl2 and increases caspase-3 activation, leading to human melanocyte apoptosis (213).

Hepatic Stellate Cells and Myofibroblast Senescence

Senescence of activated HSCs can limit the fibrogenic response to tissue damage. Cellular senescence is a stable form of cell-cycle arrest that is mediated by progressive telomere shortening and activation of a DNA damage response (214, 215). Senescent HSCs are characterized by expression of β-galactosidase; induction of p53, p21, and p16; downregulation of matrix production; and upregulation of matrix-degrading enzymes (**Figure 7**) (214). The immune system, especially NK cells, plays an important role in the clearance of senescent cells. Although senescent HSCs may have a greater susceptibility to apoptosis (199), the functional and regulatory relationship between apoptosis and senescence in this cell type remains to be clarified.

CONCLUDING COMMENTS

The fibrotic response to chronic liver injury depends on both resident and recruited cell types. Characterization of the fibrogenic cell populations, evidence of their plasticity and pluripotentiality, and characterization of their cross talk with inflammatory cells will lead to important progress in our understanding of the disease. There have been major advances in characterizing the cellular and molecular biology, fibrogenic pathways, and genetic determinants of fibrosis progression and regression. Given such substantive progress in elucidating the underlying mechanisms, our current task is to translate these findings into the development of effective and targeted antifibrotic therapies that will modify the natural history of chronic fibrosing disease.

SUMMARY POINTS

1. Hepatic fibrosis is the liver's wound-healing response to any type of acute or chronic liver injury.
2. Perpetuation of the fibrotic reaction can lead to end-stage liver disease, cirrhosis, and hepatocellular carcinoma, whose incidence is increasing worldwide.
3. Because they produce ECM following activation by liver injury, HSCs are the key effectors of the fibrogenic process. However, other cellular sources implicated in hepatic scar production were recently identified.
4. Hepatic fibrogenesis is a complex, tightly regulated process in which genetic determinants and the immune system make important contributions.

5. Advances in elucidating fibrosis pathophysiology and regulation revealed the potential reversibility of fibrosis through apoptosis and senescence of fibrogenic cells.
6. A future challenge will be developing therapeutic strategies that can modify the progression of fibrogenic disease.

DISCLOSURE STATEMENT

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