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# Liver fibrosis – from bench to bedside

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## 1. Introduction

Liver fibrosis has evolved in the past 20 years from a pure laboratory discipline to an area of great bedside relevance to practicing hepatologists. This evolution reflects growing awareness not only of the molecular underpinnings of fibrosis, but also of its natural history and methods of detection in chronic liver disease. These advances have culminated in clear evidence that cirrhosis can be reversible, and in realistic expectations that effective antifibrotic therapy will significantly alter the management and prognosis of patients with liver disease.

In view of this remarkable progress, clinicians must now view liver fibrosis in a new light as a clinical problem in its own right amenable to specific diagnostic tests and therapies that are independent of the etiology. In that spirit, this review will integrate current knowledge about the nature and prognosis of fibrosis in different forms of chronic liver disease with recent advances in elucidating its pathophysiology. These advances form the basis for rational treatment of hepatic fibrosis, which are also summarized. This article emphasizes the most recent developments, since many current reviews have focused on broader advances in pathophysiology ( [1] and articles therein, [2,3]).

## 2. General aspects of fibrosis and cirrhosis

Cirrhosis can be defined as the end stage consequence of fibrosis of the hepatic parenchyma resulting in nodule formation and altered hepatic function. A notable omission from this contemporary definition is that cirrhosis is irreversible, since ample evidence now demonstrates that reversal of cirrhosis is often possible (see below). 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 induced, cholestatic and metabolic diseases. The clinical manifestations of cirrhosis vary widely, from no symptoms at all, to liver failure, and are determined by both the nature and severity of the underlying liver disease as well as the extent of hepatic fibrosis. Up to 40% of patients with cirrhosis are asymptomatic and may remain so for more than a decade, but progressive deterioration is inevitable once complications develop including ascites, variceal hemorrhage or encephalopathy. In such patients there is a 50% 5-year mortality, with approximately 70% of these deaths directly attributable to liver disease [4]. In asymptomatic individuals, cirrhosis may be first suggested during routine examination or diagnosed at autopsy, although biopsy is still required to establish the diagnosis antemortem (see below). The overall prevalence of cirrhosis in the United States is estimated at 360 per 100,000 population, or 900,000 total patients, the large majority of whom have chronic viral hepatitis or alcoholic liver disease.

Cirrhosis affects hundreds of millions of patients worldwide. In the US, it is the most common non-neoplastic cause of death among hepatobiliary and digestive diseases, accounting for approximately 30,000 deaths per year. In addition 10,000 deaths occur due to liver cancer, the majority of which arise in cirrhotic livers, with the mortality rate steadily rising [5,6].

The molecular composition of the scar tissue in cirrhosis is similar regardless of etiology and consists of the extracellular matrix constituents, collagen types I and III (i.e. 'fibrillar' collagens), sulfated proteoglycans, and glycoproteins [7]. These scar constituents accumulate from a net increase in their deposition in liver and not simply collapse of existing stroma. Although the cirrhotic bands surrounding nodules are the most easily seen form of scarring, it is actually the early deposition of matrix molecules in the subendothelial space of Disse – so-called 'capillarization' of the sinusoid – that more directly correlates with diminished liver function (see below).

## 3. Diagnosis of hepatic fibrosis and cirrhosis

There is an urgent need for non-invasive markers of hepatic fibrosis for several reasons:

1. While millions of patients worldwide are infected with

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hepatitis C virus (HCV) worldwide, only a minority (~25%) are likely to develop significant fibrosis or cirrhosis, with prevalence predicted to peak between the years 2010 and 2015. No standard laboratory serum analyses, imaging tests or virologic assays currently can distinguish those who are at risk for progressive fibrosis. Thus, increasing numbers of patients will require assessment of fibrosis, exposing them to the potential risks, inconvenience and cost of liver biopsy and its interpretation. If a non-invasive assay were developed that reliably excludes the possibility of significant fibrosis, then such patients may not require treatment with antiviral therapies, and, moreover, could be followed regularly to confirm lack of fibrosis progression.

2. Increasing evidence that advanced fibrosis may be reversible, such that more frequent and refined analysis may render even severe disease amenable to therapy.
3. The expectation that as antifibrotic therapies are developed, there will be a need for early and regular monitoring of response in order to establish effectiveness and optimize dosing. The need for such frequent monitoring will greatly exceed what is appropriate for percutaneous or transjugular liver biopsy.

### 3.1. Current methods for assessing fibrosis

Liver biopsy analyzed with connective tissue stains has long been considered the 'gold standard' for assessing liver histology, disease activity and liver fibrosis. Biopsy is associated with potential morbidity and mortality, and has several limitations. First is the possibility of sampling error, which although possible, typically is not greater than one fibrosis stage in diffuse liver diseases. Sampling error is also possible when small biopsy samples are analyzed which may contain only one or two portal triads, or in which a nodule of hepatocytes is recovered but not the surrounding matrix. There is also inter-observer variability amongst hepatopathologists of up to ~20% in categorizing the degree of fibrosis. Moreover, a liver biopsy only provides static data, not dynamic findings reflecting the ongoing balance between matrix production and degradation, and does not sufficiently reveal underlying pathogenetic mechanisms.

Current clinical trials in which fibrosis is assessed in liver biopsies typically use either Metavir or Ishak (Knodell) scoring systems. The Metavir score is comprised of five progressive stages: F0, normal; F1, portal fibrosis; F2, few fibrotic septae; F3, numerous septae; and F4, cirrhosis [8]. The Metavir scoring system is well validated and reproducible, and the use of only four stages leads to greater concordance among pathologists than the Ishak system.

The Ishak (Knodell) score is made up of six stages that includes assessment of both fibrosis and activity (i.e. inflammation) [9]. The larger number of stages offers greater discrimination, but also may create greater discordance. It too has been validated, but is not completely linear in its original usage.

Computer morphometry can be used for more quantitative analysis of biopsies analyzed with connective tissue stains, especially picrosirius red. While quite reproducible, morphometry does not assess pathogenesis of fibrosis dynamically, and is prone to the same sampling error as any scoring system using biopsy material.

### 3.2. Serum markers of liver fibrosis

There is a compelling need for non-invasive methods of liver fibrosis given the limitations of currently available methods of fibrosis assessment. 'Serum markers' broadly refers to the measurement of one or more molecules within a blood or serum sample as a surrogate marker of fibrosis in the liver. Possible applications of non-invasive markers will include both the initial assessment and monitoring of antiviral or antifibrotic therapy, and may additionally provide new information about the natural history of fibrosis progression and regression.

There are several features required of an ideal serum marker. It should be liver-specific, independent of metabolic alterations, easy to perform, and minimally influenced by impaired urinary and biliary excretion. Serum markers should reflect fibrosis in all types of chronic liver disease, should correlate with matrix content, and must be sensitive enough to discriminate between different stages of fibrosis from chronic hepatitis to cirrhosis. They must also reflect the response to successful antifibrotic therapy.

### 3.3. Current serum markers of hepatic fibrosis

Current serum assays of single matrix molecules or their fragments are not liver-specific, may reflect impaired hepatic clearance, and often underestimate 'quiescent' cirrhosis because they correlate best with inflammation. Many assays fail to detect liver disease until late stages because fibrosis is asymptomatic for decades. They cannot discriminate between different fibrosis stages. These assays are not typically based directly on cellular pathogenesis of hepatic fibrosis, but rather on the synthetic or inflammatory state of liver. Furthermore, certain physiological states, like growth or pulmonary fibrosis are associated with increased levels of fibrosis markers.

No single marker fulfills all of the criteria sufficiently to merit routine clinical use yet. Single markers often correlate with fibrosis in large groups of patients, but do not sufficiently assess the amount of fibrosis in a single individual, especially in longitudinal use over time. However, recent efforts to assay several markers from the same serum sample promise a greater likelihood of success in discriminating minimal from severe fibrosis. Current assays are directed at measuring breakdown products of extracellular matrix (ECM) constituents and the enzymes that regulate their production or modification, including: (a) Glycoproteins including antibodies to hyaluronic acid, laminin or undulin

(type IV collagen); (b) propeptides from ECM molecules that are generated by cleavage from ECM molecules as they are incorporated into scar, for example the propeptides of types I, III and IV collagens; (c) enzymes involved in ECM synthesis including lysyl oxidase, prolyl hydroxylase and lysyl hydroxylase.

A multicenter effort is underway in Europe in cooperation with a commercial diagnostic company, to develop a panel of fibrotic markers which includes PIIINP, PICP, collagen VI, tenascin (a non-collagenous glycoprotein and presumed marker of fibrogenesis), undulin, collagen XIV, laminin P1, hyaluronic acid, TIMP-1 (tissue inhibitor of metalloproteinase-1), MMP-2, and collagen IV. Results from the initial phase of this study suggest that the combined assay may reliably distinguish between early and late stages of fibrosis but not between more intermediate stages [10]. Results are eagerly awaited from the second, longitudinal phase.

### *3.4. Other non-invasive markers*

A combined clinical and laboratory index has been reported by Poynard et al. which has a good correlation with fibrosis stage in patients with chronic HCV [11]. This index includes α2 macroglobulin, haptoglobin, gamma glutamyl transpeptidase (GGT), γ globulin, total bilirubin and apolipoprotein A. The formula used to generate this index has not been specified, however, and independent validation is necessary.

### *3.5. Potential future markers of hepatic fibrosis*

There are a number of potential imaging modalities that may prove useful in hepatic fibrosis assessment, including positron emission tomography (PET) scanning, receptor imaging and quantitation, and possibly magnetic resonance (MR). While none are yet nearing clinical evaluation, rapid growth in imaging technology may provide new avenues for non-invasive imaging in liver disease.

## 4. Liver diseases associated with hepatic fibrosis – natural history and risk factors

Fibrosis leading to cirrhosis can accompany virtually any chronic liver disease that is characterized by the presence of hepatobiliary distortion or inflammation. The vast majority worldwide have chronic viral hepatitis, or steatohepatitis associated with either alcohol or obesity, but other etiologies include parasitic disease (e.g. schistosomiasis), autoimmune attack on hepatocytes or biliary epithelium, neonatal liver disease, metabolic disorders including Wilson's, hemochromatosis and a variety of storage diseases, chronic inflammatory conditions (e.g. sarcoidosis), drug toxicity (e.g. methotrexate or hypervitaminosis A), and vascular derangements, either congenital or acquired. Of these, our understanding of natural history of fibrosis is most complete in HCV, with some information about hepatitis B virus (HBV) and steatohepatitic diseases including alcoholic liver disease and NASH. Information about fibrosis progression in other diseases is largely anecdotal, but the development of cirrhosis typically requires many years to decades, with two notable exceptions: (1) neonatal liver disease – infants with biliary atresia may present at birth with severe fibrosis and marked parenchymal distortion; (2) a subset of patients who undergo liver transplantation for cirrhosis due to HCV [12] or HBV [13] who then develop rapidly progressive cholestasis and recurrent cirrhosis within months, requiring re-transplantation. There is no clear explanation for these instances of 'fulminant fibrosis', but they underscore the possibility that fibrosis is not always a slowly progressive event. Mechanisms underlying this rapid development of cirrhosis are unknown, but could yield critical clues to understanding how to control fibrosis.

### *4.1. HCV*

Risk and natural history of fibrosis associated with HCV have been greatly clarified as a result of several large clinical studies incorporating standardized assessments of fibrosis that combine detailed historical and clinical information [8,14]. The disease can run a remarkably variable course, from decades of viremia with little fibrosis, to rapid onset of cirrhosis in 10–15 years. Remarkably, it is host, not viral factors that correlate with fibrosis progression in HCV based on the following evidence: (1) there is no relationship between viral load or genotype and fibrosis even though these factors greatly impact response to antiviral therapy; (2) human promoter polymorphisisms (e.g. TGFβ1 and angiotensin) may correlate with fibrosis risk [15], with large-scale efforts currently underway to validate these findings and to identify additional genetic markers of fibrosis risk; (3) host immune phenotype may be critical since there is more rapid progression in immunosuppressed patients, whether due to HIV or immunosuppressive drugs [16]. In mice, a Th2 phenotype strongly correlates with fibrogenic potential [17], which has led to efforts to use quantitative trait loci (QTL) mapping to identify specific fibrosis risk genes in these animals.

Identified risk factors for more rapid progression of HCV include: (a) older age at time of infection; (b) concurrent liver disease due to HBV or alcohol (>50 g/day); (c) male gender; (d) increased body mass index, associated with hepatic steatosis; (e) HIV infection or immunosuppression following liver transplantation (see above); (f) iron overload [18].

Because standard clinical indices cannot distinguish between minimal and even advanced fibrosis, knowledge about these risk factors and duration of infection can greatly inform clinical management. Thus, for chronic HCV, if the time of infection is known and a biopsy obtained at any time thereafter, the rate of progression per year based on Metavir scoring can be estimated [8], as illustrated below:

Fibrosis progression per year (according to Poynard) :

$$= \frac{\text{Fibrosis state in METAVIR units (0–4)}}{\text{Duration of infection (years)}}$$

For example, a patient has a METAVIR stage F2 biopsy who contracted HCV 8 years earlier following a blood transfusion: = 2 F/8 years = 0.250 units per year of fibrosis progression

Time to cirrhosis (i.e. stage F4) = 4/Fibrosis progression per year = 16 years

In this example above, the duration of infection is known precisely. Often the time of infection can be estimated (for example, in a middle-aged person with a brief history of drug use in their 20's), but if not, the same information can be determined if two biopsies are obtained several years apart, since this too will provide an estimate of progression rate over time.

The ability to assess the rate of progression of fibrosis can be extremely valuable to both physician and patient for at least three reasons: (a) the actual stage of fibrosis will indicate the likelihood of response to α-interferon or α-interferon/ribavirin, since advanced stages of fibrosis (F3 or F4) generally have a lower response rate to antiviral therapy; (b) if little fibrosis progression has occurred over a long interval, then treatment with antiviral therapy may be deemed as less urgent, and it may be safe to await more effective and/or better tolerated therapy; (c) the approximate time to the development of cirrhosis can be estimated. This would not, however, indicate if/when clinical liver failure would eventuate, which, as noted above, may be delayed for up to a decade or more after the establishment of cirrhosis.

It is important to note that risk estimates by Poynard are not universally accepted, as some have suggested that the risk of fibrosis may be lower in unselected populations. Moreover, it appears increasingly likely that fibrosis progression may not be entirely linear, with more advanced stages associated with accelerating, non-linear progression [19].

Once cirrhosis and its complications develop, the prognosis is predicted by widely used systems including Childs–Pugh and model for end-stage liver disease (MELD) [20], which are predictive independent of the etiology of liver disease.

### 4.2. HBV

Remarkably, few studies have assessed the progression rate of fibrosis in chronic HBV infection. In general, inflammatory activity, as influenced by viral factors including e Ag status, correlate with fibrosis [21,22]. Fibrosis progression has been correlated with HBV genotype in at least one study [23]. In a subset of patients, a rapidly progressive 'fibrosing cholestatic hepatitis' may occur [13,24], but there are neither definitive risk factors for this condition, nor are there unique etiologic, cellular or molecular determinants identified. What is clear, however, is that virologic improvement in response to a variety of antiviral regimens can effect remarkable improvement not only in serum assays and histologic inflammation, but also fibrosis as well [25–27].

### 4.3. Alcoholic liver disease

The clearest clinical determinant of fibrosis is continued alcohol abuse; patients with fibrosis who continue to drink are virtually assured of progression. In addition, two clinical features commonly seen in steatohepatitis, elevated body mass index and serum glucose, also confer increased risk of fibrosis in alcoholic liver disease [28]. Pathologically, the presence of pericentral fibrosis (central hyaline sclerosis), carries a high risk of eventual panlobular cirrhosis, which is almost certain if alcohol intake continues [29].

### 4.4. NASH

We do not yet have sufficient prospective information about natural history, risk factors for fibrosis, and rate of fibrosis progression in NASH. Patients with only steatosis and no inflammation appear to have a benign course when followed for up to 19 years [30], however, it is unclear if this lesion is completely distinct from steatohepatitis or it simply represents a precursor of NASH. In patients with sustained NASH, spontaneous histologic improvement is very uncommon. In three combined studies of 26 patients followed with sequential biopsies for up to 9 years, 27% had progression of fibrosis and 19% advanced to cirrhosis, while none had reversal of fibrosis [30]. Of interest is the recurrence of NASH following liver transplantation in some patients with cryptogenic cirrhosis, implicating an underlying metabolic defect that may account for liver disease in both the native and transplanted organs.

Risk of fibrosis and rate of progression are critical issues that will influence risk stratification and patient selection for clinical trials, since progression to cirrhosis is the most important clinical consequence of NASH. Recently developed systems to grade and stage liver disease in NASH should allow for improved, prospective collection of standardized data that can further address these vital questions.

In general, increasing obesity (body mass index >28 kg/$m^2$) correlates with severity of fibrosis and risk of cirrhosis. Other risk factors reported in three independent studies include necro-inflammatory activity with ALT > 2X normal and/or AST/ALT >1, age, elevated triglycerides, insulin resistance and/or diabetes mellitus, and systemic hypertension [31–33]. It is uncertain whether these features are comparable across the spectrum of disorders associated with NASH, including obesity with insulin-resistance, jejuno-ileal (JI) bypass, total parenteral nutrition (TPN) and rapid weight loss, among others. Whether these features represent surrogates for other risk factors (i.e. reduced antioxidant levels in older patients, increased renin-angiotensin activity in hypertensives) is unknown. Ratziu et al. have reported a clinicobiological score that combines age, BMI,

triglycerides and ALT reportedly has 100% negative predictive value for excluding significant fibrosis [32].

## 5. Fibrosis and even cirrhosis are reversible

There is now unequivocal and mounting evidence that cirrhosis can be reversible. Based originally on anecdotal evidence, this conclusion is now additionally drawn from studies involving large numbers of patients. The feature common to all cases of cirrhosis improvement is the elimination of the underlying cause of liver disease, whether due to eradication of HBV [26] or HCV [34], decompression of biliary obstruction in chronic pancreatitis [35], or immunosuppressive treatment of autoimmune liver disease [36]. Moreover, there is ample evidence of reversibility in animal models, which provide vital clues to underlying mechanisms [37,38].

Earlier studies demonstrated that fibrosis improves upon treatment of HCV [39]. A more recent study has now established that even cirrhosis can regress following HCV eradication with α-interferon/ribavirin [34]. Among a large cohort of patients successfully treated with this combination, there were 150 patients with cirrhosis, half of whom had a reduction in their fibrosis score according to Metavir staging, with several patients regressing by two or more stages [40]. Moreover, since fibrosis in HCV typically progresses over three decades, one might anticipate that an equally slow but steady regression might follow its clearance. On the other hand, more rapid regression has also been observed [26].

In this study of HCV, it is not known what distinguishes those patients whose cirrhosis reversed from those whose did not, or in fact, whether those with no reduction in fibrosis at initial follow-up might still gain a benefit over time. Nonetheless, potential factors influencing reversibility might include: (1) the duration of cirrhosis, which could reflect a longer period of cross-linking of collagen, rendering it less sensitive to degradation by enzymes over time; (2) total content of collagen and other scar molecules, which might lead to a large mass of scar that is physically inaccessible to enzymes; (3) reduced expression or enzymes that degrade matrix or sustained elevation of proteins that inhibit their function (see Section 6.11). Regardless, clinicians must now approach patients with chronic liver disease and cirrhosis with the mindset that treatments to reverse fibrosis either by attacking the primary liver disease or reducing scar accumulation are justified even when the disease is advanced.

## 6. Pathophysiology of hepatic fibrosis and cirrhosis – recent advances

The steady advances in basic research exploring mechanisms of hepatic fibrosis have fueled tremendous progress in elucidating its pathophysiology. Central among these has been the identification of hepatic stellate cells (HSC) and their myofibroblastic counterparts as key sources of an array of mediators, matrix molecules, proteases and their inhibitors that orchestrate the wound healing response in liver. In particular, methods to isolate and characterize stellate cells have provided tools to explore the pathogenesis of hepatic fibrosis in culture, animal models, and human disease.

The key features of hepatic stellate cell behavior, and the dominant cytokines in liver injury and fibrosis have been enumerated in detail in recent reviews [1,41,42]. Therefore, emphasized within the context of stellate cell activation in the following section are three areas of recent progress and importance: the role of inflammatory cells; the contribution of steatosis to hepatic fibrogenesis; the regulation of matrix degradation, and its tight linkage to stellate cell apoptosis in the resolution of fibrosis.

### *6.1. Matrix composition of normal and fibrotic liver*

The normal liver contains an epithelial component (hepatocytes), an endothelial lining, tissue macrophages (Kupffer cells) and the perivascular stellate cell (previously called Ito cell, lipocyte, perisinusoidal cell, or fat-storing cell) (see Fig. 1); stellate cells are the key fibrogenic cell (see Section 6.2). Within the sinusoid, the subendothelial space of Disse separates hepatocytes from the sinusoidal endothelium and contains a low-density, or 'basement membrane-like matrix'. This subendothelial matrix contains a defined lattice-like meshwork of ECM molecules that provide cellular support while allowing unimpeded transport of solutes and growth factors (Fig. 2A). This low-density ECM also provides signals that maintain the differentiated function of surrounding cells. During liver injury the ECM composition becomes scar-like, and hepatocellular function deteriorates, explaining why progressive liver fibrosis is manifested clinically as a decrease in serum albumin, impaired detoxification of drugs, and impaired clotting factor production (Fig. 2B). It is anticipated that if antifibrotic therapy can reconstitute the normal microenvironment of liver, normal function can be restored and clinical manifestations may regress.

'Activation' of hepatic stellate cells is the dominant event in fibrogenesis, and proceeds along a continuum that involves progressive changes in cellular function, such that at any moment following injury, there are subpopulations of stellate cells with discrete cytoskeletal and phenotypic profiles. Moreover, there is also heterogeneity of stellate cell populations even in normal liver based on expression of a number of intracellular markers, several of which are ordinarily found in neural cells, including glial fibrillary acidic protein, synaptophysin and neurotrophin receptors [43–45].

'Activation' refers to the conversion of quiescent cells vitamin A-storing cells into proliferative, fibrogenic, and contractile 'myofibroblasts'. Stellate cell activation is a complex but tightly programmed response (Fig. 3). The organization of stellate cell activation into a defined

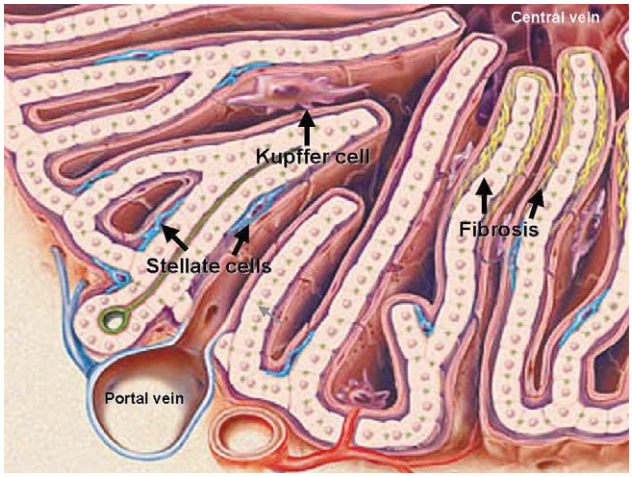


**Fig. 1. Sinusoidal architecture and the localization of hepatic stellate cells. In normal liver, cords of hepatocytes are surrounded by a fenestrated endothelial lining. In the intervening space of Disse are the hepatic stellate cells (blue). Kupffer cells (purple) are typically intrasinusoidal and are shown here as adherent to the endothelial wall. Activation of stellate cells can lead to accumulation of extracellular matrix (yellow bands on right hand side of figure). (Reprinted from [114], with permission).**

sequence provides a helpful framework for exploring specific pathways. Early events have been termed initiation (also referred to as the 'preinflammatory' stage). Initiation encompasses rapid changes in gene expression and phenotype that render the cells responsive to cytokines and other local stimuli. 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. Cellular responses following initiation have been termed perpetuation, which encompasses those cellular events that amplify the activated phenotype through enhanced growth factor expression and responsiveness; this component of activation results from autocrine and paracrine stimulation, as well as from accelerated ECM remodeling. As noted above, it is increasingly appreciated that perpetuation is a continuously dynamic process, as illustrated by sequential changes in TGFβ signaling as stellate cells progressively activate in culture, for example [46,47]. Finally, resolution of stellate cell activation represents an essential step towards reversibility of fibrosis.

### *6.2. The role of inflammation in hepatic stellate cell activation and fibrosis*

The earliest changes in stellate cells reflect paracrine stimulation by all neighboring cell types, including sinusoidal endothelium, Kupffer cells, hepatocytes, platelets, and leukocytes. Endothelial cells are also likely to participate in activation, both by production of cellular fibronectin and via conversion of TGFβ from the latent to active, profibrogenic form.

Kupffer cell infiltration and activation also play a prominent role. Influx of Kupffer cells coincides with the appearance of stellate cell activation markers. Kupffer cells can stimulate matrix synthesis, cell proliferation and release of retinoids by stellate cells through the actions of cytokines and reactive oxygen intermediates/lipid peroxides. For example, TGFβ from Kupffer cells markedly stimulates stellate cell extracellular matrix synthesis [48,49].

Another means by which Kupffer cells can influence stellate cells is through secretion of matrix metalloproteinase 9 (MMP-9; gelatinase B) [50]. MMP-9 can activate latent TGFβ, which in turn can stimulate stellate cell collagen synthesis . Lastly, Kupffer cells generate reactive oxygen species (ROS) in the liver. ROS, whether produced intern-

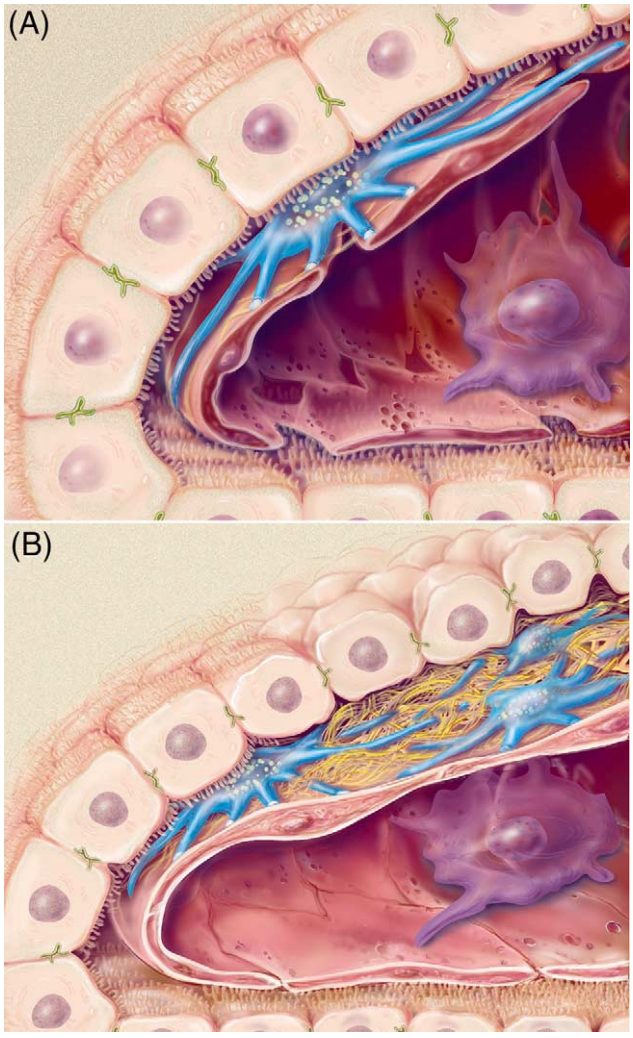


**Fig. 2. Subendothelial changes during stellate cell activation accompanying liver injury. (A) Normal sinusoidal architecture with a stellate cell (in blue) containing perinuclear vitamin A droplets and elaborating foot processes that encircle the sinusoid. Only a low density matrix is present in this state, which is not depicted in this diagram. (B) During liver injury, stellate cells multiply and are surrounded by accumulating fibrillar matrix. These events contribute to loss of hepatocyte microvilli and closure of endothelial fenestrae. (Reprinted from [114], with permission).**

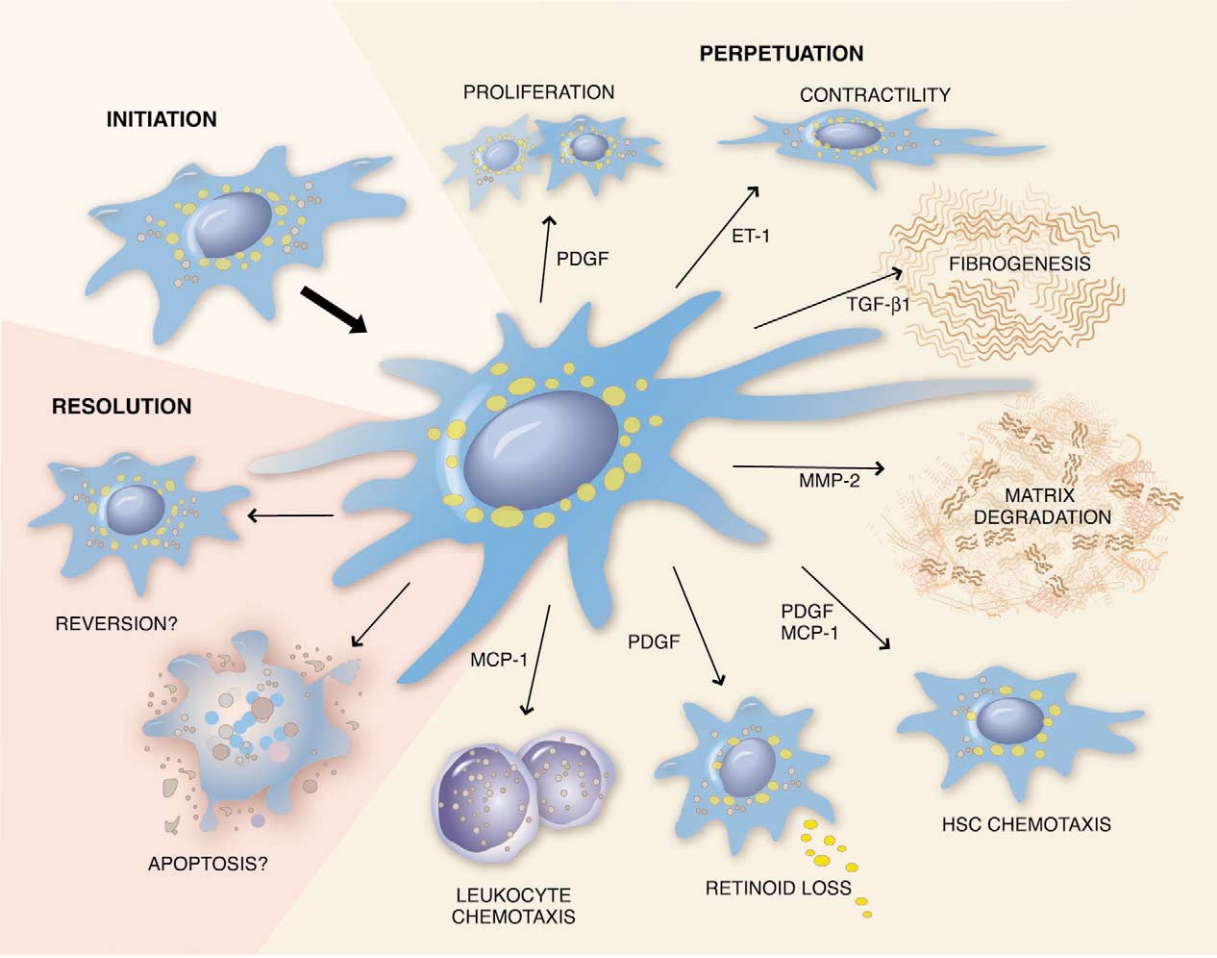


**Fig. 3. Pathways of stellate cell activation and resolution during liver injury and its resolution. Stellate cells activate in response to liver injury in a two stage process beginning with initiation, which renders the cells responsive to a host of cytokines and stimuli. This perpetuation stage is comprised of a series of events shown here that ultimately enhance degradation of normal matrix and accumulation of fibrillar, or scar matrix. As noted in the text, the process of activation is actually a continuum rather than a discrete two-step process. During resolution of liver fibrosis stellate cell numbers are diminished by apoptosis, and possibly by reversion to a more quiescent phenotype. (Reprinted from [114], with permission).**

ally within stellate cells [51] or released into the extracellular environment [52] are capable of enhancing stellate cell activation and collagen synthesis. Kupffer cells also produce nitric oxide (NO), which can counterbalance the stimulatory effects of ROS by reducing stellate cell proliferation and contractility.

Leukocytes recruited to the liver during injury join with Kupffer cells in producing compounds that modulate stellate cell behavior. Neutrophils are an important source of ROS, which may have a direct stimulatory effect on stellate cell collagen synthesis via superoxide [53]. Activated neutrophils also produce NO, which may counteract the effect of superoxide on collagen expression but does not abrogate it [53].

Lymphocytes, including CD4 T helper (Th) cells reside in the liver and may secrete cytokines. Remarkably, their role has been largely overlooked in fibrogenesis associated with chronic liver disease. Th lymphocytes help orchestrate the host-response via cytokine production and can differentiate into Th1 and Th2 subsets, a classification that is based on the pattern of cytokines produced. In general, Th1 cells produce cytokines that promote cell-mediated immunity including IFNγ, tumor necrosis factor, and IL-2. Th2 cells produce IL-4, IL-5, IL-6, and IL-13 and promote humoral immunity. Th1 cytokines inhibit the development of Th2 cells and vice versa. Thus, the host-response to infection or injury frequently polarizes to either a Th1 or Th2 response, but not both.

Several experimental models implicate Th-derived cytokines in directing the immune response and fibrosis. Generally, Th2 lymphocytes favor fibrogenesis in liver injury over Th1 lymphocytes. The response to $CCl_4$ has been examined in mice with several different lymphocyte profiles, including T-cell depletion (severe combined immunodeficiency, SCID) Th1 predominance (C57/BL6), and Th2 predominance (BALB/c) [17]. SCID mice from both C57/BL6 and BALB/c backgrounds develop liver fibrosis after treatment with $CCl_4$ for 4 weeks. The degree of fibrosis is modified significantly, however, in immunocompetent mice from both strains. Immunocompetent C57/BL6 mice, whose

lymphocyte cytokine profile includes IFN-γ, exhibit less fibrosis than SCID mice from the same background. Indeed, when C57/BL6 mice with targeted disruption of IFN-γ are treated with CCl4, fibrosis returns to the level seen in C57/BL6 SCID mice [17]. However, immunocompetent BALB/c mice, whose lymphocyte cytokine profile includes the fibrogenic compounds IL-4 and TGFβ, exhibit more fibrosis than BALB/c SCID mice.

At least one pathway directly linking immune effector cells with stellate cell activation is the CD40 ligand-receptor system [54]. Activated stellate cells express CD40 both in culture and in injured liver. By engaging its receptor, CD40 ligand from immune cells may stimulate NF-kB and jun terminal kinase (Jnk), leading to enhanced chemokine secretion [54].

### *6.3. The contribution of steatosis to hepatic fibrosis*

Steatosis is increasingly recognized as a determinant of hepatic fibrosis, both in alcoholic liver disease and in NASH. At least four pathways may contribute to steatosis-related fibrogenesis: (1) Cyp2E1/Cyp 4A-mediated oxidant stress; (2) inflammation with release of fibrogenic cytokines and mediators; (3) PPAR signaling and activity; (4) dysregulation of leptin expression and signaling. Each of these is described in more detail below.

#### *6.3.1. Oxidant stress due to Cyp 2E1 and Cyp4A*

Oxidant stress is a direct fibrogenic stimulus. In alcoholic liver disease, saturation of alcohol dehydrogenase pathways leads to induction of cytochrome 450 s that also can metabolize alcohol, in particular cytochrome P450 2E1 (Cyp2E1). Oxidation of alcohol by Cyp2E1 or Cyp4A generates reactive oxygen species (ROS). The molecular mechanisms underlying CypP450 induction in NASH are not well understood.

#### *6.3.2. Inflammation*

As noted above, leukocyte infiltration is a key feature of fibrosing steatotic liver diseases including alcoholic liver disease, HCV and NASH. In most studies, fibrosis risk and progression correlate well with the extent of inflammation assessed either pathologically or as elevation of transaminases. Inflammation-mediated fibrosis is largely attributable to fibrogenic cytokines released by infiltrating lymphocytes and neutrophils, as well as paracrine and autocrine stimulation of hepatic stellate cells. These include an array of factors including TGFβ, chemokines, interleukins and ligands for receptor tyrosine kinases such as vascular endothelial growth factor (VEGF) and platelet-derived growth factor (PDGF). Direct induction of the fibrogenic cytokine connective tissue growth factor (CTGF) in cultured stellate cells by hyperglycemia and insulin provides one direct potential link between metabolic features of NASH and fibrogenesis [55] While no inflammatory mediators are thought to be unique to steatotic liver diseases, particular attention has been paid to tumor necrosis factor alpha (TNFα) because of its role in mediating hepatocyte injury initiated by endotoxin in experimental models. Thus far, however, no direct fibrogenic role of TNFα has been documented, but paracrine activation of stellate cells by TNFα-activated Kupffer cells remains a strong possibility.

#### *6.3.3. Peroxisome proliferator activated receptor signaling and dysregulation*

There is an indirect link between peroxisome proliferator activated receptor (PPAR) biology and fibrosis, based on at least three lines of evidence. First, Cyp4A is induced by PPARα, providing a source of oxidant stress. Second, loss of PPARα leads to abnormal fatty acid oxidation and steatohepatitis in experimental models, implicating PPARα as a determinant of this lesion (but not necessarily fibrosis) [56]. Third, stellate cell activation is associated with downregulation of PPARγ signaling, and ligands of PPARγ but not PPARα downregulate stellate cell activation[57,58]. Because NASH is typically associated with insulin resistance, PPARγ ligands (e.g. thiazolidinediones such as rosiglitazone or thioglitazone) currently used for treating insulin-resistant diabetes may have the additional benefit of downregulating stellate cell activation, and therefore represent an additional rationale for testing these agents in NASH [59,60].

#### *6.3.4. Leptin activity and signaling*

Leptin, the product of the obese gene locus in mouse, is a 16 kD protein secreted primarily by adipose, although non-adipose sources, in particular stellate cells, are increasingly recognized [61]. Leptin levels are directly proportional to adipose mass, and thus obese patients have very high circulating levels of leptin. A direct fibrogenic effect of leptin on wound healing has been documented, since ob mice (which lack leptin) are protected from experimental liver fibrosis due to either carbon tetrachloride or thioacetamide, whereas fibrosis can be induced if leptin is restored [62–64].

Leptin may play a direct role in steatosis-related hepatic fibrosis as suggested by the following [65]. First, stellate cells produce leptin, and their synthesis increases with cellular activation, providing an important local source of this hormone [66]. Second, leptin is profibrogenic towards stellate cells, mediated both by an indirect upregulation of TGFβ1 in sinusoidal endothelial cells [62,63] and by a direct effect on stellate cells. Anania et al. have identified long-form leptin receptor (OB-$R_L$) on culture activated stellate cells, with recruitment and phosphorylation of Stat3 upon ligand binding [67]. Activation of primary stellate cells in culture leads to increased expression of OB-$R_L$, similar to the upregulation of many growth factor receptors during cellular activation.

### 6.4. Perpetuation of stellate cell activation

The pathways of perpetuation include proliferation, fibrogenesis, contractility, release of proinflammatory cytokines, chemotaxis, retinoid loss and matrix degradation.

### 6.5. Proliferation

Increased stellate cell numbers during liver injury [68] reflect activity of many mitogenic factors and their cognate tyrosine kinase receptors [69]. In particular, PDGF is the most potent proliferative stimulus towards stellate cells. Both PDGF [70] and its receptor [71] are upregulated following liver injury. Recently, discoidin domain receptor-2 has been identified as an upregulated receptor tyrosine kinase that has the unusual property of responding to fibrillar collagen as its ligand [72,73]. Once bound to collagen, a cascade of events is initiated that includes recruitment of src kinase and downstream signals [74], culminating in transcriptional induction of matrix metalloproteinase-2. A growing list of other stellate cell mitogens has been identified, including endothelin-1 (ET-1), thrombin, FGF, VEGF and insulin-like growth factor [41,69].

### 6.6. Fibrogenesis

Matrix production by activated stellate cells is markedly increased through the action of TGFβ1 [68,75]. Stellate cells are the most important source of TGFβ1 in liver fibrosis [76,77], but Kupffer cells and platelets also secrete this cytokine. Of the three types of TGFβ, most is known about the β1 isoform. It is secreted with the non-covalently linked latency-associated peptide (LAP). Activation of the complex can be achieved by several compounds, some with proteolytic activity such as metalloproteinases (MMP) and tissue plasminogen activator (tPA)[2].

There are three main types of TGFβ receptors. Both TβRI and RII are present in quiescent HSCs and activated HSCs with a myofibroblast-like phenotype [47]. Activation of HSCs is associated with increased responsiveness to TGFβ and in turn enhanced ECM synthesis [78]. Major advances in understanding intracellular signaling by SMADs, the intracellular effectors of TGFβ receptors, have allowed investigators to explore their role in stellate cells as well [46,47,79,80].

Connective tissue growth factor (CTGF) is a cytokine that promotes fibrogenesis in skin, lung and kidney [81–84]. It is strongly expressed by stellate cells during hepatic fibrosis [85]. Regulation of its expression in stellate cells is not defined, although it is a downstream target of TGFβ in other cellular systems [86,87].

### 6.7. Contractility

There is a marked increase in the contractility of stellate cells during activation [88] leading to increased portal resistance. Activated stellate cells impede portal blood flow by both constricting individual sinusoids and by contracting the cirrhotic liver [89]. Endothelin-1 (ET-1) is the key contractile stimulus towards stellate cells [89]. Stellate cells (as well as Kupffer and endothelial cells) also produce nitric oxide (NO), the physiological antagonist to ET-1 [90]. The net contractile activity of stellate cells in vivo reflects the relative strength of these opposing factors, with the imbalance shifted in favor of endothelin as liver disease progresses [89], because in addition to increased endothelin-1, there is decreased nitric oxide [91–93]. As with other features of stellate cell activation, increasingly complex levels of regulation are being revealed including activation of ET-1 by a converting enzyme that is regulated in stellate cells [94], providing potential new therapeutic targets.

### 6.8. Cytokine release

Autocrine cytokines play vital roles in regulating stellate cell activation. These cytokines include TGFβ1, PDGF, FGF, HGF, platelet activating factor and ET-1 [69,95] this list continues to expand as new cytokines are continually characterized. Stellate cells also release neutrophil and monocyte chemoattractants that can amplify inflammation in liver injury, including colony stimulating factor, monocyte chemotactic protein-1 [96,97] and cytokine-induced neutrophil chemoattractant (CINC)/ IL-8 [98].

Anti-inflammatory cytokines produced by stellate cells have also been identified, in particular IL-10. Upregulation of IL-10 occurs in early stellate cell activation [99,100]. Interleukin-10 knockout mice (i.e. animals genetically engineered to lack this protein) have more severe hepatic fibrosis following $CCl_4$ administration [101,102].

### 6.9. Loss of vitamin A (retinoids)

Activation of stellate cells is accompanied by loss of their characteristic retinoid (vitamin A) droplets. This process in culture is serum dependent and results in release of retinol in the extracellular space [103]. Potential effects may derive from the generation of novel metabolites of retinoic acid, but their role has not been established in vivo [104]. It is not known, however, whether retinoid loss is a requirement for stellate cell activation, and if preventing retinoid loss might alter the activation cascade. This remains an area of intense interest, in part because of growing knowledge in other tissues about the biology of retinoids and their receptors.

### 6.10. Chemotaxis

In addition to local proliferation, stellate cells accumulate in regions of injury by chemotaxis, or directed migration. Several chemoattractants have been implicated, including PDGF, IGF-I, ET and monocyte chemotactic protein 1 (MCP-1). Thus, in addition to mediating a mitogenic role, PDGF and IGF-I are also chemotactic for HSCs, with PDGF being the more potent [105,106]. MCP-1 is a member of the CC class of chemokine family, which promote leukocyte

recruitment [107]. MCP-1 production by HSCs recruits monocytes, lymphocytes and activated, but not quiescent HSCs. The effect is mediated by the PI 3-K pathways and requires $Ca^{2+}$influx. Whereas in leukocytes the CCR2 receptor is critical for MCP-1 responses, this receptor is not required in stellate cells [108]. Platelet-derived growth factor is also a chemoattractant towards activated but not quiescent stellate cells [109].

### *6.11. Matrix degradation*

Successful efforts to reverse fibrosis and cirrhosis must include the degradation of excess ECM in order for normal liver architecture to be restored. There are broadly two kinds of matrix degradation in liver, one that disrupts the low density matrix of normal liver ('pathologic matrix degradation') and may therefore worsen liver disease, the other, the degradation of excess scar that may help restore the architecture of the injured liver to normal ('restorative matrix degradation').

An expanding family of matrix-metalloproteinases (also known as matrixins) contribute to either pathologic or restorative matrix degradation. Matrix-metalloproteinases are calcium-dependent enzymes that specifically degrade collagens and non-collagenous substrates[110]. These enzymes fall into five categories based on substrate specificity: interstitial collagenases (MMP-1, -8, -13), gelatinases (MMP-2,-9), stromelysins (MMP-3, -7, -10, 11), membrane type (MMP-14, -15, -16, -17, -24, -25) and a metalloelastase (MMP-12). Metalloproteinases are regulated at many levels in order to restrict their activity to discrete regions within the pericellular milieu. Inactive metalloproteinases can be either activated through proteolytic cleavage, or inhibited by binding to specific inhibitors known as TIMPs ('tissue inhibitors of metalloproteinases'). These protein complexes typically combine in a carefully defined ratio. For example, MT1-MMP and TIMP-2 form a ternary complex with MMP-2 that is essential for optimal MMP-2 activity. Thus, net collagenase activity reflects the relative amounts of activated metalloproteinases and their inhibitors, especially TIMPs.

As noted, in liver 'pathologic' matrix degradation refers to the early disruption of the normal subendothelial matrix which occurs through the actions of at least four enzymes: matrix metalloproteinase 2 (MMP2) (also called 'gelatinase A' or '72 kDA type IV collagenase') and MMP-9 ('gelatinase B' or '92 kDa type IV collagenase'), which degrade type IV collagen, membrane-type metalloproteinase-1 or -2, which activates latent MMP2, and stromelysin-1, which degrades proteoglycans and glycoproteins, and also activates latent collagenases. Disruption of the normal liver matrix is also a requirement for tumor invasion and desmoplasia.

Failure to degrade the accumulated scar matrix is a major reason why fibrosis will progress to cirrhosis. Matrix metalloproteinase-1 (MMP-1) is presumed to be the main protease which can degrade type I collagen, the principal collagen in fibrotic liver, although it is not clear which cell(s) in liver produce this important enzyme. Alternatively, it is possible that other enzymes such as MT1-MMP and MMP-2 may also have interstitial collagenase activity – this issue is yet to be resolved. More importantly, progressive fibrosis is associated with marked increases in TIMP-1 and TIMP-2, leading to a net decrease in protease activity, and therefore more unopposed matrix accumulation. Stellate cells are the major source of these inhibitors [37,38,111] (see Section 7). Sustained TIMP-1 expression is emerging as a key reason why fibrosis progresses. Transcriptional regulation of the tissue inhibitor of metalloproteinase-1 (TIMP-1) promoter has been explored in depth by Mann et al. Its activation, which would result in increased TIMP-1 expression, inhibition of metalloproteinases, and persistent fibrosis, is dependent on JunD [112], as well as the binding of a 30 kDa protein to a specific promoter element termed UTE-1 [113].

## 7. Resolution of liver fibrosis and the fate of activated stellate cells

During recovery from acute human and experimental liver injury the number of activated stellate cells decreases as tissue integrity is restored. At least two possibilities could account for this observation, reversion of stellate cell activation, or selective clearance of activated stellate cells by apoptosis [114].

### *7.1. Reversion*

It is unknown whether an activated stellate cell can revert to a quiescent state in vivo, although it has been observed in culture. When stellate cells are grown on a basement membrane substratum (Matrigel) they remain quiescent, and plating of highly activated cells on this substratum downregulates stellate cell activation [73,115].

### *7.2. Apoptosis*

Apoptosis of HSCs probably accounts for the decrease of activated stellate cells during resolution of hepatic fibrosis[116,117]. Apoptosis might be the default mode of activated HSCs in normal liver. Thus, following injury, apoptosis might be inhibited by soluble factors and matrix components that are present during injury [37]. Indeed, spontaneous apoptosis occurs in cultured HSCs [118]. Furthermore, transdifferentiated HSCs express cell death surface receptors, Fas and its ligand, and apoptosis can be induced with Fas-activating antibodies [119]. Another death receptor, nerve growth factor receptor (NGFR), is also expressed by activated HSCs, and its stimulation with ligand drives apoptosis [120].

Survival factors also regulate the net activity of stellate cell apoptosis. IGF-I and TNF-$\alpha$ promote HSC survival via PI 3-K/c-Akt pathway and NF-kB pathway, respectively.

Molecules regulating matrix degradation appear closely linked to survival and apoptosis. The emerging story suggests that active MMP2 correlates closely with apoptosis, and in fact may be stimulated by apoptosis [121]. Inhibition of MMP2 activity by TIMP-1 blocks apoptosis in response to a number of apoptotic stimuli, which is explicitly dependent on inhibiting the protease activity of MMP2 [122].

Interactions between HSCs and the surrounding matrix also influence their propensity towards apoptosis, and this might partly explain the intriguing anti-apoptotic activity of TIMP-1. Moreover, the fibrotic matrix may provide important survival signals to activated stellate cells [123]. For example, animals expressing a mutant collagen I resistant to degradation have more sustained fibrosis and less stellate cell apoptosis following liver injury. Most recently, proof-of-principle has been generated emphasizing the importance of apoptosis during reversal of fibrosis by using gliotoxin [124], a fungal toxin that induces apoptosis in HSCs, possibly by inhibition of NF-kB. In the $CCl_4$ model of hepatic fibrosis in rats, gliotoxin decreased the number of HSCs by inducing apoptosis[124]. These data point to acceleration of stellate cell apoptosis as a potential target of antifibrotic therapy.

## 8. A new framework for developing antifibrotic therapies

### *8.1. General considerations*

The improved understanding of mechanisms underlying hepatic fibrosis makes effective antifibrotic therapy an imminent reality. Treatment will remain a challenging task, however, and thus far no drugs are approved as antifibrotic agents in humans. Therapies will need to be well tolerated over decades, with good targeting to liver and few adverse effects on other tissues. The liver offers a unique advantage as a target for orally administrated agents, since those with efficient hepatic first, pass extraction will have inherent liver 'targeting' by minimizing systemic distribution and non-liver adverse effects. Combination therapies may prove synergistic rather than additive, but agents must first be tested individually to establish safety and 'proof-of-principle'. It is uncertain whether antifibrotic therapies will require intermittent or continuous administration.

Testing of antifibrotic agents in clinical trials presents unique challenges, since efficacy cannot be simply assessed by a serum test such as viral load, and, moreover, a clinical benefit may only be apparent after a prolonged period of treatment. In contrast, for example, trials of antiviral medications for HCV, can obtain evidence of efficacy within weeks or months by a simple blood test assessing viral load. Additionally, there are no established serum markers that can substitute for obtaining tissue, obligating investigators to perform percutaneous liver biopsies at the onset and completion of therapy, which limits attractiveness to patients and providers. The costs of conducting long-term trials is considerable, which tends to discourage pharmaceutical and biotech companies from incurring the risk of such efforts.

Despite these obstacles, the future of antifibrotic therapy is extremely promising. This optimistic view results from the growing recognition that cirrhosis may be reversible, and if effective, such therapy will prevent the morbid complications of end-stage liver disease requiring transplantation. The potential market for such agents is huge, and the potential requirement for chronic therapy is particularly attractive for commercial companies seeking to recover their research and development costs.

The following section is intended only as a general framework for current directions in developing antifibrotic therapy, and not a comprehensive list. For more detailed information, the reader is referred to several excellent, up-to-date reviews [125–127]

### *8.2. Anti-fibrotic therapies – rationale and potentia agents*

The pathway of stellate cell activation provides an important framework to define sites of antifibrotic therapy. These include: (a) cure the primary disease to prevent injury; (b) reduce inflammation or the host response in order to avoid stimulating stellate cell activation; (c) directly downregulate stellate cell activation; (d) neutralize proliferative, fibrogenic, contractile and/or pro-inflammatory responses of stellate cells; (e) stimulate apoptosis of stellate cells; and (f) increase the degradation of scar matrix, either by stimulating cells which produce matrix proteases, down-regulating their inhibitors, or by direct administration of matrix proteases.

#### *8.2.1. Cure the primary disease*

As noted above, the most effective way to eliminate hepatic fibrosis is to clear the primary cause of liver disease, be it from viral, metabolic, drug induced or autoimmune causes. In essence, therefore, effective treatment for these conditions is 'antifibrotic', by either preventing the accumulation of scar or hastening its clearance as the injury resolves.

#### *8.2.2. Reduce inflammation and immune response*

A number of agents have anti-inflammatory activity in vitro and in vivo which may eliminate the stimuli to stellate cell activation. Corticosteroids have been used for decades to treat several types of liver disease. Their activity is solely as anti-inflammatory agents, with no direct antifibrotic effect on stellate cells. Antagonists to TNFα may have some rationale in inflammatory liver disease, and have shown acceptable safety profiles in rheumatoid arthritis and Crohn's disease. Ursodeoxycholic acid has a beneficial effect on fibrosis in primary biliary cirrhosis [128] possibly in part due to its anti-inflammatory activity.

#### *8.2.3. Inhibit stellate cell activation*

Reducing the transformation of quiescent stellate cells to

activated myofibroblasts is a particularly attractive target given its central role in the fibrotic response. The most practical approach is to reduce oxidant stress, which is an important stimulus to activation. Anti-oxidants, including alpha-tocopherol, (vitamin E) suppress fibrogenesis in studies of experimental or human fibrogenesis [129,130]. Other anti-oxidants also can reduce stellate cell activation in culture and provide a rationale for anti-oxidant trials in humans.

The cytokines γ interferon [131] and hepatocyte growth factor (HGF) [132,133] have inhibitory effects on stellate cell activation in animal models of fibrosis, and in fact a major controlled trial of γ interferon is underway for advanced hepatic fibrosis in patients with HCV, which is based on ongoing trials for pulmonary fibrosis [134]. The exact mechanism of HGF's antifibrotic activity is uncertain, but may include inhibition of TGFβ1 activity.

Peroxisome proliferator activated nuclear receptors (PPAR), including PPARγ, are expressed in stellate cells, and synthetic PPARγ ligands (thiazolidinediones) downregulate stellate cell activation [59,135,136]. Ongoing trials are assessing their efficacy in NASH, with the hope that a therapeutic benefit will include reduced fibrosis (see Section 18).

Herbal therapies and products derived from natural compounds that are commonly used in the Far East are increasingly being tested under controlled, scientifically rigorous conditions [137–139].

*8.2.4. Neutralize proliferative, fibrogenic, contractile and/or pro-inflammatory responses of stellate cells*

Significant advances in growth factor biology will benefit the treatment of hepatic fibrosis through the development of antagonists to cytokines and their receptors. In particular, many proliferative cytokines including PDGF, FGF and TGFα signal through tyrosine kinase receptors, inhibitors of which are already undergoing clinical trials in other diseases. Because the intracellular signaling pathways for these receptors are well understood, inhibitors to signaling models are being explored in vivo or in cultured stellate cells.

The recent success in developing a safe, effective small molecule tyrosine kinase antagonist in human leukemia and mesenchymal cell tumors [140] lends hope that this approach will work in other indications, including liver fibrosis. Small molecule compounds are under development to block cytokine receptor or intracellular signaling in liver fibrosis.

Inhibition of matrix production has been the primary target of most antifibrotic therapies to date. This has been attempted directly by blocking matrix synthesis and processing, or indirectly by inhibiting the activity of TGFβ1, the major fibrogenic cytokine. Stabilization of the collagen I mRNA is an important mode of upregulating this gene in hepatic fibrosis, which can be inhibited by using molecular decoys to inhibit mRNA levels in cultured cells [141]. TGFβ antagonists are being extensively tested because neutralizing this potent cytokine would have the dual effect of inhibiting matrix production and accelerating its degradation. Animal and culture studies using soluble TGFβ receptors or other means of neutralizing the cytokine including monoclonal antibodies and protease inhibitors to block TGFβ activation, have established proof-of-principle [142–144].

Because endothelin-1 is an important regulator of wound contraction and blood flow regulation mediated by stellate cells, antagonists have been tested as both antifibrotic and portal hypotensive agents, either by reducing endothelin or augmenting nitric oxide, its physiologic counterregulator [145,146].

Halofuginone, an anticoccidial compound has antifibrotic activity by blocking collagen expression, and has been used in a number of models of tissue fibrosis, including liver [147,148].

*8.2.5. Stimulate stellate cell apoptosis*

Attention is increasingly focused on how liver fibrosis regresses, and in particular the fate of activated stellate cells as fibrosis recedes. As noted above, a recent study using gliotoxin to induced apoptosis of stellate cells reduced fibrosis in rats with liver injury due to $CCl_4$, establishing proof-of-principle for this approach [124], (see Section 7).

*8.2.6. Increase the degradation of scar matrix*

This component of treatment is very important, because antifibrotic therapy in human liver disease will need to provoke resorption of existing matrix in addition to preventing deposition of new scar. TGFβ antagonists have the advantage of stimulating matrix degradation by downregulating TIMPs and increasing net activity of interstitial collagenase. Direct administration of metalloproteinase mRNA via gene therapy in animal models of hepatic fibrosis has begun to confirm that, in principle, matrix can be resorbed [149]; based on these data human trials of gene therapy are being planned.

## 9. Future directions

The diagnosis and treatment of fibrotic liver disease will be vastly different within the next decade as a result of the sustained progress in our understanding of its detection and pathophysiology. This can be most vividly understood by imagining a theoretical patient with HCV related fibrosis in the year 2012.

*9.1. Theoretical case history in the year 2012*

A 50 years old man with HCV not cleared by antiviral therapy (among only 5% of patients) is referred to the hepatologist.

The physician will use his palm-sized digital assistant to calculate a fibrosis risk score based on a combined index incorporating: (a) 'fibrogenic risk' genotype based on multiple single nucleotide polymorphisms; (b) length of infection; (c) alcohol intake; (d) a combined clinical and laboratory score; (e) body mass index . Next, a non-invasive assessment of fibrosis will be obtained using a novel

imaging modality that quantifies fibrogenesis, combined with a multiplex serum assay.

If all indices indicate high risk of fibrosis, a customized multi-drug antifibrotic regimen based on the patient's host genotype will be initiated. The physician will evaluate the response every 3 months using the non-invasive fibrosis assessment methods, and continue treatment intermittently 2–3 months each year, indefinitely. The patient will live to his natural life expectancy free of end-stage liver disease or its complications.

While this example may seem optimistic, such enthusiasm is clearly warranted give how far the field has advanced in 20 years. The future of liver disease management has never been brighter.

## Declaration

The authors who have taken part in this study have not a relationship with the manufacturers of the drugs involved either in the past or present and did not received funding from the manufacturers to carry out their research.

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