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Translating an understanding of the pathogenesis of hepatic fibrosis to novel therapies

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Abstract

The response to injury is one of wound healing and fibrogenesis, which ultimately leads to fibrosis. The fibrogenic response to injury is a generalized one across virtually all organ systems. In the liver, the injury response, typically occuring over a prolonged period of time, leads to cirrhosis (although it should be pointed out that not all patients with liver injury develop cirrhosis). The fact that many different diseases result in cirrhosis suggests a common pathogenesis. The study of hepatic fibrogenesis over the past 2 decades has been remarkably active, leading to a considerable understanding of this process. It has been clearly demonstrated that the hepatic stellate cell is a central component in the fibrogenic process. It has also been recognized that other "effector" cells are important in the fibrogenic process, including resident fibroblasts, bone marrow derived cells, fibrocytes, and even perhaps cells derived from epithelial cells (i.e., through epithelial to mesenchymal transition or EMT). A key aspect of the biology of fibrogenesis is that the fibrogenic process is dynamic; thus, even advanced fibrosis (or cirrhosis) is reversible. Together, an understanding of the cellular basis for liver fibrogenesis, along with multiple aspects of the basic pathogenesis of fibrosis, have highlighted many exciting potential therapeutic opportunities. Thus, while the most effective "anti-fibrotic" therapy is treatment of the underlying disease, in situations in which this not possible, specific anti-fibrotic therapy is likely to not only become feasible, but will soon become a reality. The goal of this review is to highlight the mechanisms underlying fibrogenesis that may be translated into future anti-fibrotic therapies and to review the current state of clinical development.

Introduction

The response to chronic injury is a generalized one, with features common among multiple organ systems. This feature suggests thematically related pathogenic events across organs.

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In the liver, many different kinds of injury, including viral hepatitis, alcohol, fatty liver, biliary tract disease, iron or copper overload, cystic fibrosis, and others cause fibrogenesis, and subsequently cirrhosis.

Over the past 2 decades, much has been learned about the biology and pathophysiology of fibrosis. Understanding the mechanisms underlying fibrosis has pointed out several potential therapeutic approaches. Preclinical studies have been particularly informative, and have highlighted many possible therapies. Although therapies that are directed at the underlying disease process, including anti-viral therapies for patients with hepatitis B and hepatitis C virus infection, have proven to be effective at reducing and/or reversing fibrosis, specific and effective anti-fibrotic therapy remains elusive. The objective of this review will be to emphasize fundamental concepts underlying hepatic fibrogenesis, and to review translational therapeutics.

Fibrogenesis – Pathophysiology

The fibrogenic process

A critical aspect of the fibrogenic response is that injury, typically to hepatocytes stimulates the injury response (Figure 1). Multiple forms of injury, including hepatitis, metabolic disease (i.e, in particular the metabolic syndrome) biliary injury, toxins (including alcohol), heavy metals, cause a variety of complicated and often integrated effects in the liver. For example, viral hepatitis causes activation of T cells, with recruitment of other inflammatory cells, as well as inflammatory mediators, and this leads to the fibrogenic wounding response (Figure 1). Alcohol mediated hepatocyte injury causes a classic inflammatory lesion, including TNF, which leads to hepatitis, and a fibrogenic wounding response. It should be emphasized multiple different cell types play a role in the injury mileu. For example, injury to endothelial cells, either directly or indirectly causes them to produce abnormal extracellular matrix, which in turn stimulates fibrogenesis by stellate cells [1].

A central event in the hepatic wounding response is enhanced extracellular matrix production, or fibrogenesis (Figure 1). Irrespective of the specific cause of liver injury (in both experimental models and human cirrhosis), the wound process leads to increased synthesis of extracellular matrix. The fibrogenic process is characterized by increases in a multiple matrix components, including the interstitial collagens, basement membrane collagens, proteoglycans and matrix glycoproteins such as laminin and fibronectin [2]; specific changes in matrix composition are highly similar in all forms of liver injury and hepatic fibrogenesis. Among the most prominent extracellular matrix proteins are the collagens (type I>III>IV), but increases in other matrix proteins are also prominent. It is important to emphasize that the wounding process is a dynamic one that includes aspects of matrix synthesis and deposition as well as degradation [3]. This point is exemplified by a robust body of literature data indicating that experimental [4–6] and clinical fibrosis [7–9] and even clinical cirrhosis is reversible [10–17]. In one study in patients with chronic hepatitis B infection and cirrhosis [14], 436 of 651 patients were assigned to receive lamivudine and 215 to receive placebo; 7.8 percent of patients receiving lamivudine and 17.7 percent of those receiving placebo developed hepatocellular carcinoma, spontaneous bacterial peritonitis, bleeding gastroesophageal varices, or had death related to liver disease (P=0.001).

Additionally, the Child-Pugh score increased in 3.4 percent of the patients receiving lamivudine and 8.8 percent of those receiving placebo (P=0.02). Thus, not only is advanced fibrosis reversible, but resolution of fibrosis is also associated with improved clinical outcomes.

Hepatic stellate cells and their activation in fibrogenesis

A key concept in the wounding response is that during the fibrogenic response, there is activation of effector cells. Evidence now supports the presence of a number of effector cells including stellate cells [18], peri-portal and peri-central fibroblasts [19], fibrocytes [20], myofibroblasts, and perhaps fibrogenic cells derived from hepatocytes through epithelial to mesenchymal transition (or EMT) [21, 22].

Stellate cells (also known previously as lipocytes, Ito, perisinusoidal cells), perisinusoidal, pericyte-like cells of mesenchymal origin, have garnered great attention as effectors of the fibrogenic response. In the normal liver, these cells function as a major retinoid reservoir for the body, storing much of the body's vitamin A [23, 24]. Given their pericyte-like appearance, they may also function as regulators of blood flow [23]. Notwithstanding, one of their most notable features occurs after liver injury. In this situation, stellate cells transform from "quiescent" (normal) to an "activated" (injured liver) state is a central component to the liver wounding process (Figure 1). The activation process is remarkably complex, with multiple and dynamic features. Phenotypically, it consists of many important cellular changes; characteristic features include loss of vitamin A, acquisition of stress bundles, and development of prominent rough endoplasmic reticulum (Figure 2). Perhaps the most prominent feature of activation is the striking increase in production and secretion of extracellular matrix proteins, including types I, III and IV collagens, fibronectin, laminin and proteoglycans, and others [2, 25]. An additional important feature of activation is de novo expression of smooth muscle specific proteins, such as smooth muscle α actin [26]. This feature further identifies stellate cells as liver specific myofibroblasts, a cell type typical of fibrogenesis in all organs [27, 28].

Although the most prominent features of activation include enhanced extracellular matrix production, and the expression of smooth muscle α actin, activation is also associated with other important cellular phenotypes including enhanced proliferation, release of proinflammatory cytokines [29], release of matrix degrading enzymes and their inhibitors, and recruitment and activation of other cell types such as hepatocellular cancer and cholangiocarcinoma cells [30, 31] and inflammatory cells [32]. When deisgning therapeutics focused on liver fibrogenesis, is important to emphasize that each of these features of activation (and fibrogenesis) represent a potential target for therapy. Important elements of the activation process are highlighted below.

Stellate cell fibrogenesis—Multiple factors play key pathogenic roles in stellate cell fibrogenesis. Prominent among these factors are cytokines, small peptides, and the extracellular matrix itself. Transforming growth factor beta-1 (TGF-β1) appears to be the most profibrogenic cytokine in the liver [33–35]. TGFβ1 is produced by Kupffer cells, sinusoidal endothelial cells, bile duct epithelial cells, hepatocytes and by stellate cells and

has prominent paracrine/autocrine effects on stellate cells [36, 37]. When TGFβ1 is overexpressed in the liver, it leads to promiinent fibrosis [33] and when inhibited during experimental liver injury, fibrosis is reduced [38]. TGFβ-1 signaling in stellate cells is remarkably complex [39], acting via direct (and to a lesser extent, indirect) pathways to stimulate of extracellular matrix production in stellate cells. Although none appears to be as potent as TGFβ1, a variety of other cytokines and peptides have profibrogenic effects on stellate cells (Table 1), including connective tissue growth factor (CTGF) [40, 41], endothelin-1 [6], leptin [42], angiotensin II [43], and others.

It should also be emphasized that cytokines and growth factors that drive stellate cell proliferation are important in the fibrogenic response because they help expand the total number of fibrogenic (stellate) cells. In essentially all forms of fibrosing liver injury, the number of activated effector cells is increased. Although the major mitogen driving cellular proliferation appears to be PDGF, a variety of other factors appear to be important in stimulation of stellate cell proliferation and include epidermal growth factor, fibroblast growth factor, insulin-like growth factor, thrombin, PAR agonists, monocyte chemotactic factor (MCP-1), insulin like growth factors (IGF-1 and 2), interleukin-6, CTGF, endothelin-1, angiotensin II, and others. While many of these compounds have isolated proliferative effects (i.e. PDGF), others (i.e. endothelin-1, angiotensin II, CTGF) stimulate both proliferation and fibrogenesis.

The vasoactive peptides endothelin-1 and angiotensin II, each of which have pleotrophic cell biologic and molecular effects, are notable not only because they have been emphasized in the pathogenesis of hepatic fibrogenesis [6, 43–45], but also because these compounds have vasoactive properties, and as such, may be important in the pathogenesis of portal hypertension. This raises the possibility that therapy directed at them could affect both fibrogenesis and portal hypertension. Other biologically active peptides (including unidentified compounds) may also be important in fibrogenesis. For example, dopamine beta-hydroxylase deficient mice, which cannot make norepinephrine, are resistant to fibrogenesis [46]. Thus, antagonism of these systems is attractive.

A number of cytokines and peptides appear to have anti-activation or anti-fibrogenic properties towards stellate cells. Although the number of the agents is considerably less than the number reported to be pro-fibrogenic and/or stimulate proliferation, included in this group are interferon γ [47], interferon α [48], adiponectin [49], hepatocyte growth factor [50], and possibly STAP [51].

Evolving evidence indicates that the extracellular matrix and the local environment plays an important role in modulating stellate cell activation. For example, culture of stellate cells on a basement membrane mimicking the normal basement membrane inhibits stellate cell activation and matrix synthesis [52], while culture of stellate cells on abnormal substrates such as the EDA isoform of fibronectin leads to increased activation of stellate cells [1]. Further, data suggest that stellate cells sense their surrounding environment [53]. For example, it was demonstrated that stellate cells became activated preferentially while exposed to a stiff substrate (compared to a softer substrate), and that this stiffness-dependent activation required adhesion to matrix proteins and the generation of mechanical tension [54]. It has also

been shown that integrins, which link the extracellular matrix to stellate (and other cells) play an important role in transmitting fibrogenic and contractile signals [55]. Recently, integrin linked kinase (ILK), an integrin-intracellular signaling molecule, has been shown to transmit fibrogenic signals in stellate cells [56, 57].

It should also be pointed out that fibrogenesis is a dynamic process with elements of extracellular matrix synthesis as well as degradation. During fibosis progression, there is not only increased expression of extracellular matrix protiens as highlighted above, but also metalloproteinases (MMPs) and in particular their tissue inhibitors (TIMPs). Evolving evidence suggests that early in the injury process, increases in expression of MMP-2 and membrane type 1-MMP lead to degradation of normal basement membrane matrix, which appears to facilitate stellate cell activation [58–60]. Additionally, overexpression of the TIMPs (TIMP-1 and TIMP-2) contributes to the profibrogenic phenotype [58]. This dynamic interplay of matrix synthesis and degradation is complex, but an attractive therapeutic target. As proof of concept, overexpression of MMP8 has been shown to lead to partial reversal of fibrosis [61].

Stellate cell contractility—Activation of stellate cells is accompanied by an increase in expression of proteins characteristic of contractile cells (i.e., such as smooth muscle α actin and smooth muscle myosins [26, 62]). Stellate cell contraction has been reported to be mediated by Ca++ dependent and independent mechanisms [63–66]. Stellate cell contraction has a multitude of effects in the injured liver including in perisinuoidal constriction and portal hypertension, and may also lead to the collapse and shrunken state of cirrhotic livers [45]. Stellate cell contractility is likely tied to multiple different systems, including the endothelin, angiotensin, adrenergic, and perhaps other systems [44, 45, 66–71].

Other stellate cell activation phenotypes—Beyond the phenotypes highlighted above, during liver injury and activation, stellate cells exhibit a number of important features (Figure 2). For example apoptosis (i.e., programmed cell death) is prominent in stellate cells and appears to be an important mechanism for fibrosis regression [5]. The data suggest that a balance between cell proliferation and apoptosis is important in determining the dynamics of the total overall stellate cell population in the liver. Based on these data, stimulation of stellate cell apoptosis could be an attractive therapeutic approach [72]. However, it has also been shown that stellate cell apoptosis may stimulate stellate cell activation, and thus may not be desirable [73]. Additionally, stellate cells may undergo senescence [74] or revert to a normal phenotype [75]. Recently, autophagy, a catabolic mechanism involviing cell degradation of unnecessary or dysfunctional cellular components through the lysosomal pathway, appears to play a role in stellate cell activation [76–78]. In mice with stellate cell specific deletion of autophagy-related protein 7 (Atg7), a protein important in mammalian autophagy, led to reduced activation following liver injury, leading to reduced fibrosis in vivo [78].

Approach to therapy for fibrosis

It is important to emphasize that the most effective anti-fibrotic therapies are those that target the primary stimulus to fibrogenesis (Table 2). For example, eradication or inhibition

of hepatitis B virus (HBV) [7, 9] or hepatitis C virus (HCV) [8] leads to reversion of fibrosis, and is associated with improved clinical outcomes [11, 12, 14]. Fibrosis (and cirrhosis) in patients with autoimmune hepatitis who respond to medical treatment (prednisone or equivalent) is reversible [13, 17]. Fibrosis may improve in patients with alcoholic liver disease who respond to anti-inflammatory therapy such as corticosteroids [79, 80]. Fibrosis reverts in patients with hemochromatosis during iron depletion [81, 82] and after relief of bile duct obstruction [15]. Additionally, in patients with non-alcoholic steatohepatitis (NASH) treated with the peroxisomal proliferator active receptor (PPAR) gamma agonist, rosiglitazone reduced both steatosis and fibrosis [83].

Experimental studies have demonstrated that many different interventions are capable of inhibiting (usually preventing) fibrogenesis. Such therapies have been targeted at inhibition of collagen synthesis, matrix deposition, modulation of stellate cell activation, stimulation of matrix degradation or stimulation of stellate cell death. A number of these preclinical approaches have been transitioned to clinical trials in humans (Table 3). The summary presented below indicates that as of the current writing, a specific anti-fibrotic that fits the profile of an ideal agent - one that is potent, safe, orally bioavailable, and inexpensive - is not yet available.

Specific anti-fibrotic targets and therapies

Colchicine is a plant alkaloid that inhibits polymerization of microtubules, and has anti-fibrotic properties in experimental animal models [84]. Although it has been studied in a number of clinical trials [85–88], including in primary biliary cirrhosis, alcoholic cirrhosis, as well as in miscellaneous other liver diseases [86], evidence supporting its effectiveness remains lacking.

Interleukin-10, an anti-inflammatory and immunomodulatory cytokine can down regulate production of proinflammatory cytokines, such as tumor necrosis factor-α, interleukin-1, and interleukin-2 from T cells. When administered to patients with HCV, interleukin-10 reduced hepatic inflammation and fibrosis scores (mean change from 5.0 ± 0.2 to 4.5 ± 0.3, $p < 0.05$). However, serum HCV RNA levels increased during therapy and thus has not been pursued.

Several studies have shown that interferon γ has potent inhibitory effects on stellate cells, inhibiting multiple aspects of stellate cell activation including fibrogenesis [47, 89]. A preliminary recent report in patients with chronic hepatitis C infection and fibrosis indicated that a subgroup of patients had an anti-fibrotic response [90]. However, a larger randomized study found that interferon γ failed to have an antifibrotic effect in patients with HCV and advanced fibrosis, presumably because it enrolled patients with advanced cirrhosis and treated them for too short a time period [91].

The peroxisomal proliferator activated receptor (PPAR) system has gained considerable attention in the hepatic fibrogenesis field [92–94]. PPAR γ in particular is reduced during stellate cell activation, and PPAR γ ligands inhibit activation and synthesis of extracellular matrix [92–94]. Further, the adipocytokine, adiponectin, appears to have prominent anti-fibrotic actions, and the PPAR γ effects on stellate cells are at least in part, adiponectin

dependent [95]. Because of its added putative beneficial role in the metabolic syndrome, adiponectin, is an attractive therapeutic target. Given the potential of PPAR γ agonists in treatment of patients with fibrosis and preliminary studies that demonstrated significant anti-fibrotic effects of the PPAR γ agonist, farglitazar, in animal models of fibrosis [96], a large multicenter randomized trial of farglitazar in patients with HCV was performed [97]. This well deisgned study demonstrated that farglitazar therapy for 52 weeks failed to have an effect on stellate cell activation or fibrosis in this population.

Polyenylphosphatidylcholine contains a mixture of polyunsaturated phosphatidylcholines, extracted from soybeans. Because of its presumed cytoprotective effect, it has been examined in humans [98]. Unfortunately, in a major multicenter, prospective, randomized, double-blind placebo-controlled trial study of 789 alcoholics (average alcohol intake of 16 drinks/day). comnparing either polyenylphosphatidylcholine or placebo for 2 years, there was no significant improvement in fibrosis. Of note, the majority of subjects reduced their ethanol consumption during the trial (presumably leading to an improvement in fibrosis in the control group).

Silymarin extract, derived from the milk thistle *Silybum marianum* (the major active component of which is silybinin), reduces lipid peroxidation and inhibits fibrogenesis in animal models [99–101]. In humans with fibrosis, the compound has had mixed effects [102, 103]. Thus, although silymarin appears to be safe, data supporting its use are lacking and further study is underway in patients with HCV (ClinicalTrials.gov Identifier: NCT00680342) and NASH (ClinicalTrials.gov Identifier: NCT00680407).

Ursodeoxycholic acid binds to hepatocyte membranes and appears to be cytoprotective, thereby reducing inflammation and thus fibrogenesis [104]. The aggregate data suggest that ursodeoxycholic acid may impede progression of fibrosis in primary biliary cirrhosis via effects on bile ductal inflammation, particularly if given early in the disease course [105, 106]. In a large randomized controlled trial of ursodeoxycholic acid in patients with non-alcoholic steatohepatitis over a 2-year course, examining 107 subjects who had paired biopsy data, there was no improvement in fibrosis [106]. In aggregate, rsodeoxycholic acid is safe, and while expensive, it is this author's belief that the available data justify its use at least in patients with primary biliary cirrhosis as an anti-fibrotic.

Vitamin E has gained a great deal of attention as a potential antifibrotic; it appears to be effective in animal models [107]. In humans vitamin E has has equivocal effects in patients with HCV [108], and alcoholic hepatitis [109, 110]. In patients with NASH, vitamin E led to reductions in aminotransferases, hepatic steatosis, and lobular inflammation, but failed to lead to an improvement in fibrosis [111].

A number of herbal medicines have been shown to have anti-fibrotic properties in animal models, and in some, specific mechanisms have been identified [112–115]. Herbal medicines with putative anti-viral, anti-inflammatory, and anti-fibrotic effects are being used extensively in the Far East in patients with a variety of liver diseases [116]. Medications containing herbs of the *Salvia* genus have been popular in particular as anti-fibrotics [116]. Although human trials have suggested effectiveness of specific herbal medicines in some

studies [116], data in peer-reviewed Western journals remains lacking. Since it is well appreciated that such herbal medicines may have significant toxicity, including hepatotoxicity [117], these medications should be used with caution.

The use of anti-tumor necrosis factor alpha (TNF-α) compounds in patients with alcoholic hepatitis is predicated on the rationale that TNF-α is upregulated after alcohol mediated hepatocellular injury (Figure 1), and thus these compounds should reduce inflammation, and resultant fibrosis. While early studies suggested an improvement in inflammation, [118–121], further larger studies revealed that their use was associated with an increase in the risk of serious infection [122] and mortality [123]. Pentoxyfylline appears to reduce TNF-α expression, and may also have primary antifibrotic effects [124, 125]. While data suggest an effect on certain clinical outcomes [118, 126], definitive evidence of an antifibrotic effect in humans is lacking.

Malotilate, penicillamine, methotrexate, S-adenosylmethionine, and propylthiouracil all have have been shown to exhibit some degree of anti-inflammatory and/or cytoprotective effects (presumably through their anti-oxidant properties) and as such, may have an effect on fibrogenesis [127, 128, 129]. However, evidence of an effect on fibrosis is equivocal at best [130–138]. It is important to emphasize that for many of these human studies, subjects with alcoholic hepatitis and liver injury were examined, and in these studies fibrosis was not typically measured as a specific outcome. Thus, it is may not be entirely appropriate to consider these agents as primary anti-fibrotics, but rather as compounds that could have secondary effects on fibrogenesis due to other properties.

Novel Approaches

A number of novel approaches to treat liver fibrosis exist. This includes novel mechanisms of targeting the liver, such as the use of siRNA [139, 140] or specific targeting systems [69, 141]. For example, TGF-β is well known to play a central role in the fibrogenic cascade and therefore is an important therapeutic target. Multiple proof of concept studies have demonstrated that its inhibition (through use of specific antibodies that immobilize active TGF-β or receptor antagonists) is likely to be effective in fibrosis [38, 142, 143]. However, given its important role in regulation of cell growth, global inhibition of TGF-β, or similar agents that have widespread biological effects such as PDGF or endothelin-1 could be potentially harmful. Thus, it will likley be critical to localize biological effects to fibrogenic effector cells. Early studies have provided proof of concept of this approach for stellate cells [144].

Previous and exciting new pathophysiologic studies point to further translational opportunities to treat fibrosis (Table 4). Given the central role of inflammation in chronic hepatic injury and the ensuing wound-healing process (Figure 1), it follows that bacterial products, particularly LPS may be important pathogenically. New evidence suggests that the microbiota may be important in the pathogenesis of liver inflammation [145], fibrosis [146], and even development of hepatocellular cancer [147]. In quiescent stellate cells, TLR4 (a major LPS receptor) activation not only upregulates chemokine secretion (further driving inflammation), but it also downregulates the transforming growth factor (TGF)-β

pseudoreceptor Bambi, which in turn sensitizes stellate cells to TGF-β-induced signaling [148]. In another study, liver injury was associated with early onset of increased intestinal permeability and bacterial translocation that preceded changes in the microbiota [149]. Changes in the microbiota have also been associated with fibrosis progression [146]. As such, manipulation of the intestinal flora may be an innovative approach to anti-fibrotic therapy.

MicroRNAs (miRs) have become recognized as being important in gene regulation and recent evidence suggests that a number of miRs are invovled in the pathogenesis of different forms of organ fibrosis [150] and in stellate cell function and liver fibrosis and [151, 152], and therefore may represent novel therapeutic targets.

A variety of other systems are also attractive. Among these include those related to collagen synthesis, such as the lysyl oxidase system; inhibition of this copper-dependent extracellular enzyme that catalyzes lysine-derived cross-links in collagen and elastin, could abrogate tissue fibrosis [153, 154]. Angiogenic pathways appear to be important in fibrosis, including the liver, and thus, interruption of this pathway could be an effective treatment approach. For example, a short peptide derived from endostatin, a naturally-occurring 20-kDa C-terminal fragment derived from type 18 collagen, appeared to have potent anti-fibrotic activity in skin and pulmonary fibrosis *in vivo* [155]. Nuclear factor (erythroid-derived 2)-like 2 (nrf2), a transcription factor that appears to active a number of genes involved in oxidative stress reponse appears to have protective effects for fibrosis [156, 157]. Additionaly, compounds such as pirfenidone [158], and 5′-lipoxygenase inhibitors [159] appear to have direct effects on stellate cells and/or *in vivo* effects in hepatic fibrogenesis. While there has been much interest in manipulating the balance between matrix synthesis and degradation via stimulation of collagen degrading metalloproteases, or dampening the effect of metalloprotease inhibitors, this area remains largely open.

Vascular biologic systems are intriguing because they could potentially have benefical effects both for fibrosis and for portal hypertnesion. Stellate cells express angiotensin II and endothelin receptors and stimulation of these receptors with their cognate ligands leads to prominent stellate cell effects [45].

Challenges in Developing Anti-fibrotic Therapy

Currently, a potent and effective anti-fibrotic drug or agent is not available. This is likely the result of several factors, highlighted below. Additionally, in order to develop a highly effective anti-fibrotic agent(s), several key features - as highlighted - will be important.

1. Diagnosis/Monitoring of Hepatic Fibrosis and Cirrhosis

Perhaps one of the most difficult challenges in the field of development of antifibrotic medications is monitoring the effectiveness of putative compounds. An ideal test would be one that is non-invasive and simple to perform, yet inexpensive. Currently, liver biopsy is considered to be the gold standard test for determining the extent and progression of fibrosis [160]. A quantitative measure of collagen content can be made by colorimetric assay of sirius red in liver tissue or by image analytic quantitation of collagen containing tissue [6]. Additionally, scoring systems have been developed [161–163] to quantitate fibrosis and to help

standardize the interpretation of biopsies amongst different centers; such systems are most useful for standardization and comparison of fibrosis in studies.

Unfortunately, liver biopsy, while considered the gold standard tool to assess fibrosis, is inexact. Not only is liver biopsy subject to inter-observer variability, but sampling error may be important, as evidenced by studies examining liver samples from different regions of the liver [164]. Additionally, liver biopsy is also associated with significant potential morbidity, including a significant risk of death [160] Thus, noninvasive measures that can monitor fibrogenesis would be ideal [165]. Noninvasive tools used to assess fibrosis include radiographic tests [166], combinations of routine laboratory tests [167, 168], and specific serum markers [169]. In particular, serum marker panels, including several that utilize mathematical algorithms [167, 168, 170], have been emphasized. Although some of these may even have predictive clinical value [171, 172], they have generally proven to be of limited clinical utility.

Finally, the field of molecular imaging is emerging, and with it, it is possible that effectors cells such as stellate cells may be imaged in order to more precisely quantitate their activity and or fibrogenic features [173, 174].

2. Cell Specific Targeting

As emphasized above, it would be ideal to localize therapy to only effector cells. This is particularly imporant for the targeting of systems involving systems that have widespread biological effects such as TGF-β, PDGF or endothelin-1 for example. TGF-β, in particular, is an attractive target since it appears to be the most potent stimulator of fibrogenesis. However, given its important role in regulation of cell growth, and neoplasia, it is highly likely that its global inhibition would have undesirable effects. A number of studies have provided proof of concept that at least stellate cells can be specifically targeted; by taking advantage of the expression of the mannose 6-phosphate/insulin-like growth factor II (M6P/IGF-II) receptor on stellate cells, it has been elegantly demonstrated that M6P-modified albumins conjugated to specific inhibitors or toxins reduced stellate cell mediated fibrogenesis [144, 175]. Alternatively, it is possible that physical properties of activated stellate cells may be taken advantage of, and that stellate cells could be targeted with specialized liposomes or similar compounds [176–178].

3. Length of Therapy

As emphasized above, fibrogenesis is a dynamic process that occurs over a period of time; advanced fibrosis typically develops over prolonged periods of time. Thus, it is likely that reversion of fibrosis would be expected to also occur over more prlonged periods of time. Most of the trials examining novel agents have been performed over relatively short periods of time, typically over 6 or 12 months. To see meaningful regression of fibrosis, it is likely that a trial will require longer than 1 year, and perhaps longer than 2 years.

3. Endpoints

The most appropriate endpoint for a novel treatment is a signal that the compound has antifibrotic effects. Notwithstanding this point, trials to date have used histologic assessment. This means that it is likely that the agent to be tested must be effective enough

to cause a change in histology. It may be more appropriate to use a marker or set of markers that detect a fibrogenic signal. For example, serum markers assessed over time may be acceptable. Additionally, some have suggested that an anti-fibrotic agent should have an effect on clinical outcomes. This would require a prolonged treatment, which would make the likelihood of developing an effective agent difficult.

Summary and Future Directions

The pathogenesis of hepatic fibrogenesis is now better understood than ever before. The central event in fibrogenesis appears to be activation of effector cells, most prominently hepatic stellate cells. Stellate cell activation is characterized by many important features including prominently, enhanced matrix synthesis and a contractile phenotype. The activation process is complex, leading to multiple potential sites for therapeutic interventions. A further critical concept is that the fibrogenic lesion, in particular, the extracellular matrix, is a dynamic structure; even advanced fibrosis may be reversible. These data have helped spawn interest in development of therapeutic antifibrotics. Notwithstanding, the most effective therapy for hepatic fibrogenesis is removal of the underlying disease process. While a number of challenges exist, including in the area of cell specific targeting, fibrosis monitoring, and execution of suitable clinical trials, the prospects for translation of the basic pathophysiology to therapy are bright. As for specific therapy directed primarly at the fibrotic lesion, the most effective therapies will most likely be directed fibrogenic effectors, in most cases hepatic stellate cells. In aggregate, although specific, effective, safe, and inexpensive anti-fibrotic therapies are not yet currently available, multiple potential targets have been identified, and one or more will likely emerge.

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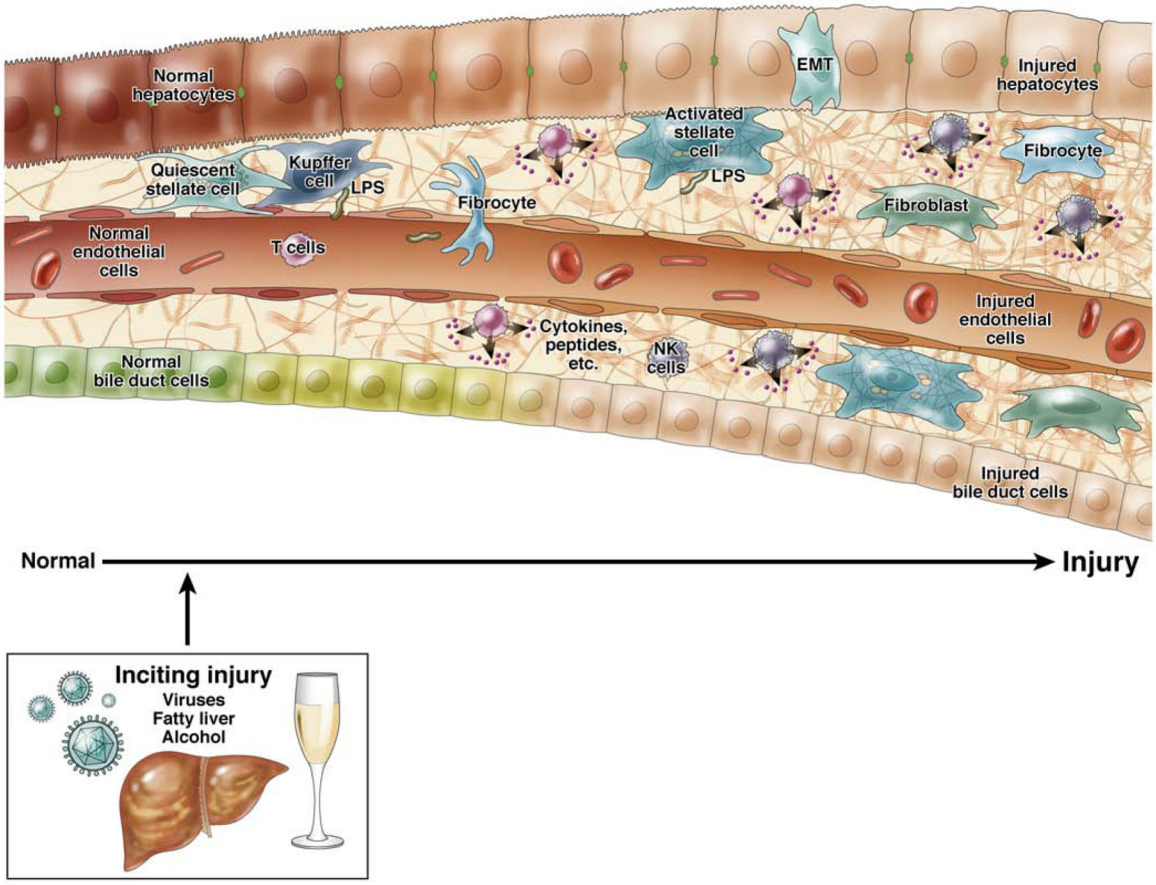

Figure 1. Liver injury and fibrogenesis

In the liver, many different types of injury (i.e., chronic hepatitis, ethanol, metabolic disease, biliary tract disease, iron, copper, etc…) lead to hepatocyte injury, and then typically an inflammatory response. This injury process is complicated, but in aggregate, it stimulates a wound healing response, which involves a number of different systems. Paramount in this process is often including recruitment of inflammatory cells. Among other properties, inflammatory cells produce a variety of mediators, cytokines, and other factors, which in turn are responsible for stimulation and/or recruitment of other cells. Key among these other cells include effector cells, highlighted in the figure and including stellate cells, fibrocytes, fibroblasts, and even fibroblasts derived though epithelial to mesenchymal transition (EMT). These effectors produce extracellular matrix proteins (see text), and importantly interact with other cells in the wounding mileu. Additionally, it is important to emphasize that many forms of injury lead to activation and transformation of other cells in the liver, such as endothelial and bile duct epithelial cells. Injury to these cells in turn leads to a variety of downstream effects. Each injured endothelial bile duct epithelial cells are capable of stimulatulation of effector cells to produce extracellular matrix consitutents.

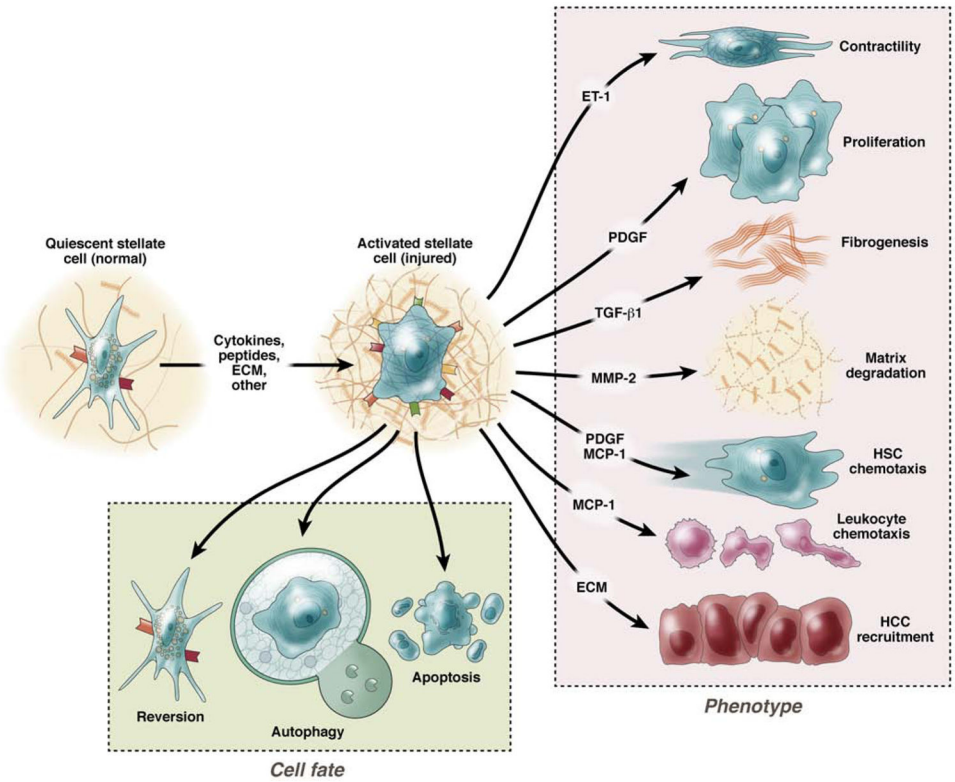

Figure 2. Stellate cell activation

A key pathogenic feature underlying liver fibrosis and cirrhosis is activation of hepatic stellate cells (note that activation of other effector cells is likely to parallel that of stellate cells). The activation process is complex, both in terms of the events that induce activation and the effects of activation. Multiple and varied stimuli participate in the induction and maintenance of activation, including, but not limited to cytokines, peptides, and the extracellular matrix itself. Recently, signaling through TLR4 on stellate cells has been identified as important in activation. Key phenotypic features of activation include production of extracellular matrix, loss of retinoids, proliferation, of upregulation of smooth muscle proteins, secretion of peptides and cytokines (which have autocrine effects on stellate cells and paracrine effects on other cells such as leukocytes and malignant cells), and upregulation of various cytokine and peptide receptors. Additionally, evidence indicates that stellate cells exhibit several cell fates, highlighted at the bottom of the figure, and each of these appear to play a role in the biology of fibrogenesis.

Table 1

Cytokines and growth factors important in stellate cell fibrogenesis

Profibrogenic	**Antifibrogenic**
Transforming growth factor-β	Interferon γ
Transforming growth factor-α	Interferon α
Connective tissue growth factor	Adiponectin
* Insulin-like growth factor (I, II)	** Endostatin
* Platelet derived growth factor	Hepatocyte growth factor
* Monocyte chemotactic factor	
* Fibroblast growth factor	
** Interleukin-1	
** Interleukin-4	
* Interleukin-6	
* Thrombin	
Endothelin-1	
Norepinephrine	
Angiotensin II	
Thrombospondin (1,2)	
Leptin	
** Lipopolysaccaride	

* Agents whose effect is largely via stimulation of proliferation

** Indirect effects on stellate cells

*** Including fragments

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Table 2

Liver Diseases in which treatment of the underlying process may reverse fibrosis

Disease	Comments
Hepatitis B	Antiviral treatment improves outcomes
Hepatitis C	Viral eradiction improves outcomes
Autoimmune hepatitis	Corticosteroids may improve outcomes
Alcoholic hepatitis	Corticosteroids may improve outcomes
Bile duct obstruction	Biliary decompression improves histology
Hemochromatosis	Iron depletion may improve outcomes
Primary biliary cirrhosis	UDCA, MTX have weak effects
Non-alcoholic steatohepatitis	PAR ligands have weak effects

See text for discussion and references.

Abbreviations: MTX = methotrexate; PPAR = peroxisomal proliferator activated receptor

Table 3

Potential anti-fibrotic therapies tested in humans

Agent	Disease	Comments	Status
Compounds with anti-inflammatory, anti-oxidant or general effects			
Interleukin-10	HCV	Increased viral load	Not suitable for therapy
PPC	ETOH	Minimal if any effect	Not recommended
SAM	ETOH	Minimal if any effect	Not recommended
Silymarin	HCV/ETOH	Further studies pending	
Anti-TNFα	ETOH	Increased mortality	Likely dangerous
UDCA	Multiple	Modestly effective, safe	May be acceptable (PBC)
Vitamin E	HCV/NASH	Modestly effective, safe	May be acceptable
Pentoxifylline	ETOH	Minimally effective, safe	May be acceptable
Compounds with specific anti-fibrotic effects			
Colchicine	Misc	Minimal if any effect	Not recommended
Interferon gamma	HCV	Minimal if any effect	Not suitable for therapy
Farglitizar	NASH	No clear effect	Not suitable for therapy
ARBs	Misc	Minimal if any effect	May be acceptable

*See the text for specific discussion of mechanism and for references.

Abbreviations: PPC = Polyenylphosphatidylcholine; SAM = s-adenosylmethionine, TNF = tumor necrosis factor; PPAR = peroxisomal proliferator activated receptor, ETOH = alcohol, HCV = hepatitis C virus, NASH = non alcoholic steatohepatitis, misc = miscellaneous, UDCA = ursodeoxycholic acid; PBC = primary biliary cirrhosis; ARB = angiotensin receptor blocker.

Table 4

Potential Anti-Fibrotic Targets

Agent or System	Mechanism
Intestinal microbiota/TLR4	TLR4 on multiple cells types, including stellate cells activates inflammatory pathways
NRF2	Transcription factor whose downstream target genes play an important role in cellular anti-oxidant defense
Loxl2	Enzyme ca thetalyzes first step in the formation of crosslinks in collagens and elastin
Adiponectin	244-amino-acid-long polypeptide regulating glucose levels as well as fatty acid breakdown that has direct effects on stellate cell fibrogenesis
Angiostatin/Endostatin	Endogenous angiogenesis inhibitors
Endothelin	21 aa potent vasoconstrictor, that also stimulates stellate cell activation

TLR4 = Toll like receptor 4

NRF2 = Nuclear factor (erythroid-derived 2)-like 2

LOXL2 = Lysyl oxidase homolog 2