Evolving challenges in hepatic fibrosis

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Abstract | Continued elucidation of the mechanisms of hepatic fibrosis has yielded a comprehensive and nuanced portrait of fibrosis progression and regression. The paradigm of hepatic stellate cell (HSC) activation remains the foundation for defining events in hepatic fibrosis and has been complemented by progress in a number of new areas. Cellular sources of extracellular matrix beyond HSCs have been identified. In addition, the role of chemokine, adipokine, neuroendocrine, angiogenic and NAPDH oxidase signaling in the pathogenesis of hepatic fibrosis has been uncovered, as has the contribution of extracellular matrix stiffness to fibrogenesis. There is also increased awareness of the contribution of innate immunity and greater understanding of the complexity of gene regulation in HSCs and myofibroblasts. Finally, both apoptosis and senescence have been recognized as orchestrated programs that eliminate fibrogenic cells during resolution of liver fibrosis. Ironically, the progress that has been made has highlighted the growing disparity between advances in the experimental setting and their translation into new diagnostic tools and treatments. As a result, focus is shifting towards overcoming key translational challenges in order to accelerate the development of new therapies for patients with chronic liver disease.


Introduction

The field of hepatic fibrosis enjoys remarkable vitality—a PubMed search with the keywords ‘hepatic fibrosis’ or ‘hepatic stellate cell’ reveals that there were approximately 3,304 publications on the topic in 2009 alone, and 15,377 publications over the past 5 years. A prior review of the topic in this journal in 2004 emphasized the emerging mechanisms of the disease and their therapeutic implications, focusing on the role of the hepatic stellate cell (HSC) as the key fibrogenic element in response to chronic liver injury. More recent advances in the cellular and molecular biology of the fibrotic wound healing response have moved into surprising new frontiers, including stem cells, immune and apoptotic signaling, and epigenetics. Complementing these advances is further proof that the liver is uniquely resilient, with evidence that cirrhosis has regressed in a growing number of patients whose underlying liver disease has been treated effectively.

Progress could not arrive a moment too soon. A recent Institute of Medicine report has imparted a sense of urgency based on the burden of chronic viral hepatitis B and C, which are the leading causes of cirrhosis and hepatocellular carcinoma (HCC) worldwide and are currently estimated to affect 1–2% of the US population. The increasing burden of liver disease in the USA in part reflects a rising prevalence of cirrhosis among those patients with chronic hepatitis C. The worldwide burden is equaly compelling, with chronic liver diseases affecting hundreds of millions of individuals, which is associated with an accelerated risk of HCC. Contributing to this burden, the explosive growth in obesity worldwide has led to a dramatic increase in the prevalence of non-alcoholic steatohepatitis (NASH), which also confers a risk of HCC once cirrhosis develops. In fact, HCC has the fastest rising cancer incidence of any neoplasm in the USA and Western Europe.

Despite all this progress, however, no drug has yet emerged as an effective antifibrotic agent in humans and, therefore, it is instructive not only to provide an update on our understanding of the pathways of fibrosis, but also to acknowledge the evolving challenges that lie ahead when translating these discoveries into new treatments. Following an update on recent advances in our understanding of the mechanisms of fibrosis, this Review highlights a few key areas of progress in the past 5 years and focuses primarily on four persistent challenges. The first challenge is to find better markers of fibrosis stage and activity. The second is to be able to predict which patients with chronic liver disease will progress to cirrhosis and which patients will reverse their fibrosis. The third is predicting which patients with cirrhosis will develop decompensated disease. Finally, the fourth challenge is to perform a proof-of-concept clinical trial that establishes the rationale for targeting fibrosis in patients with chronic liver disease.

Advances in the mechanisms of fibrosis

In the past few years, several reviews have emphasized the essential role of HSC activation into myofibroblasts...
in the pathogenesis of hepatic fibrosis,
and therefore this concept is not reviewed here in detail, but summarized in Figure 1. Instead, several new areas of progress in our understanding of the mechanisms underlying hepatic fibrosis are highlighted below. First is the identification of non-HSC cellular sources of extracellular matrix (ECM). Second is the emerging importance of chemokine, adipokine, neuroendocrine, angiogenic and NADPH oxidase signaling. Third is the role of ECM stiffness in driving fibrogenesis. Fourth is the contribution of innate immunity involving natural killer (NK) cells, dendritic cells, and Toll-like receptor (TLR) signaling. Fifth is the complexity of gene regulation in HSCs and myofibroblasts, including control by transcriptional, post-transcriptional, and epigenetic events, as well as microRNAs. Sixth is the recognition that fibrogenic cells can be eliminated during resolution of liver injury and fibrosis by the orchestrated programs of apoptosis and senescence.

Alternative sources of extracellular matrix
Several potential non-HSC sources of ECM have been identified (Figure 2). A number of studies have implicated bone-marrow-derived cells in hepatic fibrogenesis, both in experimental models and human chronic liver injury.

**Figure 1** HSC activation. The pathways of HSC activation include those that provoke initiation and those that play a part in perpetuation. Initiation is stimulated by soluble stimuli such as oxidant stress signals (reactive oxygen intermediates), apoptotic bodies, LPS and paracrine stimuli from neighboring cell types including hepatic macrophages (Kupffer cells), sinusoidal endothelium and hepatocytes. Initiation is followed by perpetuation. Proliferation, contractility, fibrogenesis, altered matrix degradation, chemokinesis and inflammatory signaling are specific phenotypic changes characteristic of perpetuation. Resolution of hepatic fibrosis, which occurs following clearance of the primary liver disease, leads to loss of activated HSCs, either through apoptosis, senescence or reversion of activated cells to a more quiescent phenotype. Abbreviations: CTGF, connective tissue growth factor; ET1, endothelin 1; FGF, fibroblast growth factor; HSC, hepatic stellate cell; LPS, lipopolysaccharide; MMP, matrix metalloproteinase; TIMP, tissue inhibitor of metalloproteinase; NO, nitric oxide; NOX, NAPDH oxidase; PDGF, platelet-derived growth factor; TGF-β1, transforming growth factor β1; TIMP, tissue inhibitor of metalloproteinase; TLR, Toll-like receptor; TRAIL, tumor-necrosis-factor-related apoptosis-inducing ligand; VEGF, vascular endothelial growth factor. Permission obtained from Elsevier Ltd © Friedman, S. L. Gastroenterology 134, 1655–1669 (2008) and the American Society for Biochemistry and Molecular Biology © Friedman S. L. J. Biol. Chem. 275, 2247–2250 (2000).
also been implicated in hepatic fibrogenesis. However, not all studies agree with these conclusions. Indeed, the overall contribution of fibrocytes to fibrosis is probably very small. Nonetheless, alternative contributions of these cells to the response to liver injury beyond fibrogenesis (for example, immunoregulation) merit further study. Although it is not clear how to reconcile these opposing views on the contribution of bone-marrow-derived cells to fibrosis, studies of this type differ in several ways—the models used, duration of injury, markers used to characterize cells, and methods of detection—so their direct comparison may be difficult.

Some evidence also implicates epithelial-to-mesenchymal transition (EMT) as a source of fibrogenic cells, a pathway that has previously been described in renal fibrosis. However, more recent studies refute this conclusion. In liver studies, experimental models have identified both hepatocytes and biliary epithelium as potential epithelial origins for EMT. Bone morphogenic protein 7 has specifically been implicated in hepatic epithelium-derived EMT, whereas the hedgehog pathway seems dominant in biliary epithelium-derived EMT. Moreover, transitions of this type may be bidirectional, with some epithelium arising from fibrogenic cells (that is, mesenchymal-to-epithelial transition [MET]). What is unclear from these studies, however, is whether cells simply express mesenchymal markers or contribute substantively to ECM production. One study indicates that their actual contribution is minimal in vivo, even though EMT can be documented in cell culture. In addition, there is intriguing evidence that either transdifferentiation between HSCs and liver progenitor cells occurs or, alternatively, that HSCs provide a uniquely supportive environment for progenitor cell survival and expansion. The implications of these findings for hepatic regeneration, fibrosis and cancer remain to be clarified.

**Signaling in hepatic fibrosis**

**Chemokine signaling**

Two elegant studies published in 2009 implicate chemokine signaling in the pathogenesis of hepatic fibrosis and complement earlier work that has been summarized elsewhere. In particular, the CC chemokine receptors CCR1 and CCR5 are both fibrogenic, but they arise from distinct cellular sources: CCR1 is derived from bone marrow cells, whereas CCR5 is derived from resident liver cells. Interestingly, the site of the CC chemokine receptor CCR2 expression evolves with progressive liver injury—CCR2 is initially expressed by bone marrow cells, but later by resident liver cells. By contrast, the activity of CXC chemokine ligand 9 (CXCL9), through its cognate receptor CXCR3, is antifibrotic, and polymorphisms in the gene encoding CXCL9 may contribute to fibrosis progression risk in patients who have chronic liver disease.

**Adipokine signaling**

Similar to chemokine signaling, signaling by adipokines—polypeptides derived from adipose tissue—has assumed increasing importance in parallel with rapid advances in understanding the role of adipokines in metabolic homeostasis outside the liver. Whereas some adipokines are strictly derived from fat, others are also produced by resident liver cells. For example, leptin and adiponectin are both derived from HSCs, and their reciprocal dysregulation (that is, elevated levels of leptin and diminished levels of adiponectin) may drive fibrogenesis primarily through local paracrine signaling.

**Neuroendocrine signaling**

Neuroendocrine activity has also been uncovered in liver, most prominently in studies of cannabinoid signaling. Interruption of cannabinoid activity is an appealing therapeutic target. Specifically, CB1 receptor signaling is profibrogenic, and thus efforts to antagonize this molecule have met with significant success in animal models, and are being evaluated in human trials. Conversely, CB2 receptor signaling is antifibrogenic—a strategy to agonize this receptor also shows promise. CB2 agonists may, however, amplify inflammation, suggesting that CB1 antagonism may be a more rational strategy to agonize this molecule also shows promise.

**Angiogenic signaling**

Angiogenic signaling is a key component of the wound healing response in hepatic fibrosis, contributing not only...
to ECM production, but also to portal hypertension.\textsuperscript{46} Classic angiogenic mediators, most notably vascular endothelial growth factor (VEGF) and platelet-derived growth factor (PDGF), drive both the angiogenic and fibrogenic responses, and may also foster a milieu that is permissive for the development of HCC (see below). On the other hand, angiogenesis may also be essential for regenerative responses to chronic liver injury, and thus efforts to antagonize angiogenic signaling must be titrated sufficiently to preserve normal growth responses while inhibiting neoplasia.

**NADPH oxidase signaling**

Oxidant stress has been implicated in fibrogenic stimulation for many years, and the intracellular pathways mediating its generation have been greatly clarified of late.\textsuperscript{49,50} Among several enzymes that regulate the generation of oxidant stress, the NADPH oxidase protein complex is particularly important, and inhibition of NADPH oxidase is an attractive target for antifibrotic therapy.\textsuperscript{50} Moreover, the fibrogenic effect of apoptotic body ingestion by HSCs is also mediated by NADPH oxidase.\textsuperscript{51}

Long-term administration of the angiotensin receptor blocker losartan in patients with chronic hepatitis C significantly decreases the expression of NADPH oxidase, collagen I, matrix metalloproteinase 2 and urokinase type plasminogen activator.\textsuperscript{52} Targeting of losartan to the liver may offer some benefit by possibly reducing its unwanted activities (for example, lowering of blood pressure), as demonstrated in an animal study that used a liver-targeted complex containing losartan.\textsuperscript{53} The promise of antioxidant therapy has received an important boost from a large-scale trial of vitamin E in patients with NASH,\textsuperscript{24,51} a disease in which oxidant stress has been consistently implicated. Future efforts with antioxidant therapies will probably attempt to enhance their potency by more targeted delivery to the liver, or by the use of formulations that are activated upon reaching the liver.

**Extracellular matrix stiffness drives fibrogenesis**

New diagnostic modalities that correlate the stiffness of the liver with the fibrosis stage (for example, transient elastography [see below]) have found a molecular correlate in studies demonstrating that the physical properties of ECM, in addition to their chemical composition, affect HSC activation.\textsuperscript{56} Moreover, stiffness may actually precede fibrosis\textsuperscript{57} as a result of both increased edema and inflammatory cell infiltration.

**The contribution of innate immunity**

Growth in our understanding of the contribution of innate immunity to hepatic fibrosis has been almost explosive. There has been rapid progress in defining the roles not only of macrophages and traditional lymphocyte subsets (for example, T and B cells), but also of NK cells, NKT cells,\textsuperscript{13,58–60} B cells, dendritic cells\textsuperscript{61} and mast cells. There are also the newly uncovered contributions of TLRs,\textsuperscript{62–65} NFoxB signaling\textsuperscript{66} and the inflamasome, a molecular complex activated upon stress that promotes inflammation.\textsuperscript{67} Moreover, bidirectional interactions between HSCs and/or myofibroblasts, and immune cell subsets, elicit specific cellular responses that modulate the composition of inflammatory infiltrates and the vigor of fibrogenesis.\textsuperscript{66,68} In aggregate, these studies point to the liver as a major immunoregulatory organ, and underscore the critical contribution of immune cell subsets in modulating fibrosis as well as liver injury.

**Gene regulation in HSCs and myofibroblasts**

Progress in understanding gene regulation in HSCs and myofibroblasts has paralleled the dramatic expansion of knowledge about both traditional mechanisms of gene regulation, including transcription factor activity, localization and modification,\textsuperscript{70} as well as epigenetic regulation of gene expression by methylation,\textsuperscript{71,72} mRNA stabilization\textsuperscript{73} and microRNA interactions.\textsuperscript{72,74,75} Elucidating the precise molecular events underlying HSC activation and fibrogenesis is translating into fruitful new therapeutic approaches.\textsuperscript{76}

The repertoire of transcription factors known to cooperatively regulate gene expression through post-translational modification of regulatory proteins, particularly through phosphorylation, is growing. For example, HSC activation provokes phosphorylation of the RelA subunit of NFκB at a specific serine residue (Ser\textsuperscript{536}) that leads to its nuclear import, resulting in increased NFκB transcriptional activity,\textsuperscript{77} which increases the survival of activated HSCs. Treatment of rodents with experimental fibrosis by using an angiotensin-converting-enzyme inhibitor or humans with hepatitis C by using an angiotensin receptor blocker, leads to reduced survival of activated HSCs and/or myofibroblasts and regression of fibrosis by inhibiting the phosphorylation of the RelA subunit of NFκB at Ser\textsuperscript{536}.\textsuperscript{78}

In an example of epigenetic regulation, myofibroblast activity can also be controlled by a microRNA, miR132, that releases a translational block on the methyl-CpG binding protein, which in turn leads to repression of the PPARγ transcription factor.\textsuperscript{72} In a final example, phosphorylation of the transcription factor C/EBPβ by the ribosomal S-6 kinase (RSK) is critical for HSC activation; this phosphorylation can be inhibited by cell-permeable peptides that block RSK and lead to cellular apoptosis.\textsuperscript{77}

**Apoptosis, senescence and resolution of fibrosis**

Exciting clinical evidence has demonstrated that cirrhosis may not only be reversible,\textsuperscript{79} but that regression of fibrosis leads to improved clinical outcomes.\textsuperscript{79} At what point cirrhosis becomes irreversible is uncertain, but irreversibility becomes increasingly likely as the scar thickens, becomes more acellular and is chemically crosslinked. Nonetheless, these successes in demonstrating fibrosis regression even in patients with advanced fibrosis have redoubled our efforts to understand the mechanistic basis for fibrosis regression in the hope of exploiting pathways that drive the clearance of HSCs in the fibrotic liver.

Complex, but remarkably well-orchestrated intracellular events conspire to sustain HSC survival and growth during progressive fibrosis, while permitting their regulated clearance during resolution of fibrosis. In support
of this concept, progression of hepatic fibrosis in patients with hepatitis C is associated with progressive loss of HSC apoptosis.\(^\text{86}\) Whereas earlier, groundbreaking studies highlighted the importance of tissue inhibitor of metalloproteinase 1 (TIMP1) in sustaining HSC survival during progressive injury, more recent efforts have focused on intracellular events that are targetable; for example, interfering with survival signals mediated by the NFκB cascade and kinase activities (see preceding section).\(^\text{76,81,82}\) The tumor suppressor protein p53 has entered the field of hepatic fibrosis through evidence demonstrating that it drives senescence of activated HSCs during resolution of experimental fibrosis.\(^\text{83}\) The functional and regulatory relationship between apoptosis and senescence of HSCs remains to be defined.

**Challenges to progress**

With the astonishing progress in clarifying the pathways of hepatic fibrosis, the greatest obstacles to translation of these advances no longer reside in the laboratory but rather rest firmly in the clinical setting. Four such translational challenges are highlighted in the following sections.

**Markers of fibrosis stage and activity**

The first challenge that we face is the need for better markers of fibrosis stage and activity (Figure 3).

**Liver biopsy**

The foundations of the discipline of hepatology were built upon histologic assessment of liver tissue, and thus percutaneous biopsy has been a bedrock for the diagnosis and staging of fibrosis. However, its limitations have become painfully clear. Apart from its invasive nature and thus the limited number of samples that can be obtained over time in an individual patient, the modality suffers from significant sampling variability, owing to the fact that the tissue obtained represents only ~1/50,000\(^\text{th}\) of the organ. Although most chronic liver diseases are relatively homogeneous, the modest variability from region to region is sufficient to yield discordance in fibrosis staging in at least one-third of biopsy samples from patients with hepatitis C\(^\text{86}\) or NASH.\(^\text{85}\) Indeed, even very large biopsy samples (>2.5 cm) will be unlikely to exceed 75% accuracy in assessing fibrosis in patients with hepatitis C.\(^\text{86}\) Fibrosis quantification in liver biopsy samples can be improved by morphometric methods rather than conventional staging\(^\text{87,88}\) but this does not overcome the problem of sampling variability when the tissue sample is inadequate.

Remarkably, only recently has a study explored the relationship between fibrosis stage and outcome, using patients enrolled in the NIH HALT-C trial of long-term treatment with interferon α for patients with chronic
hepatitis C. In this trial, which used the six-stage Ishak staging system, there was a clear relationship between fibrosis stage and both baseline markers of disease severity as well as clinical events over a 6-year period. However, among those patients with stage 6 fibrosis (that is, cirrhosis) there were no features reported within the biopsy sample that identified those patients who were more likely to develop clinical events. In addition, 25% of biopsy samples were fragmented, which significantly diminished the ability to establish an association between fibrosis stage and outcomes. Finally, the use of a discontinuous scoring system implies that the incremental changes in fibrosis content between consecutive stages are equal, when in fact there is far more fibrosis accumulated between the more advanced stages than between the early stages. This means that the amount of matrix that must be resorbed if fibrosis is reversed will be far greater if patients have advanced fibrosis at the time of treatment with either an antiviral or antifibrotic drug than if patients have mild fibrosis.

**Transient elastography**

Innovative new methodologies have begun to circumvent the need for biopsy samples to be taken in a growing fraction of patients. The most popular new modality is transient elastography, which correlates the speed of transmission of a shear wave through the liver with the organ's relative stiffness. Numerous studies have firmly established the value of transient elastography in distinguishing cirrhosis from earlier stages of fibrosis in patients with chronic hepatitis C, in whom stiffness largely reflects matrix accumulation. By contrast, stiffness in patients with acute hepatitis more likely reflects edema and inflammation, and does not correlate with fibrosis. Post-transplantation, the measurement of liver stiffness may enable stratification between a mild or severe recurrence of hepatitis C.

A more comprehensive assessment of the entire liver's stiffness can be obtained by MR elastography. Regardless of which approach is employed (bedside versus MR assessment), elastography is becoming an appealing adjunct to patient management that is currently approved for use in Western Europe and awaiting evaluation by the FDA in the USA. It remains uncertain, however, whether these technologies will enable accurate assessment of fibrosis progression over time in individual patients that is sufficient to permit their usage in patients without cirrhosis.

**Other imaging modalities**

Other imaging modalities are also gaining momentum for the assessment of liver fibrosis. Specialized MRI techniques, including diffusion-weighted imaging, perfusion imaging and functional imaging are gaining interest. A newer technology—acoustic radiation force impulse (ARFI)—is an ultrasound-based technology that seems to be comparable to transient elastography in its ability to distinguish cirrhosis from earlier stages of fibrosis in patients with chronic hepatitis C. ARFI utilizes a conventional ultrasound device, which makes the technology more readily available given the wide use of ultrasound.

**Serum markers**

Serum markers of fibrosis, measured using combinations of either common laboratory studies or proprietary matrix assays, have roughly comparable ranges of accuracies; some tests are more useful in specific diseases and at different stages than others. The two best-validated methods, Fibrotest (BioPredictive, Paris, France) and the Enhanced Liver Fibrosis (ELF) test (iQur Ltd, Southampton, UK), are even more accurate than liver biopsy in predicting clinical outcomes, whether or not they directly reflect either fibrosis accumulation or regression.

Serum markers can also distinguish between mild and progressive hepatitis C recurrence after liver transplantation, presumably by reflecting increased edema and inflammation in patients with recurrence. Like transient elastography, serum markers enable many patients to avoid biopsy in cases in which the results clearly indicate the presence of either minimal or advanced fibrosis, with up to 50% of biopsies being avoidable based on current estimates. The accuracy of serum tests may be further increased by combining them with elastography findings. Finally, serum assay values are reduced following successful treatment of hepatitis C, further reinforcing their correlation with fibrogenic activity.

The use of noninvasive markers is especially appealing in populations in whom percutaneous biopsy is more challenging, for example children and patients co-infected with HIV and HCV. Information regarding fibrosis stage is especially vital in co-infected patients, as successful therapy for HCV can greatly reduce liver-related complications.

**Markers of fibrogenesis**

Perhaps a more compelling issue is whether the amount of scar per se is the best indication of prognosis and response to therapy. As a relatively static consequence of liver injury, fibrosis content alone does not convey information about the fibrogenic activity or liver reserve. Accordingly, tests that reflect fibrogenesis (that is, the activity of scar production) are important complements to tests that assess only fibrosis content. For example, immunohistochemical markers of HSC activation in liver sections, particularly α-smooth muscle actin, are a more sensitive indicator of the rate of fibrogenesis and risk of clinical complications, particularly in patients with hepatitis C following liver transplantation.

**Measures of hepatic reserve**

Similarly, functional tests of hepatic reserve—once an appealing but cumbersome diagnostic method used in specialized centers—are making a comeback. Improved technology now enables bedside assessment of the liver's metabolic activity. Rather than simply correlating its results to the amount of scar, tests of this type provide complementary information that ultimately may be more informative than simple hepatic histologic features in predicting outcomes. Continued validation and standardization of tests of functional reserve will be essential to ensure that their results are reproducible, and...
are not confounded by external factors including diet, concurrent medications and/or comorbidities.

Hepatic venous pressure gradient assessment
Another test of hepatic function, albeit an invasive one, is the hepatic venous pressure gradient (HVPG) assessment. In fact, the test is remarkably accurate in determining which patients with cirrhosis are at risk for decompensation\(^2\) (see section below). However, the utility of assessing the HVPG is confined to patients with relatively advanced disease who are at risk of, or already have, portal hypertension.

The future of fibrosis markers
What could ultimately emerge from these broad efforts across a range of methodologies may be a multimodal approach to assess both liver structure and function. The essential advance, not yet realized, will be to define a test, or series of tests, which reliably predict changes in hepatic structure, function, fibrogenic activity, and/or prognosis over time, as well as response to therapy in individual patients. Such an advance will accelerate the ability to conduct long-term trials of antifibrotic drugs by yielding unambiguous evidence of efficacy.\(^1\)

Predicting fibrosis progression and reversal
Our second challenge is to find a way to predict which patients with chronic liver disease will progress to cirrhosis and which patients will reverse their fibrosis.

Fibrosis progression
Fibrosis progression is strongly driven by genetic factors that can be identified by simple tests already available for clinical usage. A large number of genetic markers have been uncovered that reportedly correlate with fibrosis progression rate, primarily in hepatitis C.\(^3,8,122\) Among these, a seven-gene cirrhosis risk score (CRS)—seven single nucleotide polymorphisms (SNPs) combined into a scoring system—is strongly associated with fibrosis progression,\(^126,127\) and has been validated in a separate large prospective cohort study.\(^128\)

In addition to testing genetic markers in multiple populations, a key to establishing their relevance is to link the DNA sequence changes to altered pathways of disease pathogenesis, thereby providing a mechanism to explain the impact of the SNP on fibrosis. At least one of the SNPs within the CRS has been linked to altered HSC activity.\(^74\) Several other SNPs that are not part of the CRS have also been linked to altered HSC activity.\(^8,124,129,131\)

What is the utility of determining fibrosis progression risk in a patient who has chronic liver disease? First, the knowledge that a patient may progress more rapidly, even if the liver histology findings are currently at an early fibrosis stage, may influence the frequency of follow-up evaluations and the timing of a disease-specific therapy (for example, antiviral drugs) if one is available. Second, this information is vital to designing clinical trials of antifibrotic drugs that will produce unambiguous findings (Figure 4). Specifically, if the relative distribution of fibrosis risk is not evenly distributed among placebo and treatment groups, then interpreting the potential response to a drug will be confounded by underlying, genetically determined differences in fibrosis progression rates independent of the drug's effect. Third, by enrolling only those patients who have a high risk of fibrosis progression into antifibrotic trials, clearer results will be obtained in a shorter study interval, as differences between a placebo and treatment group would emerge more rapidly if the drug were effective.

In addition to genetic determinants, the associations between environmental or behavioral risk factors and fibrosis progression have also been strengthened. For example, studies have demonstrated that both coffee\(^132,133\) and caffeine\(^134\) have a protective effect on fibrosis progression. The antifibrotic effect may be due to antagonism of adenosine receptor activity, which in animal models reduces the fibrogenic activity of HSCs.\(^135\) Moreover, until recently there were no data linking specific viral factors with fibrosis progression, but two studies have now demonstrated that hepatitis B genotype C,\(^136\) and hepatitis C genotype 3\(^137\) are both associated with more rapid fibrosis progression. The findings complement the well-known acceleration of fibrosis progression due to HIV and HCV co-infection, as underscored by the findings of a study based on liver biopsy in co-infected patients.\(^138\)

Fibrosis regression
Similar to factors that drive the rate of fibrosis progression, there are likely to be factors that determine which patients will have regression of fibrosis when the primary disease has been arrested. Ample evidence indicates that cirrhosis may regress to earlier stages in many patients who have been successfully treated with antiviral therapy, for example,\(^79,139\) yet it is unclear in whom this will occur. Moreover, the determinants of regression might be biochemical rather than genetic, for example based on the density of chemical cross linking of collagen, or the cellularity of the scar.\(^140\) Clarifying the factors that confer regression of fibrosis will become increasingly possible as a greater number of patients are successfully treated for their underlying liver disease because of improved therapies.

Predicting decompensation
The third challenge that we face is being able to predict which patients with cirrhosis will develop decompensated disease. Indeed, not all patients with cirrhosis have a comparable prognosis—some will remain stable for up to 10 years while others are at imminent risk of decompensation.\(^141\) These disparate outcomes are not sufficiently predicted by either histologic staging systems or standard clinical parameters in patients with either a low MELD or Child–Pugh score, although a large-scale analysis from the HALT-C trial indicates that common clinical and laboratory features may be predictive in patients who have more advanced disease.\(^142\) Moreover, some, but not all, studies suggest that Fibroscan® (EchoSens, Paris, France) may identify patients who have a higher likelihood of developing esophageal varices\(^143\) or HCC.\(^144\)

Why haven't better tests been developed before now to predict the risk of decompensation among patients?
with cirrhosis? The answer, in part, reflects the futility of managing cirrhosis in earlier eras—with no treatments available for either the primary disease or the consequent fibrosis, it hardly seemed critical to refine the classification and prognosis of cirrhosis. Even now, liver transplantation is only indicated for those patients who have already developed decompensated disease or who have developed potentially curable HCC that is not treatable by liver resection. Therefore, more sensitive tests to stratify the risk of decompensation among apparently stable patients who have cirrhosis are still needed.

Among the candidate modalities for predictive tests, HVPG, although an invasive test requiring technical expertise, is remarkably accurate in predicting decompensation. For example, a long-term study of patients enrolled in a trial of β-blockers to prevent variceal hemorrhage demonstrated that patients with a baseline HVPG of <10 mmHg had only an ~15% chance of decompensation during a follow-up of 8 years, whereas those patients with a baseline HVPG >10 mmHg had an ~70% risk of decompensation over the same interval. Remarkably, simple pathologic features seen on liver biopsy may provide surrogate evidence for these divergent outcomes—smaller nodule size and thicker septae both indicate a higher risk of decompensation, presumably because they reflect a higher HVPG. Interestingly, these same features are also characteristic of more advanced experimental cirrhosis, and when fibrosis regresses in these experimental models the smaller nodules begin to expand and thickened septae become thinner. Although none are available yet, a noninvasive or minimally invasive means of assessing HVPG could greatly enable the estimation of decompensation risk, and might even be useful in determining the response to antifibrotic agents.

As underscored in a review published in early 2010, the increasingly refined view of cirrhosis as being more than one stage demands a reassessment of staging systems to create better ways of predicting prognosis and anticipating complications. With new therapies on the horizon, newer models of risk stratification will enable more careful selection of patients and better prediction of outcomes.

**A proof-of-concept antifibrotic clinical trial**

We are still lacking a proof-of-principle trial that establishes the value of attacking fibrosis directly despite ongoing liver disease, and that is our fourth challenge. With all the tremendous progress that has been made in unearthing the mechanisms of hepatic fibrosis, it is reasonable to ask why there are no approved drugs for the treatment of fibrosis in patients with chronic liver disease. The first point is that animal models, although vital for creating a path to clinical development, have limitations. Fibrosis that develops in weeks in rodents does not completely mimic the slow evolution of fibrosis in humans, particularly with respect to the amount of ECM crosslinking and loss of cellularity that occurs over longer intervals.

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**Figure 4** Implications of assessing the genetic risk of fibrosis progression for designing an antifibrotic drug trial. Studies determining the genetic risk of fibrosis progression in patients with chronic liver disease provide new opportunities to refine disease-specific and generalized antifibrotic therapies. This diagram illustrates the potential implications of genetic risk information in designing a theoretical antifibrotic drug trial. **a** Unequal stratification; the potential outcome of randomization if genetic data are not considered. In this example, more patients at high risk of fibrosis progression are inadvertently randomized to the placebo arm, whereas the treatment arm contains more low risk patients. In this situation, if an antifibrotic drug was ineffective, the placebo group would progress more rapidly owing to the underlying genetic risk of disease, leading to the false interpretation that the drug was effective. **b** Equal stratification. In this situation unequal stratification is rectified by instead stratifying the genetic risk of disease equally between the treatment and placebo groups. **c** Genetic progression risk data are further exploited by first identifying only high-risk patients, who are then randomized to both placebo and treatment groups. By restricting the enrollment to only patients with high risk of fibrosis progression in this example, the number of patients and duration of therapy required to achieve a statistical difference if an antifibrotic drug is effective should be reduced.
in humans.146 Thus, animal studies have variable relevance, based on the model used, administration schedule and dosing, and the therapeutic target or targets (Box 1). The second point is that clinical trials of antifibrotic drugs are still reliant on liver biopsy as an endpoint, which obligates the trials to be conducted over lengthy intervals, increasing their cost and limiting the enthusiasm of sponsors.140 Moreover, there is growing skepticism about the reliability of liver biopsy in this setting. Thus, the third and most important point is that there is an urgent need for better noninvasive biomarkers that indicate early, robust responses to antifibrotic therapies. The field of hepatology has been conditioned by trials of antiviral agents, in which unequivocal responses to therapy are demonstrable by serum tests showing the absence of viral replication. In the fibrosis sphere, however, it is the tissue’s response to injury, rather than detection of a virus, that is being assessed. As such, responses are may be more ambiguous and require longer intervals to be established.

Despite this obstacle, the number of potential antifibrotic therapies is expanding rapidly, and their potential has been embraced by the pharmaceutical and biotech sectors, and also by the public. Dozens of antifibrotic approaches, too numerous to discuss in this article, have emerged and been reviewed.10,151–153 Moreover, exciting prospects for targeting diagnostic agents or therapies directly to HSCs may enhance efforts to improve both the diagnosis and treatment of hepatic fibrosis.53,154–157

Conclusions
In summary, the field of hepatic fibrosis continues to expand rapidly, and represents a vibrant paradigm of translational research. The persistent challenges that limit the development of antifibrotic therapies are rapidly being offset by tremendous energy, creativity and persistence in both the laboratory and clinical settings, and are certain to yield accelerating, tangible progress for patients with chronic liver diseases.

Box 1 | Determinants of relevance of animal models to human hepatic fibrosis

<table>
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<th>The model or models used</th>
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<td>Typically, the efficacy of an antifibrotic drug should be demonstrated in at least two mechanistically distinct animal models, e.g. one provoking parenchymal fibrosis (e.g. carbon tetrachloride or thioacetamide) and another associated with biliary fibrosis (e.g. bile duct obstruction, Mdr2–/– animals,147 or ANIT administration148).</td>
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<th>Administration schedule and dosing</th>
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<td>The drug should be efficacious in a ‘treatment’ model in which fibrosis is already present before the agent inducing fibrosis is started. The drug should not interfere with the toxicity or mechanism of injury of the model itself, but rather block fibrosis directly.</td>
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The dose should achieve a drug level within a range that is comparable to the safe and effective concentrations used in humans.

Target of action
The cellular or molecular target of the therapy, and its role in fibrosis, should be similar between the animal model and human disease. For example, immune pathways between animals and humans may differ substantially, and thus immune targets that are valid in animals may not have an established role in human disease. Conversely, TGF-β1, e.g. has a well-established role as a fibrogenic mediator in both humans and animal models, and thus is an appealing target to test in animal models. Similarly, the models used must mimic the key features of the human disease. For example, induction of αvβ6 integrin has been documented in biliary models,149 and thus its relevance may only extend to human biliary disease but not parenchymal diseases (e.g. viral hepatitis or NASH).

Review criteria
Articles were selected following a comprehensive review of English-language, full-text papers listed in PubMed, based on the impact and novelty of the work, relevance to the topic and overall quality. Great emphasis was placed on selecting high quality research and review articles published primarily within the past 3 years to ensure that the review was up to date. In addition to the PubMed search, the reference lists of high-impact papers and review articles were scrutinized to ensure that no significant papers were overlooked.


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