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Molecular basis of hepatic fibrosis

Alex Y. Hui and Scott L. Friedman

Hepatic fibrosis is a wound-healing response to chronic liver injury, which if persistent can lead to cirrhosis and liver failure. Activation of hepatic stellate cells (HSCs), leading to accumulation of extracellular matrix, is the central event of fibrogenesis. Exciting progress has been made in understanding the molecular basis of this process. Major advances include: (1) elucidation of the effects (and signalling pathways) of key cytokines on HSCs; (2) understanding the transcriptional regulation of HSC activation; (3) characterisation of matrix proteases and their inhibitors; (4) demonstration of apoptosis as an important event in the resolution of hepatic fibrosis, and identification of its mediators; (5) elucidation of the complex and dynamic interaction between HSCs and matrix; and (6) understanding the role of other cellular elements in hepatic fibrosis and their interaction with HSCs. Ongoing research with gene analysis using cDNA or oligonucleotide microarrays, or transcriptional profiling, will further increase our knowledge of the regulation of the process. Ultimately, advances in the understanding of the molecular biology of hepatic fibrosis are critical to the development of effective, targeted antifibrotic therapy that might benefit millions of patients with chronic liver disease worldwide.

Hepatic fibrosis is the wound-healing response of the liver to chronic injury of any type. This includes injury caused by chronic liver diseases such as chronic viral hepatitis and alcoholic liver disease that eventually lead to cirrhosis and hepatic failure. Fibrosis is characterised by accumulation of extracellular matrix (ECM), with the formation of scar tissue encapsulating the area of injury. This results in failure of the liver to perform many of its usual functions, including carbohydrate and lipid metabolism, protein synthesis, the uptake and detoxification of macromolecules from blood, and the clearance of pathogens entering the body through the gut.

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The gradual process of ECM accumulation is reversible upon removal of the primary injury. In the rat model of liver fibrosis induced by carbon tetrachloride (CCl_4), the hepatic architecture is normalised within weeks after cessation of CCl_4 administration (Ref. 1). In human studies, a sustained virological response to treatment for chronic hepatitis B or chronic hepatitis C is associated with improvement in histological scores for fibrosis (Refs 2, 3). Even cirrhosis is reversible, as shown by treatment trials for chronic hepatitis C and for chronic biliary obstruction (Refs 4, 5, 6). Secondary biliary cirrhosis resolved upon the relief of such obstruction.

The study of the molecular mechanisms underlying hepatic fibrosis is therefore important not only for understanding the pathogenesis of chronic liver diseases but also for discovering possible therapeutic targets. Research over the past 15 years has established that hepatic stellate cells (HSCs) play a central role in hepatic fibrosis (Fig. 1) (Ref. 7). This review highlights our latest understanding of the molecular basis of the biology of HSCs and their interaction with other cell types and the ECM during fibrosis. For further background on the microanatomy of liver, and how this relates to its functions in modulating immune responses to gut-derived antigens, the reader is referred to a recent review in *Expert Reviews in Molecular Medicine* (Ref. 8).

ECM and fibrosis

ECM in normal and injured liver

The ECM is a complex network of macromolecules in association with the basement membrane (BM) of the cell surface that provides cells with an extracellular scaffold (Ref. 9). The dynamic interaction between cells and signalling molecules of the ECM regulates different biological activities of the cells.

The components of ECM in liver include collagens, noncollagenous glycoproteins, glycosaminoglycans, proteoglycans, matrix-bound growth factors and matricellular proteins. The last group comprises matrix-associated nonstructural factors that mediate cell–matrix interactions, such as laminin and fibronectin. In normal liver, the dense, interstitial ECM with the so-called 'fibril-forming' collagens (types I, III, V and XI) is largely confined to the capsule, around large vessels and in the portal triad (where branches of the portal vein, hepatic artery and biliary ducts meet). The perisinusoidal matrix, in contrast, is composed of both an interstitial and a BM-like low-density ECM. The components include collagen types I, III and V, microfibrillar collagen VI, BM collagens IV and XVIII, the small proteoglycan decorin, fibronectin, laminin and heparan sulphate proteoglycan (Ref. 9).

Hepatic fibrosis is associated with a significant change in both the quantity and composition of the ECM. Total collagen content increases by three- to tenfold (Ref. 10). The perisinusoidal low-density ECM is gradually replaced by a high-density ECM, with accumulation of fibrillar collagens and electron-dense BM (Ref. 11). There is also an increase in glycoproteins, proteoglycans and glycosaminoglycans, and a shift from BM-type proteoglycans (heparan sulphate) to interstitial-type (dermatan sulphates and chondroitin sulphates) (Ref. 12).

The importance of the ECM in providing the microenvironment for normal cell function became evident when hepatocyte function was shown to be compromised by a predominantly interstitial perisinusoidal matrix (Ref. 13). A loss of hepatocyte microvilli and disappearance of endothelial fenestrations are associated with the changes in ECM (Fig. 1). Furthermore, the high-density matrix activates HSCs, which perpetuates the process of fibrogenesis.

Interaction between cellular components and ECM in hepatic fibrosis

The ECM interacts with cells via matrix receptors on the cell membrane. The best-characterised of these are the integrin family of heterodimeric transmembrane receptors (Ref. 14). Integrin receptors are composed of α and β subunits, and at least 18 α and eight β subunits are currently known. The various combinations result in over 20 functional integrin dimers with different specificities. Normal adult human hepatocytes express low levels of three integrin dimers: the collagen and laminin receptor α1β1; the fibronectin receptor α5β1; and the tenascin receptor α9β1. In addition, integrins α1β1, α2β1, α5β1 and α6β4 have been identified in cultured HSCs (Refs 15, 16). In experimental liver fibrosis, upregulation is observed of laminin-binding integrins α6β1 and α2β1, and fibronectin-binding integrin α5β1 (Refs 15, 17, 18).

In addition to ECM molecules, integrin ligands include cell adhesion molecules. Most integrin ligands contain an Arg-Gly-Asp (RGD) tripeptide sequence, which is necessary but insufficient for

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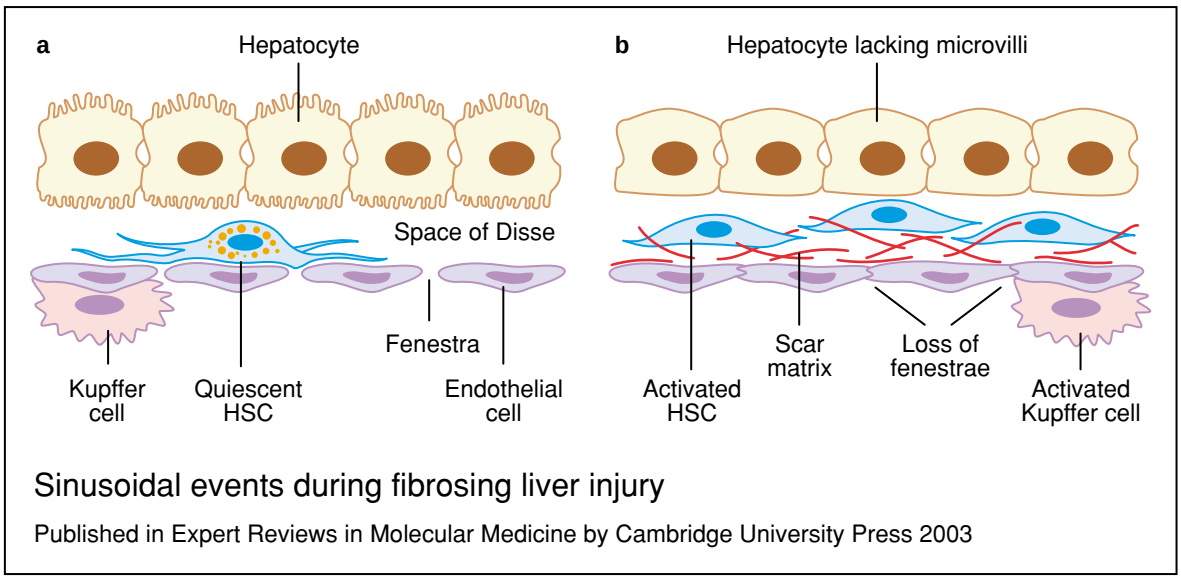


Figure 1. Sinusoidal events during fibrosing liver injury. (a) Normal liver. The cellular elements of liver are organised within the units of sinusoids, with the subendothelial space of Disse separating the epithelial component (hepatocytes) from the sinusoidal endothelium. The tissue macrophages – Kupffer cells – are found in the sinusoids, whereas the hepatic stellate cells (HSCs) are perisinusoidal in location. The hepatocyte microvilli are important for the normal physiological function of the cells. The characteristic fenestrae of the sinusoidal endothelial cells permit the passage of large macromolecules including lipoproteins. (b) Injured liver. As fibrosis develops in response to liver injury, changes occur within the subendothelial space and within the hepatic sinusoid. These changes include alterations in both cellular responses and extracellular matrix (ECM) composition. Activation of HSCs, which are the primary source of ECM, leads to the accumulation of scar (fibril-forming) matrix. This results in widening of the space of Disse and loss of endothelial fenestrae. Transport across the sinusoidal wall is hence reduced, leading to deterioration of hepatic function. Activation of Kupffer cells accompanies liver injury and contributes to paracrine activation of HSCs. For a diagram relating this microanatomy to the liver as a whole, see fig001dab in Ref. 8. Figure based on illustration in Ref. 25, with permission (Copyright © 2000 by The American Society for Biochemistry and Molecular Biology, Inc.) **(fig001sfn)**.

signalling. Upon binding of ligand, integrin becomes activated and recruits several signalling molecules in complexes called focal adhesions. Examples of such molecules are caveolin, paxillin and tyrosine kinases such as Fyn and focal adhesion kinase (FAK). Further downstream, the complexes are associated with other kinases, adaptor molecules or cytoskeletal components. FAK recruits phosphatidylinositol 3-kinase (PI 3-K) and also Grb2, an adaptor molecule that eventually leads to activation of the extracellular-signal-regulated kinase (ERK) pathway. Furthermore, during HSC adhesion, phospholipase Cγ (PLCγ) is recruited by FAK. PLCγ is a substrate of receptor tyrosine kinases, and phosphorylation of PLCγ constitutes one of the main signals that mediate platelet-derived growth factor (PDGF)-induced chemotaxis (Ref. 19) (see below). Hence, a complex web of crosstalk occurs between the integrin pathways and signalling mechanisms of the growth factor receptors. Indeed, the possibility of functional cooperation between the PDGF pathway and integrin signalling was clearly demonstrated when ligand-activated PDGF-β receptors clustered in areas corresponding to focal adhesion complexes (Ref. 20).

Another matrix receptor recently found to have a potential role in liver fibrosis is discoidin domain receptor 2 (DDR2) (Ref. 21). DDR2 is a tyrosine kinase receptor that responds to ECM ligands but not to soluble peptide factors. It is activated primarily by collagen type I, and to a lesser extent by collagen types II, III and V. DDR2 is induced during HSC activation, and the phosphorylated receptor mediates growth stimulation in response to collagen type I. Moreover, collagen type I upregulates DDR2 expression, resulting in a positive-feedback loop and further proliferation.

In addition to its structural role and direct interaction with cells, the ECM also regulates cell

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function indirectly via modulation of the availability and activity of growth factors including PDGF, transforming growth factor β (TGF-β), connective tissue growth factor (CTGF), vascular endothelial growth factor and hepatocyte growth factor (HGF) (Ref. 21). Proteoglycans such as decorin, biglycan, fibromodulin and glycosaminoglycans are the main ECM components that bind growth factors and cytokines. Fibronectin and laminin also bind tumour necrosis factor α (TNF-α), whereas collagen binds PDGF, HGF and interleukin 2 (IL-2). As well as protecting such factors from proteolysis, the ECM controls their release through the actions of proteases and their inhibitors, resulting in further modulation of their activities.

HSCs and fibrosis

The liver comprises a variety of specialised cell types required for its various functions (Ref. 8). Of these, HSCs are the primary source of ECM. They account for 15% of cells in normal liver and are found perisinusoidally. In the quiescent state, they are characterised by the presence of long cellular processes and perinuclear cytoplasmic lipid droplets containing vitamin A esters (retinoid) (Ref. 22). HSCs respond to chronic tissue injury by transdifferentiating into myofibroblast-like cells, which are devoid of vitamin A droplets, and have extended and dilated rough endoplasmic reticulum and a prominent Golgi complex. They express cytoskeletal proteins including α smooth muscle actin (α-SMA) and glial fibrillary acidic protein (GFAP) that distinguish them from fibroblasts.

It is increasingly clear that HSCs constitute a heterogeneous group of cells that are functionally and anatomically similar but different in their expression of cytoskeletal filaments, their retinoid content, and their potential for ECM production (Refs 22, 23, 24). Collectively, however, these HSC-related cell types are clearly responsible for ECM production in liver fibrosis, and are thus the primary focus of current efforts to develop antifibrotic therapies.

The activation of HSCs can be subdivided into two major phases: initiation and perpetuation (Ref. 25). Initiation refers to early changes in gene expression and phenotype that render the cells responsive to cytokines and stimuli, whereas perpetuation results from the effects of these stimuli on maintaining the activated phenotype. The concepts of 'initiation' and 'perpetuation' actually reflect a continuum of changes, such that even activated cells continually evolve in their structural and functional features.

Initiation of HSC activation

The initial activation of HSCs is likely to be a result of stimuli produced by neighbouring cells – namely hepatocytes, Kupffer cells (KCs), circulating leukocytes, platelets and sinusoidal endothelial cells (SECs) – in response to liver injury (Fig. 1 and Fig. 2). Such stimuli include reactive oxygen species (ROS)/lipid peroxides, growth factors and inflammatory cytokines.

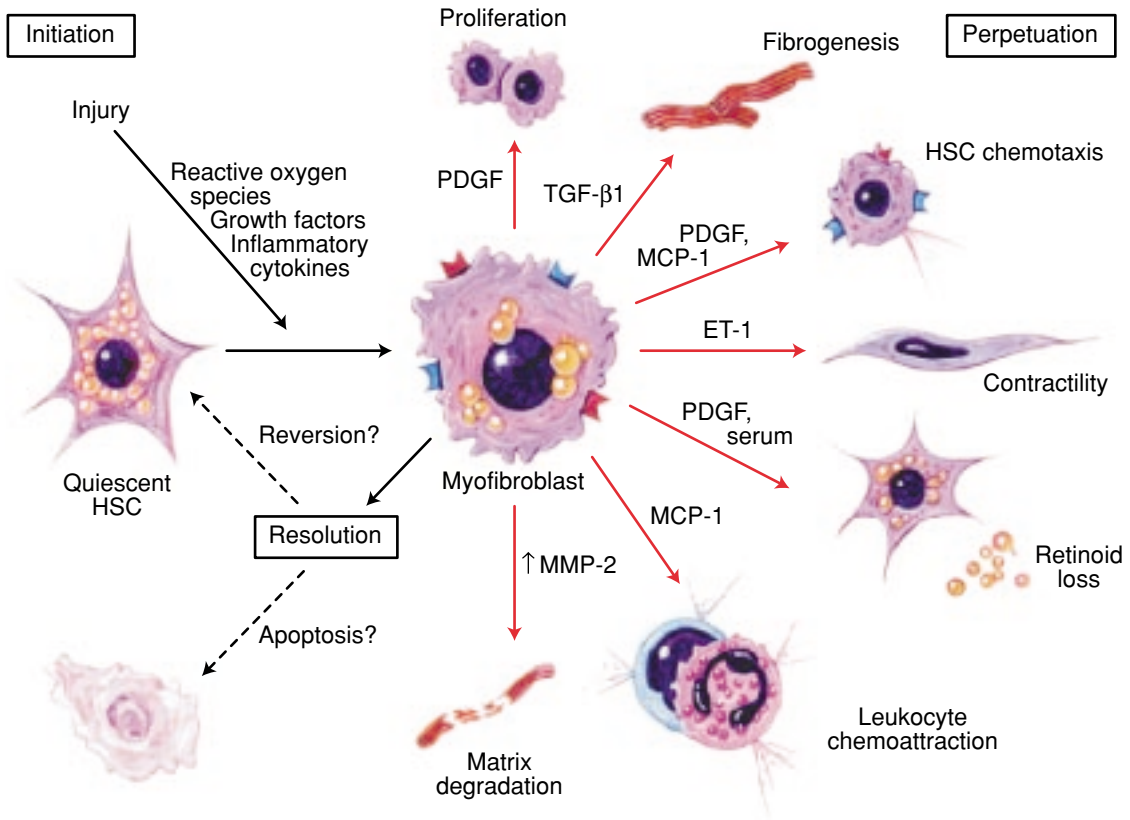
Formation of ROS and lipid peroxides by KCs, neutrophils and hepatocytes are features of liver injury and fibrosis (Refs 26, 27). In rats receiving CCl_4, HSC activation correlates temporally and spatially with superoxide production (Ref. 28). In addition, in vitro experiments demonstrated that oxidative stress enhanced activation of, and collagen synthesis by, HSCs, whereas antioxidants abrogated the stimulatory effect of free radicals (Refs 29, 30).

Transcription regulation associated with HSC activation is an area of intensive research. The effect of oxidative stress might be mediated by nuclear proteins such as c-Myb, NF-κB and CCAAT/enhancer binding protein-β (C/EBPβ) (Refs 29, 31). c-Myb is involved in transcriptional regulation and cell proliferation, since high levels of c-Myb DNA-binding activity are found in activated HSCs but not in quiescent HSCs (Refs 29, 31). Malondialdehyde, a product of lipid peroxidation, induces c-Myb expression in HSCs. One of the genes that c-Myb binds to, through the E-box motif in the promoter, encodes α-SMA. Transfection of c-Myb stimulated α-SMA expression in quiescent HSCs and, more importantly, transfection of c-Myb antisense, but not sense, RNA inhibited both expression of the endogenous α-SMA gene and HSC activation (Ref. 31). These findings imply that the HSC activation status could be directly manipulated through controlled expression of a transcription factor.

Recent studies have identified a role of peroxisome proliferator-activated receptors (PPARs), which are a family of ligand-activated transcription factors. Among the three types of PPARs (PPAR-α, -β and -γ), PPAR-γ mRNA is expressed in HSCs of normal rat liver and its expression is greatly reduced in HSCs from

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Phenotypic features of hepatic stellate cell activation during liver injury and resolution

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Figure 2. Phenotypic features of hepatic stellate cell activation during liver injury and resolution. Following liver injury, hepatic stellate cells (HSCs) undergo 'activation' mediated by reactive oxygen species, growth factors and inflammatory cytokines produced by hepatocytes, Kupffer cells, leukocytes, platelets and sinusoidal endothelial cells. This results in a transition from quiescent vitamin-A-rich HSCs into proliferative, fibrogenic, contractile myofibroblasts. The activation of HSCs can be subdivided into two major phases: initiation and perpetuation. Initiation refers to early changes in gene expression and phenotype that render the cells responsive to cytokines and stimuli, whereas perpetuation results from the effects of these stimuli on maintaining the activated phenotype. The activated HSCs or myofibroblast-like cells are devoid of vitamin A droplets, have extended and dilated rough endoplasmic reticulum and a prominent Golgi complex. The major phenotypic changes include proliferation, fibrogenesis, chemotaxis, contractility, retinoid loss, leukocyte chemoattraction and cytokine release, and matrix degradation. Key mediators underlying these changes are shown. The fate of activated HSCs during the resolution of liver injury includes selective clearance by apoptosis and/or reversal to a quiescent phenotype. Abbreviations: ET-1, endothelin 1; MCP-1, monocyte chemotactic protein 1; MMP-2, metalloproteinase 2; PDGF, platelet-derived growth factor; TGF-β, transforming growth factor β. Modified figure reproduced from Ref. 25, with permission (Copyright © 2000 by The American Society for Biochemistry and Molecular Biology, Inc.) **(fig002sfn)**.

fibrotic liver caused by bile duct ligation (Ref. 32). Furthermore, the level of PPAR-γ protein in cultured HSCs is reduced upon activation, and their ligands inhibit cell proliferation and the expression of α-SMA and collagen type 1 (Refs 32, 33, 34). It is thus possible that the PPAR-γ pathway is important in maintaining the quiescent phenotype of HSCs.

Another potentially important transcription factor in HSC activation is Kruppel-like factor 6

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(KLF6), which belongs to the family of zinc-finger transcription factors that characteristically bind to either GC-rich boxes or CACCC elements of promoters (Ref. 35). KLF6 is rapidly induced during HSC activation in vivo and in vitro as an immediate-early gene (Ref. 36). Its putative targets include collagen 1α (I), TGF-β1 and its receptors, and urokinase-type plasminogen activator (uPA), which activates latent TGF-β1 (Refs 37, 38). Remarkably, it has also been recently identified as a tumour suppressor gene in prostate cancer, and is implicated in the regulation of cell growth (Ref. 39). Thus, a paradox regarding KLF6 has not yet been resolved, since this growth suppressive gene is induced in HSCs at a time immediately preceding a large proliferative burst.

Several growth factors and cytokines have been implicated in the biology of activated HSCs. Their role in the initiation process is less clear. Potentially important mediators involved in transdifferentiation of HSCs from quiescent to activated phenotypes are PDGF, TGF-β1, epidermal growth factor (EGF), insulin-like growth factor 1 (IGF-1) and TNF. By contrast, interferon γ (IFN-γ) reduces HSC activation in both in vivo and in vitro models (Ref. 40).

There is evidence that the ECM also modulates HSC transdifferentiation. Freshly isolated HSCs become activated when cultured on a plastic surface, yet remain quiescent if cultured on matrigel (Ref. 41). Thus, a BM-like matrix might contribute to maintenance of the quiescent state of HSCs. Fibronectin, a component of ECM, activates HSCs and might be an important signal at the early stage of HSC activation (see below) (Ref. 42).

Perpetuation of HSC activation

Perpetuation of activated HSCs involves distinct changes in cell biology that result in the accumulation of ECM and fibrosis (Fig. 2): proliferation; fibrogenesis; chemotaxis; leukocyte chemoattraction and cytokine release; contractility; retinoid loss; and matrix degradation. These are each discussed in further detail below. Paracrine and autocrine stimuli with growth factors, cytokines and ROS are responsible for the regulation of these complicated and distinct processes.

Proliferation

The number of HSCs in liver increases during progressive injury and fibrosis. Several peptide growth factors and cytokines have been found to affect HSC proliferation. The mitogens known to date include PDGF, thrombin, EGF, TGF-α, angiotensin II and IGF-1. Factors that are anti-proliferative include endothelin (ET), TNF-α and IFN-γ.

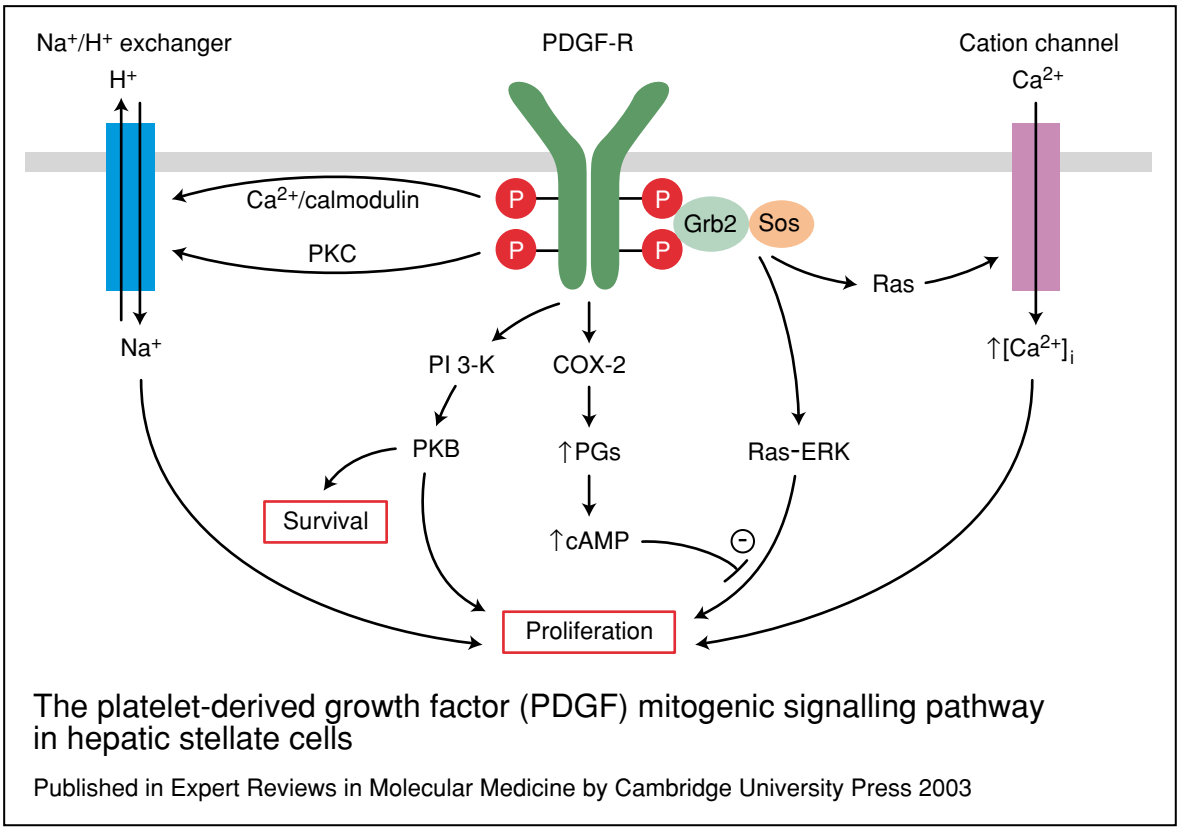
PDGF is the most potent HSC mitogen and its downstream pathways are well characterised (Ref. 43) (Fig. 3). The PDGF receptor (PDGF-R) has intrinsic tyrosine kinase activity and its expression increases with HSC activation. Of the two types of PDGF-Rs, the β isoform predominates. Upon binding of PDGF, the receptor undergoes autophosphorylation. This allows binding of adaptor molecules such as Grb2 and activation of the GTP-binding protein Ras and ERK, both of which are required for cell growth (Ref. 44). Activation of a cation channel via Ras resulting in Ca^{2+} influx also contributes to the mitogenic signal via an increased intracellular concentration of Ca^{2+}. Expression of the c-Fos proto-oncogene and binding activity of the AP-1 transcription factor are also upregulated (Ref. 45). Another kinase recruited by the activated PDGF-R is PI 3-K (Ref. 46). PI 3-K and its downstream mediators including protein kinase B (PKB) are essential for the mitogenic effect of PDGF. Wortmannin, an inhibitor of PI 3-K, abolishes PDGF-induced HSC proliferation. A third pathway involves the Ca^{2+}/calmodulin- and protein kinase C-dependent Na^+/H^+ exchanger. Inhibition of the exchanger by amiloride abolishes the mitogenic effect of PDGF (Refs 47, 48). Similarly, calphostin C, a PKC inhibitor, abrogates PDGF-induced HSC proliferation (Ref 48).

Activation of the PDGF-R also increases synthesis of the cyclooxygenase 2 (COX-2) enzyme and production of prostaglandin E_2 (PGE_2) (Ref. 49). PGE_2 increases cAMP production and inhibits ERK phosphorylation and activity, possibly by binding to one of the G-protein-coupled receptors. This provides a negative-feedback loop for the mitogenic effects of PDGF, as well as a mechanism by which the antiproliferative effects of ET and TNF-α are mediated (Ref. 50) (see below).

The Ras–ERK and PI 3-K pathways are not exclusive to PDGF signalling, as other growth factors such as IGF-1 and EGF also activate these pathways. However, unlike PDGF, the effect on HSCs of PI 3-K activation by IGF-1 is predominantly to promote cell survival and decrease apoptosis (Ref. 51).

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The platelet-derived growth factor (PDGF) mitogenic signalling pathway in hepatic stellate cells

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Figure 3. The platelet-derived growth factor (PDGF) mitogenic signalling pathway in hepatic stellate cells. PDGF binding to its receptor (PDGF-R) leads to receptor dimerisation and autophosphorylation, and thus formation of high-affinity binding sites for signalling proteins such as the adaptor molecules Shc, Shb and Grb2. Grb2 further associates with Sos to activate downstream pathways. The downstream mitogenic signalling mechanisms include the Ras–ERK (extracellular-signal-regulated kinase) pathway, the phosphatidylinositol 3-kinase (PI 3-K) pathway, and the Ca^{2+}/calmodulin- and protein kinase C (PKC)-dependent Na^+/H^+ exchanger. Activation of a cation channel via Ras resulting in Ca^{2+} influx also contributes to the mitogenic signal via increased intracellular concentration of Ca^{2+}. In addition to its mitogenic effect, the PI 3-K and protein kinase B pathway results in production of survival signals and decreases apoptosis. Negative feedback for the mitogenic effects of PDGF is provided by activation of the prostaglandin (PG) cascade. Abbreviations: cAMP, cyclic adenosine monophosphate; COX-2, cyclooxygenase 2; PKB, protein kinase B. Figure based on illustration in Ref. 43, with permission (Copyright © 2001 by Thieme Medical Publishers, Inc.) **(fig003sfn)**.

As discussed above, a role for the receptor tyrosine kinase (RTK) DDR2 has recently been uncovered in HSC activation (Ref. 21). This unusual RTK signals in response to accumulating fibrillar collagen, leading to enhanced cell growth and matrix degradation.

Regulation of the cell cycle at the molecular level in HSC is an important topic that has not been adequately addressed. However, a few facts have begun to emerge. Activation and proliferation of HSCs involve the entry of quiescent HSCs into the cell cycle (Ref. 52). Expression of G1 cyclins including D1, D2 and E has been closely associated with HSC cell cycle transition from G1 to S phase. Suppressed expression of these cyclins might contribute to the maintenance of HSC quiescence, which could be exploited therapeutically. For example, TNP-470, a semisynthetic analogue of fumagillin, has an antifibrotic effect in CCl_4-induced liver fibrosis in rats that is achieved via the suppression of expression of cyclins D1, D2 and E, thereby inhibiting HSC proliferation by blocking the cell cycle transition from G1 to S phase (Ref. 53).

Fibrogenesis

Among many known profibrogenic cytokines, TGF-β is the most potent fibrogenic stimulus to

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HSCs, resulting in production of ECM (Ref. 54). Of the three types of TGF-β, most research has been conducted on the β1 isoform. TGF-β is secreted with the non-covalently linked latency-associated peptide (LAP). The complex lacks biological activity and is further anchored by a large protein: latent TGF-β-binding protein (LTBP). LTBP has homology with ECM components and as such might sequester TGF-β for rapid release before further de novo synthesis takes place (Ref. 55). Activation of the complex can be achieved by various compounds, some of which have proteolytic activity such as metalloproteinases (MMPs) and tissue plasminogen activator (tPA).

There are three types of TGF-β receptors. TGF-β binds to the type II receptor (Tβ-RII), which subsequently recruits the type I receptor (Tβ-RI) and, together, they form a heterotrimer complex (Fig. 4). The intrinsic serine/threonine kinase activity of Tβ-RII results in the phosphorylation of Tβ-RI, which in turn is responsible for propagation of the downstream signals via its own kinase activity on the Smad proteins (Ref. 56). Smad 2 and Smad3 are phosphorylated by Tβ-RI and heterodimerise with Smad4 before migrating into the nucleus where they regulate gene expression. Smad7, in contrast, inhibits the phosphorylation of Smad2 and Smad3.

Both Tβ-RI and Tβ-RII are present in quiescent HSCs and activated HSCs with a myofibroblast-like phenotype (Ref. 57). Isolated HSCs maintained in a quiescent state by culturing the cells in suspension displayed minimal specific binding for TGF-β by affinity labelling. By contrast, activated HSCs on uncoated plastic

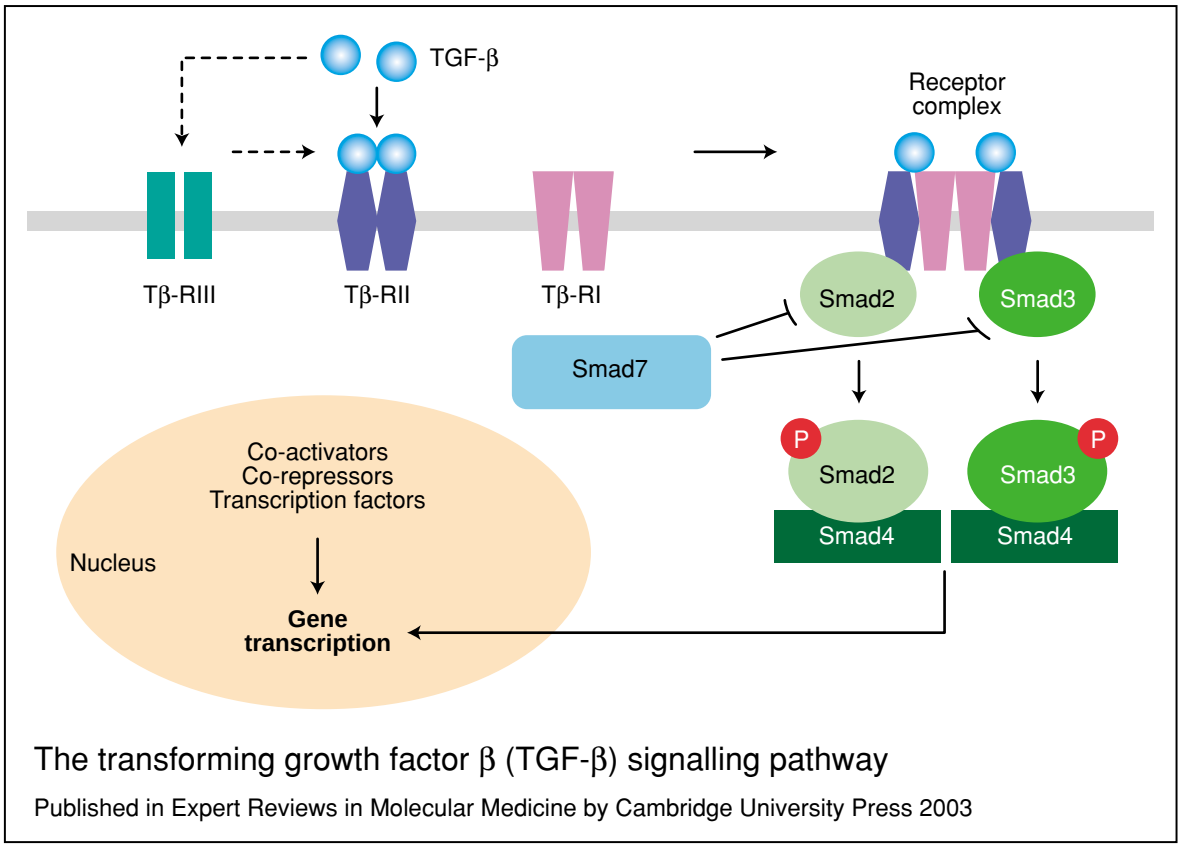


Figure 4. The transforming growth factor β (TGF-β) signalling pathway. TGF-β binds to the receptor Tβ-RII; this binding might be enhanced by the presence of Tβ-RIII. After binding to TGF-β, Tβ-RII recruits and phosphorylates Tβ-RI, leading to activation of Smad2 and Smad3 by phosphorylation (P). This process is inhibited by Smad7. Activated Smad2 and Smad3 form heterodimers with Smad4 and translocate to the nucleus. Together with co-activators, co-repressors and other transcription factors, the Smad complex regulates gene expression. Modified figure reproduced from Ref. 43, with permission (Copyright © 2001 by Thieme Medical Publishers, Inc.) **(fig004sfn)**.

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display saturable binding of TGF-β. Activation of HSCs is hence associated with increased responsiveness to TGF-β and in turn enhanced ECM synthesis (Ref. 58).

TGF-β induces ECM synthesis via multiple pathways. Collagen gene expression has been commonly used as a marker of TGF-β-induced ECM synthesis. A series of studies by Inagaki and colleagues have established the interaction between Smad3 and the transcription factor Sp1 (Refs 59, 60, 61). The binding of Sp1 to the promoter of the collagen α2(I) gene mediates the stimulatory effect of TGF-β. Meanwhile, another group demonstrated that TGF-β induced accumulation of H_2O_2 in HSCs, which upregulated α1(I) procollagen gene expression at least in part via a C/EBPβ-dependent mechanism (Ref. 62). A third group found that TGF-β activated the ERK pathway, which positively modulated the expression of the collagen gene. The action of ERK depends on AP-1, Sp1 and the NF-1-binding site of the procollagen α1(I) promoter (Refs 63, 64, 65). Therefore, TGF-β regulates ECM synthesis via multiple signalling pathways.

The cytokine CTGF is also important in fibrogenesis. Increased CTGF expression is found in liver biopsies of patients with chronic viral hepatitis and rodent rat livers after CCl_4 treatment or bile duct ligation. It is also detected in the supernatant and cell lysate of rat HSC culture (Ref. 66). In vitro, CTGF upregulates expression of type 1 collagen. Interestingly, CTGF expression is stimulated by TGF-β through a TGF-β-responsive element in the CTGF promoter (Ref. 67). This suggests that CTGF might be a downstream effector of TGF-β specifically involved in the fibrogenic effect of this growth factor.

Chemotaxis

After acute liver injury, HSCs are found in increased numbers in areas of inflammatory infiltrates (Ref. 68), and this is likely to be a consequence of both proliferation and migration of HSCs. This ability to migrate can be shown in quiescent and activated HSCs, and in vitro studies have identified several chemoattractants: PDGF, IGF-1, ET and monocyte chemotactic protein 1 (MCP-1). Thus, in addition to having a mitogenic role, PDGF and IGF-1 are also chemotactic for HSCs, with PDGF being the more potent (Ref. 51).

The growth factors PDGF and IGF-1 appear to share similar pathways in inducing HSC migration, involving both PI 3-K and Ras–ERK pathways (Ref. 51). Wortmannin inhibits PI 3-K and blocks IGF-1- or PDGF-induced migration. Likewise, chemotaxis is markedly reduced by pre-incubation of HSCs with PD-98059, a selective inhibitor of mitogen-activated protein kinase kinase. In addition, phosphorylation of both FAK and PLCγ is essential for the chemotactic effect of PDGF (Ref. 19). HSC migration is stimulated by PDGF in a dose-dependent manner and this event occurs in the presence of phosphorylated FAK. This is associated with a parallel dose-dependent PLCγ tyrosine phosphorylation. Herbimycin A, a tyrosine kinase inhibitor, completely abolishes the process.

ET was demonstrated to be important in HSC migration in response to wounding in experiments in vitro. The Rho-kinase pathway downstream of the ET receptors mediates the effect by acting on the actomyosin cytoskeleton (Ref. 69) (see later).

MCP-1 belongs to the CC class of chemokine family. This family promotes leukocyte recruitment, with different members having different relative specificities for leukocyte subpopulations (Ref. 70). Secretion of MCP-1 by HSCs results in the recruitment of monocytes and lymphocytes (discussed in further detail below). In addition, MCP-1 causes migration of activated, but not quiescent, HSCs. The effect is mediated by the PI 3-K pathway and requires Ca^{2+} influx. In contrast to the MCP-1 pathway in leukocytes, the chemokine receptor CCR2 is not responsible for signal transduction in HSCs (Ref. 71).

Leukocyte chemoattraction and cytokine release

One of the roles of HSCs during liver injury is the secretion of chemoattractants that facilitate recruitment of leukocytes to the site of injury as part of the inflammatory response. Several cytokines secreted by HSCs control different steps of the inflammation cascade (Table 1).

Early studies found that macrophage colony-stimulating factor (MCSF) was produced by HSCs upon stimulation with PDGF. MCSF maintains differentiation of monocytes/macrophages and might be important in KC homeostasis during liver injury (Ref. 72). Subsequent to this discovery, it was also found that HSC-conditioned medium contained chemotactic factors for monocytes, and MCP-1 was found to account for 80% of this activity (Ref. 73).

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Table 1. Hepatic stellate cells secrete several cytokines and cell adhesion molecules that regulate different steps of the inflammatory process (tab001sfn)

Inflammatory effect	Regulatory cytokine or cell adhesion molecule
Leukocyte recruitment and activation	Chemokines, e.g. MCP-1; PAF; complement; osteopontin; IL-8
Leukocyte maturation and survival	MCSF, SCF
Leukocyte adhesion	ICAM-1, VCAM-1, NCAM, PAF
Mast cell recruitment and activation	SCF
Acute phase protein synthesis	IL-6
Inhibition of inflammation	IL-10, TGF-β

Abbreviations: ICAM-1; intercellular adhesion molecule 1; IL, interleukin; MCP-1, monocyte chemotactic protein 1; MCSF, macrophage colony-stimulating factor; NCAM, neural cell adhesion molecule; PAF, platelet-activating factor; SCF, stem cell factor; TGF-β, transforming growth factor β; VCAM-1, vascular cell adhesion molecule 1.

MCP-1 recruits leukocytes and activates T cells and basophils. Interestingly, it also exerts chemotactic effects on HSCs. Its expression is enhanced in conditions of liver damage, such as in patients with chronic viral hepatitis (Refs 74, 75). In an animal model of liver injury, the majority of MCP-1 expression in liver occurred in HSCs. In vitro, proinflammatory cytokines such as IL-1, TNF-α and IFN-γ upregulate MCP-1 gene expression in HSCs. Other inducers of MCP-1 include TGF-β, thrombin, lipopolysaccharide (LPS) and oxidative stress. The intracellular pathways that mediate the effect of these stimuli include NF-κB and the PG cascade (Refs 76, 77). The fact that DNA binding of NF-κB is present in activated HSCs but not in quiescent cells might explain why activated cells are more responsive to some of these inducers (Refs 29, 78).

Migration of leukocytes might also be assisted by cell adhesion molecules expressed by HSCs. Intercellular adhesion molecule 1 (ICAM-1) is expressed in activated but not quiescent HSCs (Ref. 79). It binds integrin receptors on lymphocytes, monocytes and neutrophils and its expression in HSCs can be upregulated by TNF-α, IFN-γ and LPS. Similarly, neural cell adhesion molecule (NCAM) expression is not detected in freshly isolated HSCs but only after at least three days of culture (Ref. 80). NCAM might be important in lymphocyte migration.

HSCs are not only sources of proinflammatory mediators, they are also producers of cytokines with anti-inflammatory activity. A keenly studied example is that of IL-10. IL-10 inhibits cytokine synthesis by T helper 1 (Th1) cells and macrophages (Ref. 81). IL-10 mRNA becomes detectable after culture activation of HSCs and its gene expression is enhanced by TNF-α, TGF-β and LPS (Refs 82, 83). In vivo studies also confirm that IL-10 RNA and protein are upregulated in HSCs from rats injured with bile duct ligation, as compared with those from sham-operated animals (Ref. 83). Expression of IL-10 in rat liver is upregulated after intraperitoneal CCl_4 injection, and in IL-10-gene-deleted ($IL\text{-}10^{-/-}$) mice CCl_4 treatment results in more severe fibrosis (Ref. 84). The potential antifibrotic effect of IL-10 was explored in a pilot human study on patients with chronic hepatitis C (Ref. 85). IL-10 reduced liver fibrosis in a large proportion of patients receiving treatment, but results from a controlled trial have not been reported.

Finally, TGF-β might also function as an immunoregulatory factor by suppressing lymphocyte and macrophage activity, in addition to serving as an autocrine and paracrine profibrogenic cytokine (Refs 86, 87).

Contractility

In vitro and in vivo studies provide ample evidence that HSCs are contractile. Activated HSCs express intermediate filaments typically associated with smooth or skeletal muscle cells, including α-SMA, desmin, nestin, vimentin and

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skeletal muscle myosin heavy chain isoforms (Refs 22, 88). GFAP, an intermediate filament normally found in cells of astroglial lineage, is also expressed. As mentioned above, the heterogeneity of HSCs is reflected in the differential expression of these proteins in different subpopulations of HSCs. For example, periportal HSCs express more desmin than pericentral cells do (Ref. 89). The complete functional and/or development significance of HSC heterogeneity is not fully appreciated. One implication might be that tissue-specific transcription factors might subtly regulate HSC differentiation in a temporally and/or spatially dependent manner. For example, MyoD, a myogenic transcription factor that controls skeletal muscle differentiation, has been identified in activated HSCs. Regulation of a discrete set of target genes by this transcription factor might in turn determine the myogenic and contractile phenotypes in subpopulations of HSCs (Refs 88, 90).

The contractile filaments and the long perisinusoidal and intersinusoidal cytoplasmic processes allow HSCs to fulfil the physiological role of sinusoidal contraction, which in turn regulates hepatic blood flow (Ref. 91). In fibrotic or cirrhotic liver, HSCs are activated and increased in number. They are also found in large numbers in the fibrous bands typical of end-stage cirrhosis. As such, they raise the intrahepatic resistance to blood flow by constricting the sinusoids and contracting the whole cirrhotic liver. Activation and transdifferentiation of quiescent HSCs into the myofibroblast phenotype is associated with enhanced contractility. This is attributed to expression of the cytoskeletal proteins α-SMA and GFAP, and activation of voltage-operated Ca^{2+} channels and signalling cascades that stimulate contraction.

Several molecules provoke HSC contraction: thrombin, angiotensin II, vasopressin, $PGF_{2\alpha}$ and ET. The ET cascade is particularly well studied, both in vitro and in vivo. Physiologically, ETs are potent vasoconstrictors produced by endothelial cells (Ref. 92). Their function is the regulation of local blood flow, although other roles in development and cell proliferation have also been discovered. In studies in vivo, an ET receptor antagonist, bosentan, reduced portal pressure in cirrhotic liver in rats, presumably by acting on HSCs (Refs 93, 94, 95).

Among the three ETs, ET-1 is the most important in hepatic fibrosis. ET-1 is produced by HSCs and endothelial cells after liver injury (Ref. 96) and, among the different cell types in injured liver, HSCs express the most ET receptors (Ref. 97). An implication of these findings is that the effect of ET-1 is both paracrine and autocrine. There are at least two types of ET receptors – ET_A and ET_B – and both are G-protein-coupled. ET_A is preferentially activated by ET-1, whereas ET_B has equal affinity for ET-1, ET-2 and ET-3. Although both types of receptors are found on HSCs, there is a shift in predominance of ET_A to ET_B as HSCs transdifferentiate from the early activated phase to the myofibroblast phenotype (Ref. 98). Furthermore, the sensitivity of HSCs to ET-1 increases with activation, as shown by experiments measuring the ET-1-induced intracellular Ca^{2+} transient (Ref. 99).

ET-1 induces HSC contraction at least partly via cascades that lead to increased intracellular Ca^{2+} concentration (Fig. 5). Ligand binding to the ET receptor results in activation of PLCβ and release of messenger inositol 1,4,5-trisphosphate (IP_3). IP_3 stimulates release of Ca^{2+} from the endoplasmic reticulum store. Cellular contraction occurs when the Ca^{2+}/calmodulin-dependent myosin-light-chain kinase (MLC-K) is activated (Ref. 91). Some of the effect of ET-1 on contraction might be mediated via a Ca^{2+}-independent mechanism, involving the small G-proteins RhoA and Rho-kinase (Ref. 69). This pathway is important in ET-1-modulated wound-induced migration of rat HSCs.

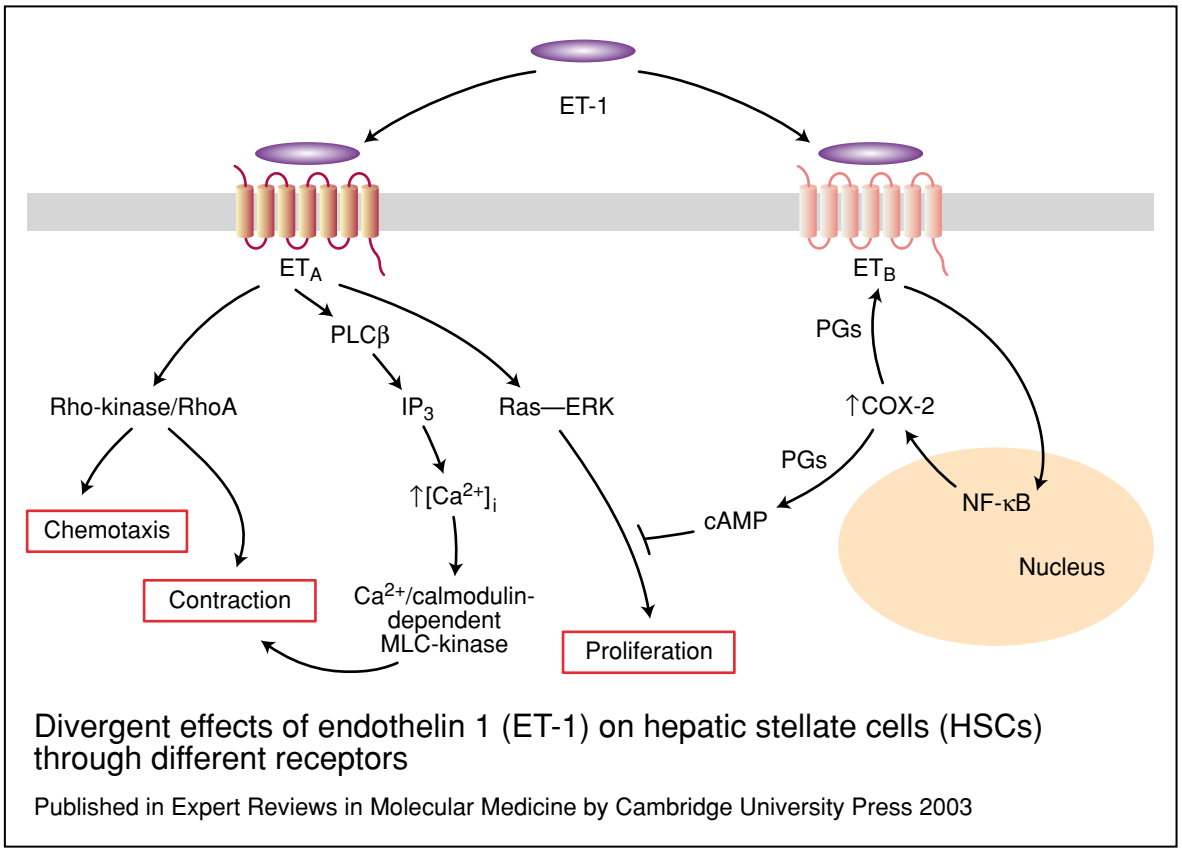
Use of an ET_A antagonist and ET_B agonist indicates that ET_A mediates the contractile and proliferative effects of ET-1, whereas ET_B receptors mediate the antiproliferative effects of ET-1 in myofibroblasts. Following ET-1 binding to ET_B, the COX-2 enzyme is induced via NF-κB, resulting in elevated PG production and activation of G-protein-coupled PG receptors. This, in turn, leads to increased cAMP production, which inhibits the activation of JNK (c-Jun N-terminal kinase) and ERK, two important signalling pathways in HSC proliferation (Refs 50, 100).

Retinoid loss

Quiescent HSCs are characterised by the presence of perinuclear lipid droplets that contain the vitamin A derivative retinoid in the form of retinyl esters. Upon activation, the droplets are lost and retinoid is released as retinol, a transport form in plasma. The causal relationship between retinoid loss and HSC activation is unclear. In vivo studies

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Divergent effects of endothelin 1 (ET-1) on hepatic stellate cells (HSCs) through different receptors

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Figure 5. Divergent effects of endothelin 1 (ET-1) on hepatic stellate cells through different receptors. The procontractile effect of ET_A receptors is mediated downstream via both Ca^{2+}-dependent and Ca^{2+}-independent mechanisms. ET_A activates phospholipase Cβ (PLCβ) causing release of messenger inositol 1,4,5-trisphosphate (IP_3). IP_3 stimulates release of Ca^{2+} from the endoplasmic reticulum store. Cellular contraction occurs when the Ca^{2+}/calmodulin-dependent myosin-light-chain kinase (MLC-K) is activated. A Ca^{2+}-independent mechanism involves the small G-proteins RhoA and Rho-kinase, via their effects on the actomyosin cytoskeleton. The proliferative effect of ET_A is a result of activation of the Ras–ERK (extracellular-signal-regulated kinase) pathway. By contrast, ET_B receptors mediate antiproliferative effects via prostaglandin (PG) and generation of cAMP. Following ET-1 binding to ET_B, the cyclooxygenase 2 (COX-2) enzyme is induced via NF-κB, resulting in elevated PG production and activation of G-protein-coupled PG receptors. This, in turn, leads to increased cAMP production, which interferes with the Ras–ERK pathway. Prostaglandins and cAMP further upregulate ET_B binding sites, suggesting a positive-feedback regulatory loop. In addition, activated hepatic stellate cells synthesise and secrete ET-1, which in turn acts in an autocrine fashion. Abbreviations: cAMP, cyclic adenosine monophosphate; NF-κB, nuclear factor κB. Figure based on illustration in Ref. 43, with permission (Copyright © 2001 by Thieme Medical Publishers, Inc.) **(fig005sfn)**.

also yield conflicting results. On the one hand, vitamin A deficiency promotes CCl_4-induced liver fibrosis (Ref. 101). On the other hand, retinoid accelerated porcine-serum-induced liver fibrosis in rats (Ref. 102). In culture experiments, retinoic acid (RA), the active metabolite of retinoids, increases cellular plasminogen activator and plasmin levels (Ref. 102). Plasmin, in turn, activates latent TGF-β and fibrogenesis.

Recently, the main focus of studies documenting retinoid loss in HSCs has been nuclear retinoid receptors and their role in transcription regulation. RA binds to cellular RA-binding protein (CRABP) in the cytoplasm and exerts its biological functions through two nuclear receptors: RA receptor (RAR) and retinoid X receptor (RXR) (Ref. 103). One of the nuclear receptors bound by RXR is the PPAR-γ receptor. Interestingly, reduction of RXR and PPAR-γ has been associated with transdifferentiation of HSCs into the activated phenotype (Refs 33, 104). Treatment of activated HSCs with PPAR-γ ligand

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restores the level of PPAR-γ mRNA to amounts typical of quiescent HSCs (Ref. 32). Furthermore, combined use of PPAR-γ and RXR agonists leads to a more pronounced inhibition of HSC proliferation in vitro (Ref. 34). It is thus conceivable that vitamin A metabolites and PPAR-γ agonists cooperate in maintaining the quiescent phenotype of HSCs in normal liver.

Matrix degradation

The progression or resolution of hepatic fibrosis is a reflection of the net result of fibrogenesis and ECM degradation. Activated HSCs express several MMPs and specific tissue inhibitors of the MMP family (TIMPs) (Ref. 105). The integral activity of these proteins determines the rate of ECM degradation. The MMP family comprises 25 members divided into five categories: interstitial collagenases, gelatinases, stromelysins, membrane-type MMPs (MT-MMPs) and a metalloelastase (Ref. 105). This functional classification is somewhat arbitrary as there is overlap in activities among the different enzyme groups.

In early primary culture, HSCs express MMP-3 (a stromelysin) and MMP-2 but not TIMPs (Refs 106, 107, 108). Although this matrix-degrading phenotype could be a result of the isolation process, it might also reflect the early phase of 'pathological' matrix degradation in vivo after injury, during which the normal subendothelial matrix and BM are damaged. With prolonged culture of HSCs, MMP-1/MMP-13 are downregulated whereas expression of TIMP-1, TIMP-2, MMP-2 and MT1-MMP is enhanced (Ref. 105).

According to one proposed model, increased MMP2 and MT1-MMP production by HSCs provokes degradation of the pericellular matrix. This results in an altered HSC–matrix interaction and further HSC activation (Ref. 105). Meanwhile, as the expression of TIMP is increased, degradation of newly synthesised collagen is inhibited, with consequent accumulation of ECM. The role of TIMP-1 in liver fibrosis was tested in transgenic mice overexpressing human TIMP-1 in liver under the control of the albumin promoter/enhancer (Ref. 109). Without any treatment, overexpression of TIMP-1 did not induce liver fibrosis. With CCl_4 treatment, transgenic mice had significantly more severe liver fibrosis than nontransgenic mice. These results suggest that TIMP-1 does not by itself cause liver fibrosis, but strongly promotes its development in the presence of chronic injury. Regulation of TIMP-1 expression is mediated via the AP-1 family proteins JunD, Fra2 and FosB, which bind to an AP-1-binding site at the 5' end of the TIMP-1 promoter. There is also a cis-acting regulatory DNA element named upstream TIMP-1 element 1 (UTE-1) located downstream of the AP-1 site that is essential for the transcriptional activity of the promoter in HSCs (Refs 110, 111).

Resolution of fibrosis, in contrast, is associated with a marked reduction in TIMP activity, as seen in animal models of reversible liver fibrosis (Ref. 1). MMP1/MMP13, derived either from HSCs or KCs, together with MT1-MMP and MMP2, then contribute to the degradation of fibrillar ECM typical of fibrotic liver, leading to restoration of normal liver histology. The importance of TIMP-1 in this process was demonstrated by studies in the TIMP-1 transgenic mice model cited above, where overexpression of TIMP-1 attenuated the spontaneous resolution of liver fibrosis through a combination of a net reduction of the MMP activity and suppression of apoptosis in HSCs (Ref. 112) (see next section).

Apoptosis of HSCs and resolution of hepatic fibrosis

Whereas the number of HSCs increases with progressive liver injury, it decreases with resolution of fibrosis. Apoptosis accounts for this process – at least in rodent models of liver fibrosis (Refs 113, 114). In other models of progressive fibrosis, a simultaneous proliferation and apoptosis of HSCs occurs, such that the number of activated HSCs reflects the balance of the two parallel processes (Ref. 115). As the injurious stimulus is removed, apoptosis overtakes proliferation as the predominant event. The trigger and timing of HSC apoptosis is critical, as HSCs are also an important source of MMPs that degrade the fibrotic tissue. Premature apoptosis of HSCs might thus be counterproductive to resolution of fibrosis. Timely termination of HSCs by apoptosis, however, removes not only the primary source of ECM but also the producers both of TIMPs that inhibit ECM degradation and of various cytokines/growth factors that perpetuate the fibrotic process.

Apoptosis might be the default pathway for activated HSCs in normal liver. Thus, following injury, apoptosis might be inhibited by soluble factors and matrix components that are present during injury (Ref. 116). Indeed, spontaneous

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apoptosis occurs in cultured HSCs (Ref. 117). Furthermore, transdifferentiated HSCs express the cell death surface receptor Fas and its ligand, and apoptosis can be induced with Fas-activating antibodies (Ref. 118). Another death receptor, nerve growth factor receptor (NGFR), is also expressed by activated HSCs (Ref. 119). Stimulation of NGFR with its appropriate ligand results in apoptosis. Another recently discovered pro-apoptotic factor in HSCs is cyclopentenone of the J series, especially 15-deoxy-$\Delta^{12,14}$-PG J2 (15-d-PGJ_2) (Ref. 120). It induces apoptosis in activated human HSCs via oxidative stress. Interestingly, although 15-d-PGJ_2 is an endogenous ligand of PPAR, the apoptotic effect is not mediated by this pathway.

IGF-1 and TNF-α are two soluble factors known to promote HSC survival (i.e. are anti-apoptotic). IGF-1 activates the PI 3-K–PKB pathway and the downstream targets that promote cell survival. Inhibition of PI 3-K with wortmannin abolishes the effect. TNF-α exerts its effect by activation of NF-κB, which is known to have anti-apoptotic activity in HSCs as well as other cell types. NF-κB occurs in either a homodimeric (p50–p50 or p65–p65) or a heterodimeric (p50–p65) form. In quiescent HSCs, nuclear NF-κB is predominantly the p50–p50 form, which represses transcription because p50 lacks an activation domain (Ref. 37). Upon HSC activation, the transcriptionally active p65–p65 and p50–p65 dimers are translocated into the nucleus. The persistent expression of p50 and p65 proteins in activated HSCs is accompanied by the dramatic downregulation of IκB-α, the natural inhibitor of NF-κB (Ref. 121). In addition, activated HSCs express a hyperphosphorylated form of IκB-β that can compete with IκB-α for NF-κB but lacks the inhibitory properties of IκB-α and instead maintains NF-κB in a transcriptionally active state. The anti-apoptotic effect of TNF-α is mediated by the upregulation of p50–p65, which, in turn, transactivates downstream target genes, among them inhibitors of the cellular apoptotic machinery (the caspase cascade).

Interestingly, TIMP-1 was also found to have anti-apoptotic activity in a recent study (Ref. 122). Serum deprivation, cycloheximide exposure or NGF-induced apoptosis of cultured HSCs was inhibited by TIMP-1. Furthermore, the effect was shown to be dependent on MMP inhibition and involve upregulation of Bcl-2 expression, which enhances cellular resistance to apoptosis.

Interactions between HSCs and the surrounding matrix also influence their propensity for apoptosis, and this might partly explain the intriguing anti-apoptotic activity of TIMP-1. Activated HSCs are more prone to apoptosis, possibly because of the altered ECM composition (Refs 116, 123). Yet, even the interstitial collagen in hepatic fibrosis might provide a survival signal to the activated HSCs, as shown in a study using animals with fibrotic liver expressing mutated collagen I that is resistant to degradation (Ref. 124). In these animals, HSC apoptosis was reduced, presumably as a result of sustained accumulation of mutant type I collagen.

A proof-of-concept study using gliotoxin has established the potential for stimulating HSC apoptosis as a means to reduce fibrosis (Ref. 125). Gliotoxin is an epipolythio-dioxopiperazine fungal toxin that induces apoptosis in HSCs, possibly by inhibition of NF-κB. In the rat model of hepatic fibrosis induced by CCl_4, gliotoxin decreased the number of HSCs by inducing apoptosis. The resolution of hepatic fibrosis was enhanced by one single injection of the chemical.

The roles of other cellular components in liver fibrosis

Lipid peroxidation products and ROS are important stimuli of HSC activation and their role in vivo has been shown in studies on human and animal models of liver fibrosis (Refs 126, 127, 128, 129). Hepatocytes are potent sources of such stimuli as well as growth factors and cytokines at the time of injury. In vitro, HSC proliferation is enhanced by hepatocyte-conditioned medium (Refs 130, 131), and expression of the collagen gene in HSCs is upregulated by oxidative stress (Ref. 132). In co-cultures of HSCs and HepG2 cells, hepatocytes containing cytochrome P450 2E1 (CYP2E1) release mediators including ROS that stimulate HSC activation and collagen production (Refs 133, 134). Since CYP2E1 is alcohol-inducible, ROS might be an important mechanistic link between alcoholic hepatocellular injury, HSC activation and fibrogenesis.

KCs produce a host of regulatory compounds that influence HSC behaviour (Ref. 135). Such compounds include TNF-α, ILs, TGF-α and TGF-β, MMPs, PGs, ROS and nitric oxide (NO). Co-culture experiments suggested that TGF-α from KCs is responsible for the proliferative effect of KC-conditioned medium on HSCs (Ref. 136). In addition, IL-6 from KCs under conditions of

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acute insult induces expression of ECM proteins in HSCs. MMP-9 produced by KCs activates latent TGF-β, which, in turn, stimulates collagen synthesis by HSCs (Ref. 137). Contractility of HSCs and sinusoidal tone could be influenced by PGs secreted by KCs. In normal KCs, PGD_2 is the predominant eicosanoid and its derivative, 15-d-PGJ_2, could have an important role in HSC activation/apoptosis as discussed above.

Circulating neutrophils, lymphocytes and platelets are all producers of ROS, cytokines and growth factors that might affect HSC activity. The exact roles of these cells are not well defined but it is likely that, in hepatic injury, infiltration of such cells and secretion of the regulatory factors contribute to the overall stimulatory effect on fibrosis.

SECs secrete ET, which stimulates HSC contractility, sinusoidal tone and pressure as reviewed above. SECs also express NO synthase (NOS), which catalyses the enzymatic reaction that produces NO from L-arginine. NO is a potent vasodilator that regulates portal pressure in liver (Ref. 91). In vitro, NO diminishes the procontractile effect of ET-1 on HSCs (Ref. 138).

As well as their role in regulation of portal pressure, SECs also produce ECM components, although to a lesser extent than HSCs. In normal liver, SECs produce collagen types I, III and IV, laminin, syndecans and fibronectin (Refs 42, 139, 140, 141). During fibrosis, there is a fivefold increase in collagen type I mRNA. Moreover, during the initial phase of fibrosis, SECs release a specific cellular isoform of fibronectin ('cFN' or 'EIIIA Fn'; the product of alternative splicing of the primary transcript) that stimulates activation of HSCs in vitro (Ref. 142). The activated HSCs in turn produce more of the variant of fibronectin, thereby contributing to HSC perpetuation.

Finally, SECs express TGF-β receptors, which, following TGF-β1 stimulation, increase secretion of functionally active plasminogen activator inhibitor type 1 (Ref. 142). This serves to reduce ECM degradation indirectly via its action on the plasmin system (Ref. 141).

Clinical implications

The improved understanding of the molecular biology of hepatic fibrosis has increased the number of potential therapeutic targets (Ref. 143). Although not the primary focus of this review, many potential treatments are undergoing intense evaluation in animal models, some of which have reached clinical testing. Antioxidants have been analysed in both animal and human studies with the aim of reducing the deleterious effects of ROS in promoting fibrosis; however, so far, the results have been mixed (Refs 144, 145, 146). Modulation of cytokine activity has also been studied as an alternative strategy. The TGF-β pathway has attracted most attention and attempts have been made to inhibit its signalling mechanism by using antagonists such as anti-TGF antibody, soluble TGF-β type II receptor, antisense oligonucleotides and recombinant LAP (Refs 147, 148, 149, 150). A caveat in interfering with the TGF-β pathway is that it could lead to growth dysregulation and increased risk of neoplasia, since TGF-β is both fibrogenic and a growth inhibitor. Such concerns need to be addressed should any human study be considered. ET receptor antagonists have also been tested as antifibrotic agents and appear promising (Ref. 151).

Agents that reduce ECM accumulation or interaction with cells have been tested as antifibrotics. For example, halofuginone, a potent inhibitor of collagen synthesis, prevents liver fibrosis and cirrhosis in rats caused by administration of dimethynitrosamine or thioacetamide (Refs 152, 153). Another approach is the use of an RGD analogue, which interferes with binding between ECM and integrins (Ref. 154). Several intracellular pathways are also potential targets. For example, an inhibitor of the PDGF-R signalling pathway such as pentoxyphylline could be used (Ref. 155). Meanwhile, in vitro study has demonstrated the potential benefit of ligands to PPAR-γ receptors in hepatic fibrogenesis (Refs 34, 156).

Research in progress and outstanding research questions

Although major progress has been made over the past decade, we still lack a complete understanding of the molecular biology of hepatic fibrosis. In vitro studies with HSCs have provided valuable information, but some of their complex biological activities require further elucidation. For example, the mechanism and biological significance of retinoid loss during HSC activation are unclear. Furthermore, the key genes initiating HSC activation and apoptosis during the resolution of fibrosis are not fully appreciated. Likewise, how different stimuli such as oxidative stress, cytokines and ECM influence the expression of these genes in vivo is also not

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known. In other words, a better understanding of the crosstalk between signalling pathways and integration of such signals is needed.

Recent advances in genomics and the advent of new technologies such as gene microarray offers new opportunities to resolve these outstanding issues. These techniques, combined with the use of viral vectors to introduce DNA into HSCs, have allowed precise tracking and manipulation of specific genes. Establishment of transgenic mice that express or lack genes of interest in HSCs will provide valuable in vivo data. Ultimately, efforts in unravelling the molecular biology of hepatic fibrosis will lead to successful antifibrotic therapy.

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Features associated with this article

Figures

Figure 1. Sinusoidal events during fibrosing liver injury (fig001sfn).
Figure 2. Phenotypic features of hepatic stellate cell activation during liver injury and resolution (fig002sfn).
Figure 3. The platelet-derived growth factor (PDGF) mitogenic signalling pathway in hepatic stellate cells (fig003sfn).
Figure 4. The transforming growth factor β (TGF-β) signalling pathway (fig004sfn).
Figure 5. Divergent effects of endothelin 1 (ET-1) on hepatic stellate cells through different receptors (fig005sfn).

Tables

Table 1. Hepatic stellate cells secrete several cytokines and cell adhesion molecules that regulate different steps of the inflammatory process (tab001sfn).

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