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Review

Molecular mechanism of hepatic stellate cell activation and antifibrotic therapeutic strategies

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Activation of hepatic stellate cells (HSCs) is the dominant event in liver fibrosis. The early events in the organization of HSC activation have been termed initiation. Initiation encompasses rapid changes in gene expression and phenotype that render the cells responsive to cytokines and other local stimuli. Cellular responses following initiation are termed perpetuation, which encompasses those cellular events that amplify the activated phenotype through enhanced growth factor expression and responsiveness. Multiple cells and cytokines play a part in the regulation of HSC activation. HSC activation consists of discrete phenotype responses, mainly proliferation, contractility, fibrogenesis, matrix degradation, chemotaxis and retinoid loss. Currently, antifibrotic therapeutic strategies include inhibition of HSC proliferation or stimulation of HSC apoptosis, downregulation of collagen production or promotion of its degradation, administration of cytokines, and infusion of mesenchymal stem cells. In this review, we summarize the latest advances in our understanding of the mechanisms of HSC activation and possible antifibrotic therapeutic strategies.

Key words: hepatic stellate cells, activation, liver fibrosis, molecular mechanism

Introduction

Fibrosis and cirrhosis represent the consequences of a sustained wound healing response to chronic liver injury from a variety of causes, including viral, autoimmune, drug-related, cholestatic, and metabolic diseases. The excessive accumulation of extracellular matrix (ECM) occurs in most types of chronic liver disease. Activated hepatic stellate cells (HSCs) have been identified as major collagen-producing cells in injured liver. Other cell types such as bone marrow-derived fibrocytes and portal and septal fibroblasts also participate in this process.[1,2] Following liver injury of any etiology, HSCs undergo a response known as "activation," which is the transition of quiescent cells into proliferative, fibrogenic, and contractile myofibroblasts (activated HSCs). Thus, the correlation between HSC activation and liver fibrosis is close. Most recently proposed antifibrotic therapeutic approaches are based on the clarification of molecular mechanism of HSC activation.

Hepatic stellate cell activation

HSC activation is a remarkably pleiotropic yet tightly programmed response occurring in a reproducible sequence, which can be roughly divided into two phases—initiation and perpetuation.[3] Initiation is associated with rapid gene induction resulting from paracrine stimulation by inflammatory cells and injured hepatocytes or bile duct cells, and from early changes in ECM composition. Perpetuation is a continuously dynamic process, which encompasses those cellular events that amplify the activated phenotype through enhanced growth factor expression and responsiveness; this phase of activation results from autocrine and paracrine stimulation, as well as from accelerated ECM remodeling.

Initiation

The earliest changes in HSCs reflect paracrine stimulation by all neighboring cell types, including Kupffer cells, hepatocytes, lymphocytes, leukocytes, and sinusoidal endothelium. A complex interplay among

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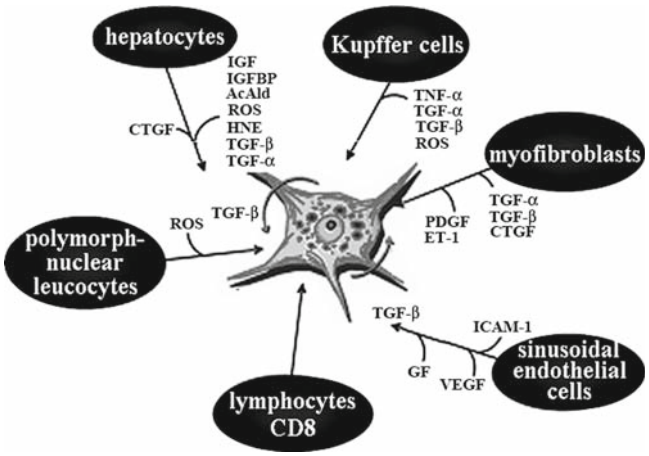

Fig. 1. The most important paracrine mediators that activate hepatic stellate cells: synopsis of cellular interactions of resident liver cells and immigrated inflammatory cells with hepatic stellate cells in the process of activation and transdifferentiation to myofibroblasts. *IGF*, insulin-like growth factor; *IGFBP*, insulin-like growth factor binding protein; *ROS*, reactive oxygen species; *HNE*, hydroxynonenal; *TGF*, transforming growth factor; *TNF*, tumor necrosis factor; *PDGF*, platelet-derived growth factor; *ET*, endothelin; *CTGF*, connective tissue growth factor; *GF*, growth factor; *ICAM*, intercellular adhesion molecule; *VEGF*, vascular endothelial growth factor

different hepatic cell types takes place during hepatic fibrogenesis[4] (Fig. 1).

Kupffer cell infiltration and activation play a prominent role in HSC activation. An influx of Kupffer cells coincides with the appearance of HSC activation markers such as α-smooth muscle actin (α-SMA). On the one hand, Kupffer cells can stimulate cell proliferation, matrix synthesis, and release of retinoids by HSCs through the actions of cytokines, such as transforming growth factor (TGF)-β,[5,6] tumor necrosis factor (TNF)-α, and matrix metalloproteinase (MMP)-9.[7] On the other hand, Kupffer cells generate reactive oxygen species (ROS) in the liver, which are capable of enhancing HSC activation and collagen synthesis. In addition, Kupffer cells also produce nitric oxide (NO), which can counterbalance the stimulatory effects of ROS by reducing HSC proliferation and contractility.

It has been reported that damaged hepatocytes release ROS during alcohol metabolism via cytochrome P450 2E1 (CYP2E1). Using a coculture of CYP2E1-transfected HepG2 cells and HSCs, Nieto and colleagues[8] demonstrated that ROS generated in hepatocytes can increase collagen production in HSCs, and that the administration of antioxidants, CYP2E1 inhibitors, and hydrogen peroxidase can reduce HSC activation. Another source of ROS from damaged hepatocytes is NADPH oxidase, which produces superoxide anions in phagocytes. In addition, hepatocyte apoptosis might also be a profibrogenic stimulus; Canbay and colleagues[9] have shown that the engulfment of apoptotic bodies drives HSC fibrogenesis.

Lymphocytes, especially CD4 T-helper (Th) lymphocytes, help orchestrate the host response via cytokine production and can differentiate into Th1 and Th2 subsets. In general, Th1 cells produce cytokines promoting cell-mediated immunity, including interferon (IFN)-γ, TNF, and interleukin (IL)-2. Th2 cells produce IL-4, IL-5, IL-6, and IL-13 and promote humoral immunity. Results from previous experimental models imply that Th2 lymphocytes favor fibrogenesis in liver injury over Th1 lymphocytes.[10] However, recent studies of Wynn[11] suggest that more than two T-cell subsets underlying a highly complex, orchestrated response are involved, and they also provide us a more important paradigm for how these intersecting pathways may regulate fibrosis. In animal models, IL-13 has emerged as a key mediator because it increases TGF-β1 and MMP expression by macrophages, whereas IL-4 has a limited role. One study examined the activity of IL-13 in cultured HSCs and suggested that IL-4 and IL-13 directly affect HSCs by increasing collagen production and suppressing HSC proliferation.[12]

Leukocytes recruited to the liver during injury join with Kupffer cells in producing compounds that modulate HSC behavior. Neutrophils are an important source of ROS, which may have a direct stimulatory effect on HSC collagen synthesis via superoxide.[13] Activated neutrophils also produce NO, which may counteract the effect of superoxide on collagen production but not abrogate it.[13]

Endothelial cells are also likely to participate in HSC activation, both by production of cellular fibronectin and via conversion of TGF-β from the latent to the active, profibrogenic form.

HSCs also participate in inflammatory signaling through expression of Toll-like receptor 4 (TLR4),[14] part of a family of "pattern recognition receptors" driving the innate immune response.[15] Both Kupffer cells and HSCs express TLR4, and the genetic deletion of TLR4 reduces macrophage infiltration, injury, and fibrosis in TLR4-knockout animals with experimentally induced liver damage. When a ligand such as lipopolysaccharide (LPS) binds to TLR4, it induces an intracellular signaling pathway, including activation of nuclear factor κB (NFκB).

Early alterations in the ECM are the consequences of the following transcription events. GC-rich sequences and related GT or CACCC boxes are found in the regulatory regions of many profibrogenic genes expressed in the activated HSCs, including the α1(I) collagen, TGF-β_1, and type I and II TGF-β receptor genes. These sequences are recognition sites for the Krupple-like transcription factor (KLF) family, which share identical

C_2H_2 C-terminal DNA-binding domains. This large and expanding family of transcription factors characteristically regulates expression of ubiquitously expressed or housekeeping genes, but also controls the transcription of tissue-specific genes. Activated HSCs express at least three members of the family, Sp1, basic transcription element binding protein (BTEB1). and KLF6, all of which have the ability to regulate transcription of the α1(I) collagen gene. KLF6 is rapidly induced as an immediate–early gene during HSC activation in vivo and in culture.[16] Findings show that KLF6 is alternatively spliced,[17,18] and that growth-promoting short isoforms rather than full-length isoforms are overexpressed during HSC activation. Sp1 is a potent transcriptional regulator of α1(I) collagen expression, and it is associated with two GC-rich regions (FP1 and FP2) of the α1(I) collagen gene promoter in activated HSCs.[19,20] Sp1 DNA-binding activity and protein expression are induced during HSC activation by an undefined post-transcriptional mechanism.[20] The BTEB1 gene is a target for activation protein-1 (AP-1), and induction of BTEB1 expression in acetaldehyde-treated HSCs is mediated via activation of c-jun N-terminal kinase (JNK) and elevation of AP-1 activity.[21]

Perpetuation

Paracrine stimuli continue during "perpetuation," and, simultaneously, autocrine cytokines play a prominent role at this stage, including cytokines promoting HSC activation such as TGF-β_1, platelet-derived growth factor (PDGF), fibroblast growth factor (FGF), and endothelin (ET)-1, and cytokines inhibiting HSC activation such as hepatocyte growth factor (HGF). HSCs also release neutrophil and monocyte chemoattractants, which can amplify inflammation in liver injury, including colony stimulating factor, monocyte chemotactic protein-1 (MCP-1),[22,23] and cytokine-induced neutrophil chemoattractant/IL-8.[24] Anti-inflammatory cytokines produced by HSCs have also been identified, in particular IL-10.

ECM remodeling continues during this phase. The low-density subendothelial matrix is progressively replaced by one rich in fibril-forming collagen. This fundamental shift in ECM composition affects the behavior of hepatocytes, sinusoidal endothelium, and HSCs. In advanced stages, the liver contains approximately six times more ECM than normal, including collagens (I, III, and IV), fibronectin, undulin, elastin, laminin, hyaluronan, and proteoglycans. Fibril-forming ECM also accelerates HSC activation. Fibrillar collagens can bind and stimulate HSCs via discoidin domain receptor 2 (DDR2) and integrins. DDR2 has been identified as an upregulated tyrosine kinase receptor that has the unusual property of responding to fibrillar collagen as its ligand.[25,26] Once bound to collagen, a cascade of events is initiated that includes recruitment of *src* kinase and downstream signals,[27] culminating in transcriptional induction of MMP-2.

Phenotypic responses of activated HSCs and the underlying mechanisms

Activation of HSCs takes the form of discrete phenotype responses, which include proliferation, contractility, fibrogenesis, matrix degradation, chemotaxis, and retinoid loss (see Fig. 2).

Proliferation

Increased numbers of HSCs in injured liver arise in part from local proliferation in response to polypeptide growth factors. PDGF is the most potent mitogen for HSCs; its production is upregulated in the fibrotic liver, and its inhibition attenuates experimental liver fibrogenesis.[28] PDGF has been shown to activate mitogen-activated protein kinase (MAPK) signaling, specifically JNK, extracellular signal-regulated kinase (ERK), and p38. Both JNK and ERK activations induce HSC proliferation. However, the activation of p38 inhibits the proliferative response.[29] The phosphatidylinositol 3-kinase (PI3-K) signaling pathway, which is also stimulated following PDGF treatment in activated HSCs, leads to Akt and $p70^{s6}$ kinase activation, resulting in increased HSC proliferation and chemotaxis.[30] The list of identified HSC mitogens has been growing and now includes ET-1, thrombin, FGF, vascular endothelial growth factor, and insulin-like growth factor (IGF).[31]

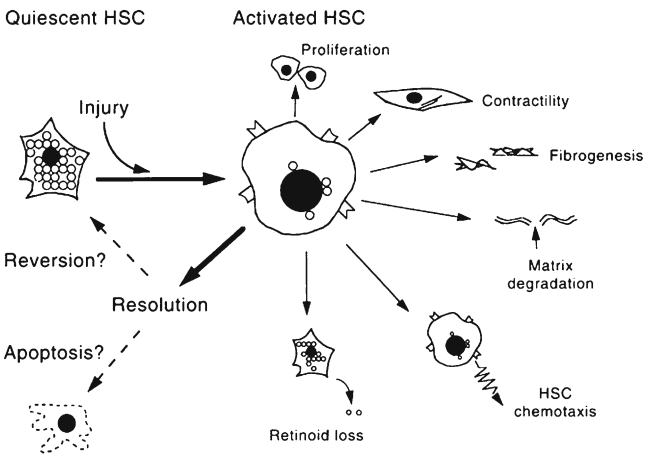

Fig. 2. Phenotypic features of hepatic stellate cell (*HSC*) activation during liver injury and resolution

Contractility

Contractility of activated HSCs represents an important mechanism underlying increased portal resistance during liver injury. ET-1 and other contraction-stimulating cytokines secreted by HSCs are the key contractile stimuli directed toward HSC. Upregulation of ET-1 production is accompanied by increased ET-converting enzyme 1, which activates the latent ET-1.[32] At least two G-protein-coupled receptors—ET receptor types A and B (ETA and ETB)—mediate the effects of ET-1 and are expressed on both quiescent and activated HSCs.[33] Shi-Wen et al.[34] have shown that ET-1 induces collagen matrix contraction through the ETA but not the ETB receptor by using specific ET-1 receptor inhibitors. Blocking ET-1 or the PI3-kinase/Akt cascades might be beneficial in reducing scar formation in pulmonary fibrosis.[34] NO produced by HSC is the physiological antagonist to ET-1.

Fibrogenesis

Upregulation of collagen synthesis during activation is among the most striking molecular responses of HSCs to injury and is mediated by both transcriptional and posttranscriptional mechanisms. Transcriptional activation of type I collagen has been extensively characterized. Fibrogenic growth factors, vasoactive substances, and adipokines are each required for the development of fibrosis.[35] TGF-β_1 is the most potent fibrogenic factor for HSCs; matrix production by activated HSCs is markedly increased through the action of TGF-β_1.[36,37] HSCs are the most important source of TGF-β_1 in liver fibrosis,[38,39] and Kupffer cells and platelets also secrete this cytokine.[21] TGF-β is consistently increased during liver fibrosis, and stimulates both Smad and MAPK signaling and α1(I) collagen gene expression in HSCs.[40,41] Additional studies have indicated that TGF-β treatment increases the α1(I) collagen mRNA half-life, mediated by increasing stability of α1(I) collagen mRNA through p38 MAPK but not Smad signaling.[42] The half-life of collagen α1(I) mRNA increases 20-fold in activated HSCs compared with quiescent HSCs.[43] A conserved stem–loop structure at the 5′-end of the collagen α1(I) mRNA mediates this enhanced mRNA stability through interaction with the 3′-untranslated region.[44] A role for TGF-β_1 in perpetuating rather than initiating HSC activation has been established by examining the behavior of HSCs in TGF-β_1-knockout mice with acute liver injury. Disrupting TGF-β synthesis or signaling pathways prevents scar formation in experimental liver fibrosis.[45]

Leptin, one of the major adipokines, is a 16-kDa nonglycosylated protein secreted primarily from the adipocytes of white fat; a minor level of regulated leptin expression also occurs at other sites such as placenta, skeletal muscle, and stomach fundus, and in culture-activated HSCs.[46] Leptin has been recognized as a profibrogenic hormone in the liver. Niu et al.[47] reported that leptin acts directly on liver fibrogenesis by stimulating α1(I) collagen production in activated HSCs. The process appears to be mediated by the PI3K/Akt pathway through activated JAK1.[47]

Matrix degradation

Increased collagenolytic activity is a major mechanism of fibrosis resolution. Fibrillar collagens (I and III) are degraded by interstitial MMPs. HSCs express virtually all of the key components required for matrix degradation. In particular, they are a key source of MMP-2 as well as stromelysin/MMP-3. During fibrosis resolution, MMP activity increases owing to a rapid decrease in the expression of tissue inhibitor of metalloproteinase (TIMP)-1. Through upregulation of TIMP-1 and TIMP-2, activated HSCs can inhibit the activity of interstitial collagenases, which additionally favors the accumulation of scar tissue.[48] Available data indicate that increases in the expression of MMP-2 and membrane type 1 MMP as well as TIMP-1 and TIMP-2 are prominent during fibrogenesis.[49] TIMP expression is localized to activated HSCs/myofibroblasts. Hepatic macrophages are an important source of proteases, including MMP-13.[50] Interestingly, overexpression of MMP-8 lead to partial reversal of fibrosis, providing proof of concept for a therapeutic role for overexpression of MMPs.[51] Moreover, resolution of fibrosis in CCl_4-induced liver injury is attenuated in mice lacking MMP-13,[52] yet fibrosis is accelerated in these knockout animals with biliary fibrosis.[53] The discrepant findings from these two models may indicate that the same proteases play different roles depending on the type and duration of injury. Additionally, B lymphocytes, which make up as much as 50% of the entire lymphocyte pool in the liver, are also implicated in liver fibrosis. Mice genetically deficient in B cells ($JH^{-/-}$ strain) suffer the same extent of injury after acute administration of CCl_4 but can markedly reduce collagen deposition, raising the possibility that B cells may be able to interact with fibrolytic pathways in resolution of liver fibrosis.[54]

AP-1 regulates TIMP- and ECM remodeling-related genes, and shows increased and persistent activity in activated HSCs. AP-1 is composed of at least one member of the Jun family of proteins (c-Jun, JunB, JunD), which can form homodimers, or heterodimers with another Jun protein or with a related Fos family member. JunD is the most important member of the AP-1 proteins in activated HSCs as it is required for both TIMP-1 and IL-6 gene expression.[55] TIMP-1 gene transcription is regulated by JunD homodimers, which

appear to operate in concert with a 30-kDa nuclear protein (RUNX protein).[56] The protein is induced in culture-activated HSCs and binds to a novel and essential DNA-binding site in the TIMP-1 promoter called upstream TIMP-1 element 1. Therefore, shifts in the fine balance of the expression and activity of different forms of AP-1 and their target genes (MMP and TIMP genes) in response to cues from the microenvironment promote the ability of the activated HSCs to remodel the hepatic ECM.

HSC chemotaxis

The directed migration of activated HSCs enhances their accumulation in areas of injury. Several chemoattractants have been implicated, including PDGF, IGF-1, ET, and MCP-1. MCP-1 is a member of the CC class of the chemokine family, which promotes leukocyte recruitment.[57] MCP-1 production by HSCs recruits monocytes, lymphocytes, and activated but not quiescent HSCs. The effect is mediated by the PI3-K pathway and requires Ca^{2+} influx.

Retinoid loss

Loss of intracellular retinoid is a notable feature of HSC activation, yet it remains unknown whether retinoid loss is required for HSCs to activate or whether retinoids might accelerate or prevent activation in vivo. Milliano and Luxon[58] have concluded that exposure of HSCs to extracellular retinoids diminishes some activation markers but does not prevent HSC activation.

Destiny of activated HSCs during fibrosis resolution

During tissue recovery from acute human and experimental liver injury, the number of activated HSCs decreases as tissue integrity is restored. This observation has two possible explanations: activated HSCs may be reversed to quiescent HSCs or they may be cleared. The answer calls for further exploration.

Reversion

Peroxisome proliferator-activated receptor γ (PPARγ) is a member of the steroid/thyroid hormone nuclear receptor superfamily that has been is implicated in metabolic diseases (e.g., diabetes) and operates as an obligate heterodimer with the retinoid X receptor to regulate gene transcription and cellular differentiation. Expression of PPARγ protein is dramatically reduced in activated HSCs both in vitro (human and rat cells) and in vivo.[59] Culture-activated HSCs can be phenotypically and functionally reversed to cells with the quiescent phenotype by forced expression of PPARγ.[60] Treatment of culture-activated HSCs with PPARγ ligands ($15dPGJ_2$ and BRL49653) reversed biochemical features of HSC activation. In particular, the ligands inhibited α1(I) procollagen promoter activity, its mRNA expression, and collagen production by the cells, and the inhibition by $15dPGJ_2$ was shown to be blocked by a PPARγ antagonist, confirming the involvement of PPARγ.[59] These results suggest that PPARγ plays pivotal roles in the molecular regulation of HSC activation in liver fibrogenesis.

Apoptosis

Apoptosis of HSCs probably accounts for the decreased number of activated HSCs during resolution of hepatic fibrosis.[61,62] HSC apoptosis associated with reduced TIMP-1 expression has been documented during the recovery phase of experimentally induced liver injury.[63] TIMP-1 protects activated HSCs from apoptosis,[64] and blocking of TIMP-1 with specific monoclonal antibody reverses CCl_4-induced hepatic fibrosis.[65] Partial degradation of fibrillar collagen occurs, and the altered interaction between activated HSCs and ECM favors apoptosis.[66] In the meantime, HSCs also undergo apoptosis during spontaneous activation, in parallel with increased expression of CD95 L (Fas ligand), Bcl-2, and p53.[67] Removal of activated HSCs by apoptosis precedes fibrosis resolution. Although apoptosis of activated HSCs may well be critical in fibrosis resolution, other work suggests that apoptosis of activated HSCs may stimulate activation, and thus could be detrimental with regard to fibrogenesis.[9] More recent studies combining in vitro and in vivo work have provided definitive evidence of a role of NFκB in protecting HSCs from apoptosis,[68] and its inhibition can accelerate recovery from liver fibrosis. The mechanism by which NFκB prevents HSC apoptosis may involve inhibition of the JNK cascade and AP-1 pathway. There is increasing interest in the AP-1 pathway as a modulator of cell death and as a target for therapeutic manipulation.[69]

Antifibrotic therapeutic strategies

Currently there is no standard treatment for liver fibrosis. Although experimental studies have revealed targets for preventing fibrosis progression in rodents, the efficacy of most treatments has not been proven in humans. The removal of the causative agent is the most effective intervention in the treatment of liver fibrosis. Meanwhile, potential effective antifibrotic therapeutic strategies include downregulating HSC activation, neutralizing antiproliferative, fibrogenic, and contractile responses of HSCs, promoting matrix degradation, promoting

Table 1. Potential antifibrotic strategies and agents

Strategy	Mechanism	Agents
Inhibiting HSC activation	Antioxidative stress;	Vitamin E, silymarin, phosphatidylcholine, *S*-adenosyl-L-methionine
	Inhibiting key signal transduction pathways	Pentoxifylline, dickkopf-1
Antiproliferative, fibrogenic, contractile responses of HSCs	Cytokine receptor antagonists; Reduction in the contraction and growth of HSCs	Imatinib mesylate, camostat mesylate Losartan, prostaglandin E2 and NO donors
Promoting matrix degradation	Stimulating collagen degradation	MMP-8 and urokinase-type plasminogen activator
Promoting HSC apoptosis	Inducing HSC apoptosis	Bortezomib, gliotoxin, tetrandrine, curcumin, adiponectin
Cytokine therapy		IGF, HGF, and cardiotrophin
Cell therapy		Infusion of mesenchymal stem cells
Other therapy		Pentoxifylline–M6PHSA, downregulation of CTGF using siRNA

HSC, hepatic stellate cell; NO, nitric oxide; MMP, matrix metalloproteinase; IGF, insulin-like growth factor; HGF, hepatic growth factor; CTGF, connective tissue growth factor

HSC apoptosis, cytokine therapy, and cell therapy (Table 1).

Inhibiting HSC activation

Oxidative stress stimulates HSC activation; thus, reducing oxidative stress is a possible antifibrotic target. Antioxidants such as vitamin E, silymarin, phosphatidylcholine, and *S*-adenosyl-L-methionine can inhibit HSC activation, protect hepatocytes from undergoing apoptosis, and attenuate experimental liver fibrosis. Our research has also shown that the antioxidant indole-3-carbinol (I3C)[70] has inhibitory effects on activated HSCs induced by acetaldehyde.

Substances that inhibit key signal transduction pathways involved in liver fibrogenesis also have potential for use in treating liver fibrosis. Pentoxifylline, a methylxanthine derivate, has been reported to have antifibrotic properties by a mechanism dependent on IκB-α degradation, which in turn blocks NF-κB activation.[71] Wnt signaling has been implicated in pulmonary and renal fibrosis, and some investigators have reported that Wnt signaling promotes hepatic fibrosis by enhancing HSC activation and survival, suggesting that Wnt antagonism has therapeutic potential for liver fibrosis.[72]

Neutralizing antiproliferative, fibrogenic, or contractile responses of HSCs

Neutralizing of the proliferative, fibrogenic, or contractile responses of HSCs is another approach targeting a potential antifibrotic compound. Cytokine receptor antagonists may be used as potential antifibrotic agents. Imatinib mesylate (STI-571, Gleevec, Novartis, Basel, Switzerland), a clinically used PDGF receptor (PDGFR) tyrosine kinase inhibitor, markedly attenuated PDGF-BB-induced proliferation and migration, and α-SMA and α2-(I)-procollagen mRNA expression of activated HSCs in a dose-dependent manner.[73] It may provide an effective new strategy for antifibrosis therapy.

Camostat mesylate prevents porcine serum-induced rat hepatic fibrosis via reduced generation of active TGF-β.[74] Studies including use of a dominant-negative type II TGF-β receptor, the expression of truncated type II receptor, and the construction of a soluble type II receptor have demonstrated antifibrotic efficacy.[75] There is concern, however, that with long-term TGF-β antagonism in humans, the modulation of inflammation and the immune response and the loss of TGF-β mediated growth suppression could stimulate hepatocellular growth, thereby promoting cancer.

Inhibition of the renin–angiotensin system might be the most promising strategy for treating liver fibrosis. Renin–angiotensin inhibitors are widely used as antifibrotic agents in patients with chronic renal and cardiac diseases, and appear to be safe when administered for prolonged periods of time. Preliminary pilot studies in patients with chronic hepatitis C and nonalcoholic steatohepatitis suggest that renin–angiotensin blocking agents may have beneficial effects on fibrosis progression.[76] Transplanted patients receiving renin–angiotensin system inhibitors as antihypertensive therapy show less fibrosis progression than patients receiving other types of drugs. However, this approach cannot be recommended in clinical practice until the results of ongoing clinical trials become available.

The blockade of ETA and the administration of vasodilators (prostaglandin E2 and NO donors) exert anti-

fibrotic activity in rodents, but their effects in humans are as yet unknown.[77]

Promoting matrix degradation

An alternative approach is the promotion of collagen degradation. MMP-8 and urokinase-type plasminogen activator stimulate collagen degradation in vivo.[78] However, the efficacy of these drugs in humans is unknown.

Promoting HSC apoptosis

Emerging experimental and clinical evidence suggests that liver fibrosis, even cirrhosis is potentially reversible. More importantly, apoptosis of HSCs initiated this process by removing the cell type responsible for both the production of ECM as well as protection of MMPs through the production of TIMP. Thus, it is anticipated that the induction of HSC apoptosis in a selective manner is an ideal strategy for curing liver fibrosis. These discoveries have attracted increasing interest of researchers and provide a novel strategy for antifibrotic therapy.

A few pharmacological agents have been reported to induce HSC apoptosis. The best investigated of these is the fungal metabolite gliotoxin. Gliotoxin induces apoptosis in HSCs by mitochondrial cytochrome *c* release, caspase-3 activation, and ATP depletion, which attenuated the fibrotic response in a rat model.[79]

One of the other best characterized agents is the polyphenol compound curcumin, which is the main yellow pigment in the popular spice turmeric. Treatment of activated HSCs with curcumin induces apoptosis. This effect is mediated by downregulation of PPARγ.[80] To date, however, no trials have shown a beneficial effect of curcumin on hepatic fibrosis in vivo. The proteasome inhibitor bortezomib, which blocks NFκB activity by increasing the half-life of its inhibitor, is also a potential therapeutic strategy for inducing HSC apoptosis and inhibiting hepatic fibrogenesis.[81]

Cytokine therapy

Administration or delivery of growth factors (e.g., IGF, HGF, and cardiotrophin) by gene therapy attenuates experimental liver fibrosis. Levels of IGF-1, which is a crucial regulator of intermediary metabolism, decrease in liver cirrhosis; thus, IGF-1 treatment might improve liver function, and it has been shown to decrease oxidative liver damage and histopathological findings in experimental liver cirrhosis-induced common bile duct ligation.[82] In the dimethylnitrosamine rat model of liver cirrhosis, administration of HGF suppressed proliferation while promoting apoptosis of α-SMA-positive cells in the liver, events that were associated with reduced hepatic expression of α-SMA and histological resolution of liver cirrhosis. Growth inhibition and enhanced apoptosis in portal myofibroblasts by HGF are newly identified mechanisms aiding resolution of liver fibrosis/cirrhosis by HGF.[83] However, these approaches have not been tested in humans and may be carcinogenic.

Cell therapy

The liver has been a major target of bone marrow- and stem cell-based regenerative therapy. Indeed, bone marrow and stem cells have been shown to differentiate into hepatocytes and endothelial cells in several liver injury models.[84,85] Bone marrow-derived mesenchymal stem cells (MSCs) have been reported to prevent the development of liver fibrosis in a number of preclinical studies. Parekkadan and colleagues[86] showed that IL-6 secretion from activated HSCs induces IL-10 secretion from MSCs, suggesting a dynamic response of MSCs to the HSCs in the microenvironment. Blockade of MSC-derived IL-10 and TNF-α abolished the inhibitory effects of MSCs on HSC proliferation and collagen synthesis. In addition, release of HGF by MSCs was responsible for the marked induction of apoptosis in HSCs, as determined by antibody-neutralization studies.[86] These findings demonstrate that MSCs can modulate the function of activated HSCs via paracrine mechanisms and provide a plausible explanation for the protective role of MSCs in liver inflammation and fibrosis, which may also be relevant to other models of tissue fibrosis.[87,88] Infusion of mesenchymal stem cells ameliorates experimentally induced fibrosis,[87] which suggests that this approach has potential for the treatment of chronic liver diseases.

Other therapy

A limitation of the current antifibrotic approaches is that antifibrotic drugs are not efficiently taken up by activated HSCs and may produce unwanted side effects. Promising preliminary results have been recently obtained by using different carriers (e.g., cyclic peptides coupled to albumin-recognizing collagen type VI receptor and/or PDGFR).[89] Pentoxifylline-M6PHSA (neoglycoprotein mannose-6-phosphate-albumin) is a new type of platinum-based linker that conjugates pentoxifylline with M6PHSA and accumulates efficiently in HSCs during liver fibrosis via coordination chemistry rather than a covalent linkage. When incubated in plasma or in the presence of thiol compounds, free pentoxifylline is released from pentoxifylline–M6PHSA at a sustained slow rate.[90] This allows sustained delivery of the drug to HSCs in the fibrotic liver.

Conclusion

In conclusion, progress in the technology of functional genomics will allow more in-depth exploration of the mechanism of HSC activation and bring us hope for novel therapies for solving the puzzle of liver fibrosis.

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