REVIEW

"Let my liver rather heat with wine" - a review of hepatic fibrosis pathophysiology and emerging therapeutics

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Abstract: Cirrhosis is characterized by extensive hepatic fibrosis, and it is the 14th leading cause of death worldwide. Numerous contributing conditions have been implicated in its development, including infectious etiologies, medication overdose or adverse effects, ingestible toxins, autoimmunity, hemochromatosis, Wilson's disease and primary biliary cholangitis to list a few. It is associated with portal hypertension and its stigmata (varices, ascites, hepatic encephalopathy, combined coagulopathy and thrombophilia), and it is a major risk factor for hepatocellular carcinoma. Currently, orthotopic liver transplantation has been the only curative modality to treat cirrhosis, and the scarcity of donors results in many people waiting years for a transplant. Identification of novel targets for pharmacologic therapy through elucidation of key mechanistic components to induce fibrosis reversal is the subject of intense research. Development of robust models of hepatic fibrosis to faithfully characterize the interplay between activated hepatic stellate cells (the principal fibrogenic contributor to fibrosis initiation and perpetuation), hepatocytes and extracellular matrix components has the potential to identify critical components and mechanisms that can be exploited for targeted treatment. In this review, we will highlight key cellular pathways involved in the pathophysiology of fibrosis from extracellular ligands, effectors and receptors, to nuclear receptors, epigenetic mechanisms, energy homeostasis and cytokines. Further, molecular pathways of hepatic stellate cell deactivation are discussed, including apoptosis, senescence and reversal or transdifferentiation to an inactivated state resembling quiescence. Lastly, clinical evidence of fibrosis reversal induced by biologics and small molecules is summarized, current compounds under clinical trials are described and efforts for treatment of hepatic fibrosis with mesenchymal stem cells are highlighted. An enhanced understanding of the rich tapestry of cellular processes identified in the initiation, perpetuation and resolution of hepatic fibrosis, driven principally through phenotypic switching of hepatic stellate cells, should lead to a breakthrough in potential therapeutic modalities.

Keywords: fibrosis, cirrhosis, hepatic stellate cell, reversal, transdifferentiation, senescence, apoptosis

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Introduction

The role of the liver in human physiology has long fascinated physicians and philosophers. Interestingly, its regenerative properties were alluded to in the Greek myth of Prometheus and the Roman myth of Tityus, in which birds of prey feasted on the livers of the respective protagonists, only to have their livers regenerate overnight. In the 5th century BCE, Hippocrates noted associations between jaundice and behavioral anomalies in his "Prognostics and Prorrhetics of

Hippocrates."[1] The Roman physician Galen saw the liver as the predominant organ from which all other organs originated, conjecturing that it was "the principal instrument of sanguification," the site from which blood was derived.[2] Indeed, given the contemporaneous theory of four bodily humors, the liver, spleen and gallbladder were thought to be the sites of production and storage of three of the four humors. However, the liver was not uniformly ascertained to play such a principal role by all philosophers and physicians; Aristotle ascribed a central role to the heart,[3] a view shared by the Islamic medical scholar Avicenna, who nonetheless noted that the liver was "the seat of the nutritive or vegetative faculties" and the "seat of manufacture of the dense part of the humors."[4]

In 1685, an English surgeon named John Browne described a "liver appearing glandulous," which is thought to be one of the first surgical descriptions of cirrhosis.[5] The term cirrhosis was initially used by Rene Laennec in 1819 in his seminal work on auscultation after his invention of the stethoscope.[6] The term was not mentioned in the medical literature again until William Osler used it as the eponym Laennec's cirrhosis in his essential medical textbook "Principles and Practice of Medicine," first published in 1901.[7] The prolific and renowned British hepatologist Sheila Sherlock published the seminal "Diseases of the Liver and Biliary System" in 1955,[8] along with over 600 articles on hepatic pathology, with momentous contributions to the field of hepatology, including the first serologic test for primary biliary cholangitis.[9]

Cirrhosis epidemiology, diagnosis and treatment

Estimates of the epidemiology of the various etiologies of cirrhosis vary significantly. Overall, the prevalence and incidence of cirrhosis is likely underestimated given its asymptomatic initial stages. Alcoholic liver disease is thought to account for almost half of liver-related deaths worldwide.[10] Currently, alcohol use disorder affects nearly 1 in 6 Americans, an increase of ~50% between 2001 and 2012.[11] Of the viral etiologies, there are approximately 2.2 million US adults with HBV,[12,13] and more than 2.5 million US adults with HCV, a significant proportion of whom are unaware of their infection.[12–15] The US prevalence of non-alcoholic fatty liver disease (NAFLD) has a broad range of between 29 and 113 million; of these, between 3.2 and 9.7 million have non-alcoholic steatohepatitis (NASH).[16–18] Inherited metabolic disorders as etiologies

of cirrhosis have a lower prevalence with α-1 antitrypsin (α1AT) deficiency present in 20 per 60,000 to 100,000 patients,[19–21] hemochromatosis in 300 per 100,000 patients[22–24] and Wilson's disease occurring in 3 per 100,000 patients.[25] Cholestatic etiologies such as primary sclerosing cholangitis and primary biliary cholangitis are prevalent in up to 40.2 per 100,000 patients.[26] Autoimmune hepatitis is seen in 10 per 50,000 to 100,000 patients.[27] The prevalence and/or incidence of vascular etiologies such as Budd-Chiari have not been determined.

The impact on the health care system is profound, as cirrhosis is the 14th leading cause of death in the world[28] and the 10th leading cause of death in men,[29] resulting in the yearly deaths of over 1 million persons globally, and over 33,000 persons in the US in 2015.[30] Treatment and management of its complications cost ~$9.5 billion dollars in one 2018 study,[31] of which $5.3 billion was due to alcoholic cirrhosis alone. Combined direct and indirect costs for care of cirrhosis and its complications exceed $12 billion in the US yearly.[32] From 2006 to 2011, over 3 million emergency department visits in the US were due to cirrhosis and its complications,[33] while in 2010, about 100,000 US inpatient hospitalizations were due to cirrhosis and chronic liver disease.[34] Between 2002 and 2014, there were 1.24 million inpatient admissions for cirrhosis and its complications in the US, with an aggregate cost of $28 billion, rising to $2.8 billion in 2014 alone.[35] Further, patients affected by cirrhosis tend to be in lower socioeconomic strata, have adverse effects on employment, and are negatively affected by medical expenditures.[36] Additionally, these patients report low health-related quality of life.[37]

The initial suspicion for hepatic pathology is usually borne out of symptoms, signs, or during routine evaluation with a panel of liver function tests. Work-up for acute or chronic liver damage typically includes imaging, serologies to look for viral or autoimmune etiologies, evidence of ingestion, levels of ceruloplasmin and α1AT serologies, among other tests. There are several well-validated combinations of non-invasive markers of cirrhosis in clinical use. While the gold standard for diagnosis remains a liver biopsy, imaging modalities including ultrasound-based transient elastography have emerged as validated alternatives for diagnosis of cirrhosis.[38] Staging of fibrosis, from stage I (early) to IV (previously thought to be irreversible cirrhosis) can be attained from either invasive or imaging-based non-invasive modalities to risk-stratify patients. The well-known model for end-stage liver disease (MELD) score[39] relies on multiple serologic measurements to

produce a score that is used as the principal ranking parameter on transplantation lists. Mortality is quite high when cirrhosis decompensates, as the portal hypertension driven by the diffuse fibrotic tissue in the hepatic parenchyma has multiple downstream repercussions that often involve other tissues such as lung (portopulmonary hypertension, hepatopulmonary syndrome), kidney (hepatorenal syndrome) and heart (cirrhotic cardiomyopathy, portopulmonary hypertension, hepatopulmonary syndrome), not to mention splenomegaly, portal vein and splenic vein thromboses, ascites, hepatic encephalopathy and varices. Sequelae of portal hypertension can be recognized on physical exam by presence of hepatosplenomegaly, spider angiomas, palmar erythema, Dupuytren's contractures, and Terry's nails.

Treatment of cirrhosis is complicated by its previously assumed irreversibility, which has been challenged by multiple in vivo and in vitro lines of evidence showing reversibility in various settings. Typically, cessation of the offending agent, if ingestion is the etiology, is of paramount importance. If of a viral etiology, treatment with antiviral therapy can result in cessation of progression, as outlined below. The only curative treatment for cirrhosis remains orthotopic liver transplantation. Mortality is estimated based on Child-Pugh and MELD scores. Child-Pugh provides information about life expectancy and perioperative mortality for abdominal surgeries,[40] while MELD provides estimated 3-month mortality rates based on score ranges.[39] Management of decompensated cirrhosis is largely aimed at prophylaxis and surveillance of variceal bleeding, mitigation of hepatic encephalopathy, therapeutic paracenteses for excessive ascites and prophylaxis of spontaneous bacterial peritonitis. Preventative strategies are limited to screening for esophageal varices by endoscopy and radiologic screening for hepatocellular carcinoma.

Pathophysiology of cirrhosis

A recent drive toward alternative treatment modalities for cirrhosis has focused on the pathophysiologic mechanisms undergirding the development and exacerbation of fibrosis. Much has been gleaned from study of isolated hepatocytes in addition to various in vivo systems of induced cirrhosis in mice and rats, using carbon tetrachloride (CCl_4), bile duct ligation (BDL) and thioacetamide (TAA) treatment. Numerous studies in mice have examined accelerated or attenuated cirrhosis induced by CCl_4 in transgenic mice with various modifications, including dietary components. Additionally, in

vitro models of liver fibrosis, while challenging to develop, have nevertheless been bolstered recently by co-culture of human hepatocyte lines (HepaRG) with hepatic stellate cell-like cells derived from induced pluripotent stem cells (iPSC-HSCs).[41] These "3D cultures" have recently been validated[42] by the composition of cells within the culture, as well as by the response of constituent cells to various factors, including activation of hepatic stellate cells (HSCs) with subsequent fibrogenic activity. Such advancements in development of in vitro models of cirrhosis will yield further insights into the mechanisms of fibrosis reversal.

The liver parenchyma is primarily composed of epithelial hepatocytes, making up 60–80% of the total liver mass and arranged in lobules composed of a portal triad at the periphery and a central vein. Other cell types include Kupffer cells, which are tissue macrophages that reside in the lumen of sinusoids and are members of the reticuloendothelial system; HSCs of mesenchymal lineage that comprise 10–15% of the resident liver cells and which reside in the subendothelial space of Disse; liver sinusoidal endothelial cells with prominent fenestrae that line the sinusoids; bile ductular cells, also known as cholangiocytes, into which bile canaliculi empty; and the hepatic and portal vasculature.[43] As the disease process advances to frank cirrhosis (Figure 1), the liver undergoes widespread fibrogenesis with formation of fibrotic septa surrounding areas of diffuse nodular regeneration. As these changes progress, they ultimately lead to parenchymal extinction with subsequent necroinflammation, as well as distortion and collapse of vascular architecture.[44] There is resultant increased resistance to portal blood flow, resulting in portal hypertension and its stigmata, as well as decreased hepatic synthetic function. This process is principally driven by HSCs, which are activated or transdifferentiated into contractile, migratory, proliferative and fibrogenic myofibroblast-like cells that secrete copious extracellular matrix components into the space of Disse.

The pathophysiology of portal hypertension (recently reviewed by Gracia-Sancho and colleagues)[45] can be mostly attributed to increased hepatic resistance, though splanchnic vasodilation and the formation of portosystemic collaterals including varices also contribute. Increased hepatic resistance to portal blood flow has two primary contributors, namely anatomic aberrations and functional abnormalities. As mentioned earlier, the altered anatomy of hepatic parenchyma accounts for about 70% of the hepatic vascular resistance, with loss
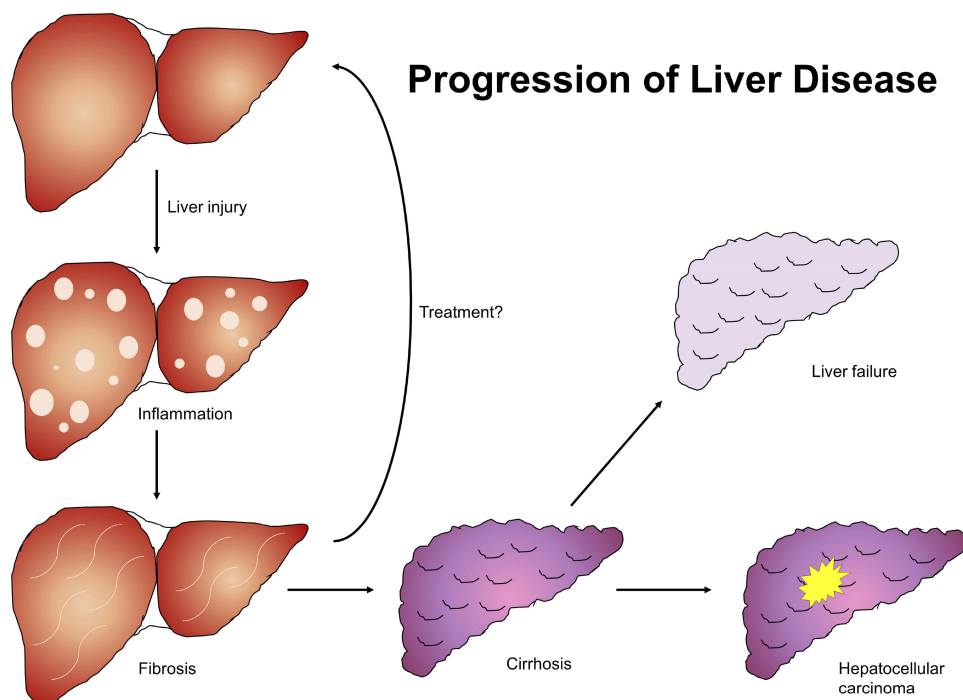
Figure 1 Natural progression of liver disease. Liver insult of any etiology results in inflammatory changes in hepatic parenchyma which progress to fibrosis and ultimately cirrhosis if unaddressed. Cirrhosis culminates in liver failure and is a principal risk factor for hepatocellular carcinoma.

of endothelial fenestration resulting in sinusoidal capillarization, as well as fibrogenesis and angiogenesis, leading to formation of intrahepatic shunts. Functional abnormalities in the form of endothelial dysfunction, increased response to vasoconstrictors and increased hepatic vascular tone account for about 30% of the total portal pressure. Splanchnic vasodilation is primarily an adaptive response to altered intrahepatic hemodynamics and opposes the increased hepatic vascular tone. The resultant increased blood flow into the portal system exacerbates portal pressure. Not infrequently, it can become so pronounced that in advanced cirrhosis it causes a hyperdynamic splanchnic and systemic circulation, contributing to ascites and to hepatorenal syndrome.[46] Systemic vasodilation exacerbated by hyperdynamic circulation also leads to pulmonary ventilation/perfusion mismatch, which in advanced cases can lead to hepatopulmonary syndrome with arterial hypoxemia.[47] In this setting, portopulmonary hypertension due to pulmonary vasoconstriction can also develop; the underlying pathophysiology is thought to be due to pulmonary vasculature endothelial dysfunction.[48] Further, development of varices due to portosystemic collateral formation can lead to hematemesis in the case of esophageal varices, as well as portal

hypertensive gastropathy and bleeding in the setting of dilated gastric mucosal vasculature.[49] Hepatic encephalopathy can ensue via portosystemic collaterals shunting blood from the portal circulation into the systemic circulation.[50] Lastly, intrahepatic shunting adversely impacts hepatocyte perfusion, a significant contributor to hepatic failure.

Evaluation of liver fibrosis and inflammation

The gold standard for evaluation of grade (degree of inflammation, corresponding to active liver injury) and stage (established fibrosis) of liver pathology is a liver biopsy. The most common histologic scoring systems to grade and stage liver pathology include METAVIR,[51] the International Association for Study of the Liver (IASL),[52] and Batts and Ludwig.[53] Other systems, such as Ishak[54] and Knodell,[55] are more complex and are usually used in research settings, including in clinical trials. Liver biopsies can be obtained via percutaneous, transjugular and laparoscopic routes. Given their invasive nature, liver biopsies are not without complications; further, sampling error and interobserver variability can lead to incorrect staging of fibrosis. As such, there are various noninvasive modalities to predict fibrosis, including direct and indirect laboratory markers, and radiologic modalities. Direct laboratory markers

include procollagens I, III and IV, matrix metalloproteinases (MMPs), cytokines and chemokines. The FIBROSpect II assay combines hyaluronic acid, tissue inhibitor of metalloproteinase-1 (TIMP-1) and α2-macroglobulin to predict fibrosis at stages F2-F4.[56] There are various indirect markers of fibrosis, including platelet count, prothrombin time, albumin, total bilirubin, ALT and AST, as well as hyaluronic acid and α2-macroglobulin. Many algorithms exist that use these markers to predict fibrosis with varying degrees of sensitivity and specificity, including AST to platelet ratio index (APRI),[57] FIB-4,[58] FibroIndex,[59] Forns Index,[60] HepaScore,[61] FibroTest and ActiTest,[62] Lastly, radiologic modalities that can estimate fibrosis include liver ultrasound, transient elastography (FibroScan), shear wave elastography (used less often than transient elastography in the US) and magnetic resonance elastography.[38]

Cellular mechanisms of hepatic fibrosis

A common feature to the chronic fibro-proliferative disorders affecting liver, kidney and lung is the presence of the myofibroblast. These are cells of mesenchymal lineage which have contractile, proliferative, secretory and migratory properties. In liver, quiescent HSCs are activated by a variety of intracellular, microenvironment, extracellular and extrahepatic processes, resulting in transdifferentiation into myofibroblast-like cells that exhibit pro-fibrotic transcriptional and secretory properties. These activated HSCs secrete excess extracellular matrix (ECM) components into the space of Disse, resulting in their accumulation as well as loss of endothelial fenestration.

There are other potential sources of myofibroblasts, including bone marrow-derived fibrocytes and mesenchymal stem cells. Resident epithelial cells, including hepatocytes and cholangiocytes, are capable of differentiating into a mesenchymal phenotype by losing epithelial markers and gaining mesenchymal markers. This well-characterized process is known as epithelial-to-mesenchymal transition (EMT)[63] and likely represents only a minor source of myofibroblasts. Similarly, endothelial cells are capable of a phenotypic switch to mesenchymal cells in a process known as endothelial-to-mesenchymal transition (EndMT),[63] although the extent of contribution via this mechanism to the myofibroblast pool is unclear. Mesothelial cells, which make up ~15% of the resident liver cells and which express *Wt1* (Wilms' tumor 1), are another potential source of myofibroblasts through mesothelial-to-mesenchymal transition (MMT).[64] In mice, chlorhexidine gluconate-induced liver fibrosis has

been shown to trigger MMT.[65] Animal studies have suggested that HSC transdifferentiation is the primary source of myofibroblasts involved in fibrogenesis. A cell fate tracing study in rats, in which HSCs were genetically labeled to express fluorescent Cre reporter proteins under the control of the lecithin-retinol acyltransferase (LRAT) promoter, found that 82–96% of the myofibroblasts originated from HSCs in CCl_4, BDL and TAA models of cirrhosis.[66] A murine study found that in CCl_4-induced cirrhosis, HSCs were the predominant source of myofibroblasts, while in cholestatic BDL-induced cirrhosis, portal fibroblasts were the major source of myofibroblasts.[67] The data thus far suggest that HSCs are the predominant source of myofibroblasts; however, these rodent studies have not yet been shown to recapitulate the human condition(s).

There are various mechanisms whereby HSCs become activated, then initiate and perpetuate hepatic fibrosis. A variety of extracellular and intracellular events contribute to HSC activation, encompassing a wide range of cellular processes. Histologically, a prominent feature of quiescent HSCs is the presence of retinoid droplets in the cytoplasm, which are lost during transdifferentiation.[68,69] Many different marker transcripts and proteins specific for HSCs have been identified over the past decade. Together, they have advanced research into histologic detection, cell fate tracing, genetic targeting, imaging and ultimately therapeutic targeting through identification of relevant mechanisms. The paradigm of fibrogenesis and its perpetuation encompass the hallmarks of HSC activity, notwithstanding its initial description ~20 years ago.[70] Initiation refers to an initial phenotypic switch favoring contractility and fibrogenicity, transcription and translation of growth factor receptors, and modulation of growth factor signaling. Perpetuation encompasses processes that amplify the phenotypic switch, including paracrine, autocrine, juxtacrine and matricrine interactions. Lastly, clearance of HSCs includes pathways such as apoptosis, necroinflammation and reversion to a quiescent state.

Extracellular mechanisms of HSC activation

There are numerous events occurring extracellularly that contribute to activation of HSCs (Figure 2). Parenchymal damage to hepatocytes due to processes such as NASH and viral hepatitis can result in the release of various ligands and intracellular proteins, nucleic acids and molecules that are able to elicit a non-infectious "sterile"
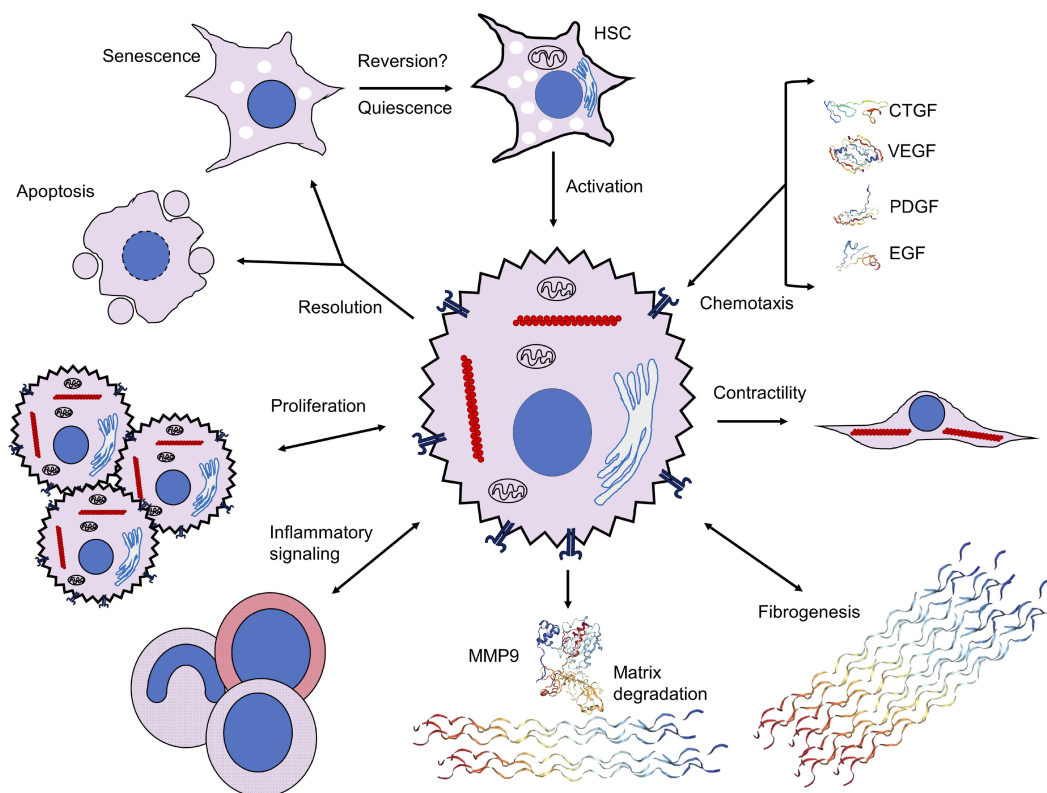
Figure 2 Extracellular signaling mechanisms initiated by and contributing to hepatic stellate cell activation. Activation of HSCs and initiation of fibrogenic, contractile, proliferative and chemotactile processes facilitate perpetuation of fibrogenesis. Chemotaxis through synthesis and secretion of various growth factors, such as CTGF (SWISS-MODEL accession P29279), VEGF (PDB ID 1TZH),[226] PDGF (PDB ID 4QCI)[227] and EGF (PDB ID 2KV4),[228] promote proliferation, secretion of ECM components and maintenance of a profibrotic milieu via juxtacrine, paracrine and autocrine interactions. Upregulation of fibrogenic genes, including αSMA (red chains) to enable contractility and collagen III (PDB ID 6A0A),[229] leads to expansion of ECM and fibrotic septa, and integrin-dependent matricrine interactions facilitate perpetuation of the activated state. Dysregulation of matrix degradation through differential expression of various MMPs, including MMP9 (PDB ID 1L6J),[230] enables accumulation of ECM components and sustenance of a profibrotic microenvironment. Inflammatory signaling recruits various WBCs and macrophages with profibrotic downstream effectors through T_h1 and T_h17 juxtacrine and paracrine signaling. Activated HSCs proliferate via TGF-β-, Ras- and Hedgehog-dependent pathways. Resolution of HSC activation proceeds via apoptosis, senescence or reversal to a quiescent state; it is unclear whether reversal to the deactivated state fully occurs. Crystal structures rendered with NGL Viewer.[231]

Abbreviations: αSMA, alpha-smooth muscle actin; CTGF, connective tissue growth factor; ECM, extracellular matrix; EGF, epidermal growth factor; HSC, hepatic stellate cell; MMP, matrix metalloproteinase; PDB, Protein Data Bank; PDGF, platelet-derived growth factor; TGF-β, transforming growth factor beta; VEGF, vascular endothelial growth factor; WBC, white blood cell.

inflammatory and profibrotic milieu. Damage-associated molecular patterns (DAMPs), such as mitochondrial and nuclear DNA, ATP, heat shock proteins and S100 proteins, bind to pattern-recognition receptors such as Toll-like receptors (TLRs) including TLR9, TLR4 and purine P2X7 receptors.[71] Murine models with constitutively active inflammasome components (NLRP3) exhibited increased rates of hepatocyte caspase-1-dependent pyroptosis and HSC activation,[72] underscoring the role of cellular death in activation of HSCs. IL-33 is a cytokine released by hepatocytes during liver injury, which activates innate lymphoid cells. These cells are known to trigger HSCs and promote liver fibrosis.[73] Phagocytosis of apoptotic bodies by HSCs also promotes HSC activation.[74,75] These hepatocyte-mediated mechanisms of HSC activation highlight the role of released intracellular components and apoptotic bodies in enabling a pro-inflammatory microenvironment.

About 80% of all macrophages in the body reside in the liver.[76] Liver macrophages, including Kupffer cells, monocyte-derived macrophages and resident macrophages, activate HSCs during hepatic injury through production of cytokines and chemokines, including TGFβ, PDGF, TNF, IL-1β, MCP1, CCL3 and CCL5. There are subsets of macrophage populations that are profibrotic and pro-inflammatory (M1 polarization) and associated with T_h1 responses, as well as some that down-modulate inflammation, promote resolution of inflammation and fibrosis (M2 polarization) and are associated with T_h2 responses. Expression of fibrolytic MMPs including MMP12 and MMP13 is a key component of fibrosis resolution.[77,78] A specific population of murine macrophages has been

identified ($CD11b^{hi}/F4/80^{int}/LY6C^{low}$) that is derived from a phenotypic switch of pro-inflammatory and profibrogenic $LY6C^{hi}$ macrophages.[79] This population is restorative, with enhanced MMP expression and upregulation of CX3CR1, a chemokine receptor whose interaction with its ligand CX3CL1 inhibits pro-inflammatory properties in Kupffer cells and macrophages, resulting in decreased hepatic fibrosis in a murine CCl_4 model.[80]

The subendothelial ECM, composed primarily of type IV collagen, heparan sulfate proteoglycan and laminin, appears to be crucial in maintaining the function of all types of resident liver cells. As hepatic fibrosis progresses, both the amount and type of ECM changes; both collagenous and non-collagenous ECM components increase 3- to 5-fold, and type IV collagen is replaced by fibril-forming type I and III collagens.[81,82] These changes result in increased density and stiffness of ECM and serve as mechanical stimuli to further activate HSCs in part via integrin signaling pathways, resulting in a positive feedback loop.[83,84] HSCs express integrins as well as discoid domain-containing tyrosine kinase receptors (DDRs) that bind to triple helical collagen on HSCs and promote further fibrosis.[82,85] ECM that has expanded during liver injury can also function as a reservoir for various growth factors, including platelet-derived growth factor (PDGF), epidermal growth factor (EGF), fibroblast growth factor (FGF), hepatocyte growth factor (HGF) and vascular endothelial growth factor (VEGF). Such growth factors promote expansion of HSCs.[86,87]

Other cell types are also either directly or indirectly involved in HSC activation. Platelets promote fibrosis in various hepatic disorders, including hepatitis B and C, alcoholic liver disease (ALD) and NASH, through their release of platelet-derived growth factor beta (PDGFβ) and transforming growth factor (TGFβ).[88,89] Natural killer (NK) cells target activated HSCs for apoptosis via NK-derived interferon-γ (IFNγ)[90] and death receptor-induced apoptosis.[91] NK cells also promoted resolution of fibrosis by targeting senescent activated HSCs.[92] Liver sinusoidal endothelial cells (LSECs) in their differentiated, fenestrated phenotype were stimulated by VEGF to produce nitric oxide (NO), which prevented HSC activation and also promoted reversal of HSC activation, resulting in quiescence.[93] However, LSECs can act in opposing fashion depending on whether there is acute or chronic liver injury through action of pro-regenerative CXCR7 and profibrotic CXCR4 receptors.[94] Lastly, HSCs appear to influence innate B cell activity via retinoic acid- and

MyD88-dependent B cell recruitment of dendritic cells and monocytes in mice.[95] A study with B cell-deficient mice showed decreased fibrosis induced by CCl_4, suggesting a role for B cells in the perpetuation of hepatic fibrosis.[96]

Metabolic and molecular dysregulation of hepatic stellate cells
Membrane receptors
The HSC intracellular processes regulating initiation and perpetuation of fibrosis are complex and multifactorial, involving various and disparate signaling pathways and cellular biologic mechanisms (Figure 3). Multiple transcription factors are implicated in modulation of HSC activation, including ERAS, GIV, YAP, SOX9, GATA4, Kruppel-like factors, AhR, MRTF-A and NR4A1/2. Various membrane receptor-signaling pathways have been implicated. TGFβ is secreted in a latent form by activated HSCs and promotes hepatic fibrosis via an autocrine positive feedback loop through the action of SMAD2/SMAD3 transcription factors.[97,98] PDGF is a chemoattractant to HSCs and myofibroblasts,[68] and expression of its receptor, PDGFRβ, was upregulated in mice with HSC activation.[99,100] Connective tissue growth factor (CTGF) knockdown in mice resulted in decreased CCl_4-induced fibrosis.[101] EGF receptor antagonism was associated with reduced hepatic fibrosis and HCC in rodent models,[102] while transgenic mice lacking macrophage EGF receptor showed a decrease in HCC.[103] As noted previously, integrins play a key role in driving fibrogenesis through interactions with ECM and the activation of latent TGFβ.[68,86] Of note, deletion of αv integrin in activated HSCs reduced CCl_4-induced fibrosis.[84] The Hedgehog pathway is a well-characterized signal transduction pathway implicated in development, stem cell maintenance and carcinogenesis. Rodent hepatocyte Hedgehog ligand expression induced HSC activation, resulting in fibrogenesis and HCC.[104,105] Inhibition of this pathway by forskolin reduced CCl_4-induced fibrosis in rats,[106] and by vismodegib in both BDL-induced and NASH-induced fibrosis.[107] Cannabinoid receptors CB1 and CB2 are neuromodulatory and immunomodulatory GPCRs; CB1 has pro-fibrogenic function, and CB2 displayed hepatoprotective properties.[108] Agonism of CB2 induced HSC quiescence and/or apoptosis, and also reduced IL-17 production by T_h17 cells, leading to a reduction in hepatic fibrosis.[109]
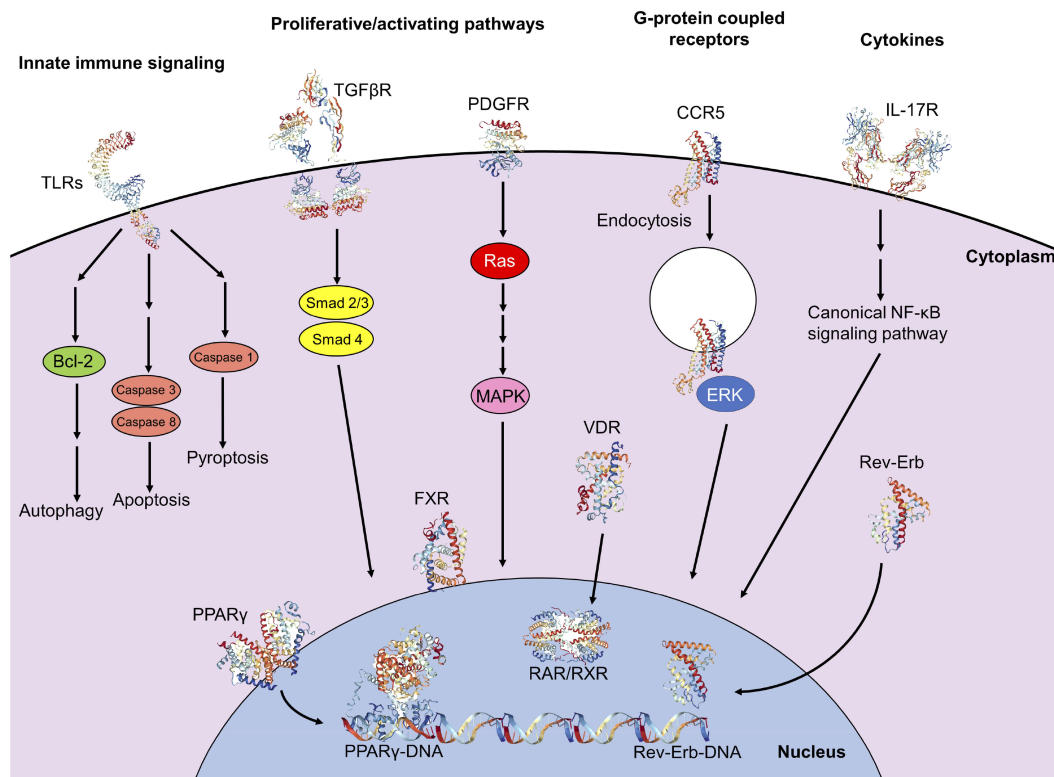
Figure 3 Intracellular signaling pathways involved in initiation and perpetuation of hepatic stellate cell activation. Initiation of the activated state involves engagement of proliferative and activating pathways, including TGFβR (PDB IDs 1KTZ, 3TZM, 5E8U, 5E8V)[232–234] binding and downstream nuclear translocation of Smad2/3 and Smad 4 proteins, resulting in transcription and translation of profibrotic genes. Binding of PDGF to its receptor PDGFR (PDB ID 5GRN)[235] results in activation of cytoplasmic Ras and MAPK effectors, with subsequent nuclear translocation and expression of proliferative genes. Engagement of the GPCR CCR5 (PDB ID 6AKX)[236] results in clathrin-mediated endocytosis, activation and nuclear translocation of ERK1/2, and expression of proliferative genes, also allowing for migratory properties. Cytokines also have significant modulatory properties on fibrogenesis; for example, binding of IL-17 to its receptor IL-17R (PDB ID 4HSA)[237] results in activation of the canonical NF-κB pathway, resulting in pro-inflammatory gene expression. Programmed cell death can be facilitated via innate immune signaling; engagement of TLRs (PDB IDs 6NIH, 2MKA, 2J67)[238–240] with various TLR ligands propagates downstream signaling culminating in autophagy, apoptosis or pyroptosis. Nuclear membrane receptors also have large roles in modulating profibrogenic properties; engagement of ligands, including fatty acids and thiazolidinediones, with receptors of the PPAR family, including PPARγ (PDB ID 2Q8S),[241] results in nuclear translocation, heterodimerization with RXR and binding with DNA (PDB ID 3DZU),[242] with subsequent expression of antifibrogenic genes. Binding of obeticholic acid to FXR and of vitamin D to VDR results in receptor nuclear translocation, heterodimerization with RXR and expression of antifibrogenic genes. Meanwhile, binding of heme to Rev-Erb upregulates Rev-Erb expression, accumulation of Rev-Erb in the cytoplasm and promotion of a profibrogenic, contractile HSC phenotype. Crystal structures rendered with NGL Viewer.[231]
Abbreviations: CB1, cannabinoid receptor type 1; CCR5, C-C chemokine receptor type 5; CX3CR1, C-X3-C motif chemokine receptor 1; DNA, deoxyribonucleic acid; EKR1/2, extracellular signal-regulated protein kinases 1 and 2; FXR, farsenoid X receptor; GPCR, G-protein coupled receptor; HSC, hepatic stellate cell; IL-17, interleukin 17; IL-17R, IL-17 receptor; MAPK, mitogen-activated protein kinase; NF-κB, nuclear factor kappa-light-chain-enhancer of activated B cells; PDGF, platelet-derived growth factor; PDGFR, PDGF receptor; PPARγ, peroxisome proliferator-activated receptor gamma; RXR, retinoid X receptor; TGFβR, transforming growth factor beta receptor; TLR, Toll-like receptor; VDR, vitamin D receptor.

Nuclear receptors

Various nuclear receptors are implicated in the activation of HSCs and perpetuation of fibrosis (Figure 3). Peroxisome proliferating activated receptors (PPARs), which recognize fatty acids and various derivatives, are involved in regulation of lipid homeostasis.[110] In HSCs, PPARγ suppresses PDGFRβ signaling and TGFβ1 by virtue of the β-catenin pathway.[111,112] Various PPAR agonists are currently under investigation in clinical trials (ClinicalTrials.gov identifier NCT02704403, Clinical Trial Registry India CTRI/2015/10/006236).[113,114] Signaling through the farsenoid X receptor (FXR), which is activated by bile acids, increased

sensitivity to insulin and enhances β-oxidation of fatty acids in hepatocytes.[115] A phase II clinical trial of a synthetic FXR agonist (obeticholic acid) demonstrated reduced liver fibrosis in NASH patients without cirrhosis,[116] and this agent is also under investigation in a phase III trial of NASH patients with fibrosis (REGENERATE, ClinicalTrials.gov identifier NCT02548351). Vitamin D receptor (VDR) activation reduced TGFβ and SMAD3 target gene transcription, thereby inhibiting fibrosis.[117] TGFβ1 rearranged VDR binding sites throughout the genome in HSCs at SMAD3 target genes via chromatin remodeling. VDR signaling promoted by p62/SQSTM1 suppressed HSC activation, decreasing fibrogenesis and HCC. The effect was offset by VDR

knockout in a murine model.[117] Retinoid receptors, including retinoic acid receptors (RARs) and retinoid X receptors (RXRs), participate in various metabolic processes. Through interaction with *all-trans* retinoic acid (ATRA), RARs inhibited expression of various genes, including α-smooth muscle actin (αSMA, a marker of HSC activation), TGFβ, IL-6, procollagen I, III and IV, fibronectin and laminin, with a resultant inhibitory effect on HSC activation.[118] RXRs heterodimerize with VDR, thereby transducing cytoplasmic signals to the nucleus in HSCs with profibrogenic, pro-inflammatory and carcinogenic effects.[119] Other nuclear receptors, including Rev-Erb and liver X receptors, also modulated HSC activation.[120,121] Retinoids activated latent TGFβ in rat HSCs, with the predicted profibrotic effects.[122]

Epigenetic dysregulation of hepatic stellate cell activation

Activation of HSCs results in global epigenomic and local epigenetic dysregulation, promoting fibrogenesis.[123] DNA methyltransferase (DNMT) expression appears to play a role in HSC activation, and suppression of DNMTs dampened HSC activation.[124–126] DNA hypermethylation by DNMT1 of phosphatase and tensin homolog (PTEN) protein resulted in HSC activation.[127] Inheritable adaptation might also play a role, where family history of liver damage in rats was associated with epigenetic modifications resulting in increased expression of PPARγ, reduced TGFβ expression and fewer myofibroblasts.[128] Dysregulation of various micro-RNAs (miRNAs) and other non-coding RNAs has been implicated in HSC activation and inactivation, via post-translational modifications. Profiling of miRNAs using high-throughput methods has identified profibrogenic miRNAs that are upregulated in activated HSCs, as well as anti-fibrotic miRNAs.[129–131] For example, miR-378 appears to inhibit HSC activation through suppression of Gli3, a downstream effector and transcription factor in the Hedgehog pathway.[132] Overexpression of miR-200a suppressed translation of αSMA and inhibited TGFβ by various mechanisms associated with reduced fibrogenesis.[133–135] There is also evidence that ethanol induces lysine methyltransferase 2A (KMT2A), resulting in transcriptional activation of elastin and other ECM genes,[136] underscoring the role of toxins in epigenetic regulation of fibrogenesis.

HSC activation results in dysregulation of cellular energy homeostasis, as the energy required for various intracellular processes is shunted toward the transcriptional and translational demands of HSC transdifferentiation. Autophagy in HSCs is induced by endoplasmic reticulum (ER) and oxidative stress in response to chronic liver injury[137] and provides ATP to fuel HSC activation.[138] Specifically, the knockdown of essential autophagy genes *Atg5* and *Atg7* not only inhibited autophagy but also suppressed HSC activation.[139] Another ER stress effector pathway, the unfolded protein response (UPR), is implicated in HSC activation through stimulation of the TGFβ pathway in protein kinase R-like ER kinase (PERK)-mediated UPR.[140–142] Chaperone proteins involved in protein folding are also implicated in ER stress and HSC activation; deletion of collagen-specific heat shock protein 47 (Hsp47), which mediates folding of collagen I, resulted in collagen I accumulation, induction of autophagy and reduced levels of apoptosis.[143] Various other pathways, effectors and receptors, such as advanced glycation end products (AGE), inflammasome (NLRP3), Rev-Erb nuclear receptors, LXR, PPARγ and hypoxia-inducible factor-1α (HIF1α) have been implicated in the regulation of autophagy, ER stress and HSC activation. Further, retinoids appeared to activate latent TGFβ in rat HSCs, exacerbating hepatic fibrosis.[122] Alcohol dehydrogenase 3-mediated oxidation of retinol to retinoic acid promoted expression of TGFβ and collagen in HSCs, while retinol secreted by HSCs promoted HSC survival via suppression of NK cells.[144] Lastly, protein ubiquitination was inhibited during HSC activation through upregulation of ubiquitin C-terminal hydrolase 1 (UCHL1), a deubiquitinase, resulting in proliferation of activated HSCs in CCl₄ and BDL mice.[145] Many pathways are currently under investigation, including YAP1 (Hippo pathway), endosialin, BRD4, galectin 3, GATA4, BMP6, GAS6 and AXL.

Cytokines also play an important role in HSC activation. IL-17, secreted by T$_h$17 cells, activated the STAT3 signaling pathway, inducing synthesis of type I collagen and promoting fibrosis.[146] IL-20 is a pro-inflammatory cytokine that promotes TGFβ expression and activates HSCs, and antibody neutralization of either IL-20 or its receptor inhibited expression of TGFβ, activation of HSCs and hepatic fibrosis.[147] Meanwhile, IL-15 and IL-15 receptor α (IL-15Rα) have a hepatoprotective role by downmodulating progression of hepatic fibrosis via inhibition of collagen expression in HSCs, as well as by promoting NK cell homeostasis.[148] IL-22 has been shown to mitigate hepatic fibrosis via induction of HSC senescence. An in vivo study of increased hepatic IL-22 expression in transgenic mice resulted in accelerated fibrosis resolution following liver

injury, through reduced αSMA expression and increased numbers of senescent HSCs.[149] Murine administration of IL-22 improved BDL-induced fibrosis,[146] while deletion of IL-22 worsened fibrosis.[149]

Deactivation pathways of hepatic stellate cells

Cessation of chronic liver injury can lead to fibrosis reversal, even at the cirrhotic stage, whereas previously it was thought to be an end-stage and irreversible condition. There are three predominant mechanisms that contribute to clearance of activated HSCs and resolution of fibrosis, namely induction of HSC apoptosis, senescence and reversion/transdifferentiation to an inactivated state, all ostensibly culminating in a non-fibrogenic state (Figure 3). There is evidence that antiviral treatment for HBV and HCV results in some degree of fibrosis reversal, as detailed below.

HSC apoptosis

Induction of apoptosis serves to decrease the number of activated HSCs, leading in part to resolution of fibrosis. In the activated state, HSCs express various "death" receptors, including those for TNF receptor 1 (TNFR1), first apoptosis signal (FAS), p75 neutrophin (p75NTR) and TNF-related apoptosis-inducing ligand (TRAIL).[150] In a transgenic mouse model lacking the p75NTR ligand-binding domain, there was decreased resolution of fibrosis and decreased apoptosis of myofibroblasts.[151] Exogenous pegylated TRAIL improved CCl$_4$-induced fibrosis and induced apoptosis of activated HSCs in a rat model.[152] Notwithstanding this seemingly increased susceptibility to apoptosis, activated HSCs do display increased resistance to apoptosis. There are various pathways of programmed cell death (PCD); apoptosis, autophagy, oncosis leading to necrosis, and pyroptosis.[153] PCD pathways, including apoptosis, are suppressed by NF-κB, which induces transcription of various antiapoptotic genes, such as Bcl-2 family members like Bcl-X$_L$ and Bfl-1,[154] as well as TRAF1, TRAF2, XIAP, FLIP and c-IAP.[155,156] TNF and IL-1β activate NF-κB and promote resistance to HSC apoptosis.[157] In rats, inhibition of NF-κB by the small molecule BAY 11-7082 reduces hepatic fibrosis in vivo.[158] Further, proteasome inhibition by small molecules bortezomib and MG132 induced apoptosis via three mechanisms that included inhibition of (i) nuclear translocation of RelA/p65 protein; (ii) I-κBα degradation (preventing

NF-κB nuclear translocation)in and (iii) NF-κB DNA binding activity, thus preventing expression of Bcl-2 family genes involved in apoptosis.[158] Additionally, TIMP1 and TGFβ have been shown to have anti-apoptotic action and to promote survival of activated HSCs.[159] Inhibition of histone deacetylation by the small molecule nilotinib was shown to induce apoptosis and autophagy, exemplifying epigenetic regulation of PCD.[160] Lastly, the Akt anti-apoptotic pathway, when activated, suppressed apoptosis mediated by Jun N-terminal kinase (JNK).[161] Inhibition of several tyrosine and Raf kinases, including Akt pathway kinases, induced both apoptosis and autophagy in HSCs.[162]

Senescence

Cellular senescence is typically associated with some type of cell damage, as well as with developmental differentiation. There are various damage-induced senescence mechanisms, through which cells lose proliferative markers, express tumor suppression gene products, DNA damage markers, secrete various signaling molecules and express cell cycle inhibitors.[163] Replicative senescence is the result of telomere shortening due to consecutive cycles of cell division, which is detected as a form of DNA damage. Induction of senescence is achieved through multiple signaling pathways, most of which activate p53, that ultimately converge on activation of cyclin-dependent kinase (CDK) inhibitors p16, p15, p21 and p27. Such activation results in proliferative arrest, mostly mediated by hypophosphorylated retinoblastoma (RB) protein.[164] Stress-induced senescence is mediated through increased levels of reactive oxygen species (ROS). As an example, the RAS-RAF-MEK-ERK cascade, which can lead to high intracellular ROS levels, activates p38 MAPK, which upregulates p21 and increased transcription of p53.[165] Activation of oncogenes can also induce senescence, an observation first made when an oncogenic isoform of RAS was overexpressed in human fibroblasts.[166] Other oncogenes that can result in induction of senescence include von Hippel-Lindau disease tumor suppressor (VHL), neurofibromin (NF1) and PTEN.[167] Murine activation of the p53/p21 pathway inhibits activated HSC proliferation and attenuates hepatic fibrosis.[92,168] Senescence results in a pro-inflammatory phenotype, the senescence-associated secretory phenotype (SASP)[169–172] that upregulates TGFβ, IL-6, IL-8, various chemokines and macrophage inflammatory proteins, proteases, growth factors and granulocyte-macrophage colony-stimulating factor (GM-CSF).[173,174] Such an

environment is conducive to recruitment of phagocytic macrophages and NK cells, ultimately leading to clearance of senescent activated HSCs.

Reversal/transdifferentiation to an inactivated state

In rodents, there is some evidence that activated HSCs have the capacity to undergo reversion into an inactivated phenotype not unlike the quiescent, pre-activation phenotype. It was further observed that these reverted HSCs have a lowered threshold for reactivation. While profibrogenic genes such as *Col1a1, Acta2, Timp1* and *Tgfbr1* are downregulated, genes associated with quiescence, including *Gfap, Adfp* and *Adipor1*, are not expressed.[175,176] A murine study with genetically labeled activated HSCs showed that after stopping CCl$_4$ administration, expression of labeled genes decreased significantly, with some apoptosis and regression to the inactivated but primed state.[175] Similarly, single-cell PCR and genetic tracing in mice found that upon withdrawal of the offending agent (CCl$_4$), HSCs reverted back to a quiescent-like phenotype, yet remained "semi-activated" and primed to reactivation.[176] In vitro, human fetal limb fibroblasts were induced to transdifferentiate to hepatocyte-like cells using lentiviral expression of *FOXA3, HNF1A* and *GATA4*.[177] This study was replicated in vivo via lentiviral induction of fibroblasts into hepatocyte-like cells via ectopic expression of *FOXA3, GATA4, HNF1A* and *HNF4A*.[178] Though advances have been made in identifying relevant mechanisms in this partial reversal to a non-fibrogenic, inactivated state, much work remains to be done to identify targets amenable to therapeutic intervention.

Clinical evidence to reverse fibrosis
Reversal of HBV fibrosis following antiviral treatment

There is growing evidence that antiviral treatment of HBV is associated with hepatic fibrosis reversal. The standard of care prior to the newer generation of antivirals, including nucleoside/nucleotide analog reverse transcriptase inhibitors, was pegylated IFNα (PEG-IFNα), which has been shown to inhibit TGFβ, reduce HSC activation and stimulate HSC apoptosis in vitro.[179] A case series of 110 patients with chronic HBV (CHB) treated with PEG-IFNα showed a 27% histologic improvement in hepatic fibrosis at 16 months.[180] In a small case study of patients with CHB, treatment with the nucleoside analog lamivudine was associated with fibrosis reversal in 68% of the patients,

including complete regression of fibrosis or cirrhosis in 21% of the patients.[181] An earlier case series of CHB patients treated with lamivudine demonstrated histologic cirrhosis reversal in 73% of the patients at 36 months.[182] A larger case study of 348 CHB patients treated with the reverse transcriptase inhibitor tenofovir showed a 51% improvement in fibrosis at 5 years' follow-up.[183–185] A cohort of 57 patients with CHB were treated with entecavir, another reverse transcriptase inhibitor, and showed a remarkable 88% histologic improvement in fibrosis.[186,187] A randomized controlled trial (RCT) of 125 patients with CHB treated with adefovir, a nucleotide analog of adenosine, showed histologic fibrosis improvement in 71% of the patients at 240 weeks (Table 1).[188] A study of 57 patients with CHB treated with the thymidine nucleoside analog telbivudine demonstrated significant improvements in Ishak fibrosis scores after long-term treatment (mean duration 261 weeks).[189] Improvement in histologic fibrosis stage has been associated with, and perhaps dependent on a reduction in HBV viral load.[190,191]

Reversal of HCV-induced fibrosis via DAA treatment

Patients with chronic hepatitis C (CHC) differentially benefited from PEG-IFNα treatment based on genotype, displaying viral eradication rates from 40% with genotype 1 to 70% with genotypes 2 or 3. A study of 60 patients with CHC treated with PEG-IFNα and ribavirin showed an improvement in fibrosis of 82% at 60-month follow-up.[192] The newer generation of oral direct-acting antivirals (DAA) for HCV has shown excellent efficacy in curing HCV. Indirect measurements of fibrosis and cirrhosis made by transient elastography indicated that patients that achieved a sustained virologic response (SVR) following therapy with DAA exhibited lower FibroScan scores, as well as indirect biochemical markers of fibrosis.[193] More recently, short-term evaluation of 51 patients with CHC treated with DAA and who underwent SVR showed moderate improvement in necroinflammation, though no histologic improvement in fibrosis was observed at a mean of 41 weeks after completion of DAA treatment.[194] In a recent prospective study of 260 patients with CHC treated with DAA, 94.6% of whom achieved SVR, 40% underwent significant regression in fibrosis measured indirectly via transient elastography. Interestingly, the change was more pronounced in patients with higher baseline fibrosis scores.[195] The use of angiotensin receptor blockers such as

Table I Summary of published and ongoing phase II/III randomized controlled trials (RCTs) of therapeutic agents for hepatic fibrosis reversal

Agent	Etiology	Findings	Reference
Adefovir	CHB	71% fibrosis improvement at 240 weeks	188
Farglitazar	CHC	No improvement in fibrosis at 18 months	197
Candesartan	Alcohol-related cirrhosis	33% fibrosis improvement vs 12% (placebo)	199
UDCA	PBC	Slower disease progression (RR =0.28)	201
UDCA	PBC	5-fold lower progression to fibrosis/cirrhosis	202
Pioglitazone	NASH	Reduced fibrosis and improved NAS scores	208
Pioglitazone	NASH	No improvement in NAS scores or fibrosis	113
Vitamin E	NASH	Improved NAS scores, no improvement in fibrosis	113
Rosiglitazone	NASH	No improvement in fibrosis	209
Pentoxifylline	NASH	Improved NAS scores and fibrosis	210
Obeticholic acid	NASH	Improvement in fibrosis at 24 weeks	116
Simtuzumab	NASH	No improvement in hepatic collagen content	212
Cenicriviroc	NASH	Improvement in NAS scores	214
Emricasan	Cirrhosis	Reduced MELD, Child-Pugh scores	215
Elafibranor	NASH	Improved fibrosis	114
Selonsertib	NASH	Improved fibrosis	216

Abbreviations: CHB, chronic hepatitis B; CHC, chronic hepatitis C; MELD, Model for End-Stage Liver Disease; NAFLD, non-alcoholic fatty liver disease; NAS, NAFLD activity score; NASH, non-alcoholic steatohepatitis; PBC, primary biliary cholangitis; RR, relative risk; UDCA, ursodeoxycholic acid.

losartan, thought to inhibit NADPH oxidase-induced oxidative stress and resultant fibrogenesis, has also been shown to reduce hepatic fibrosis. In a small case study of 14 patients with CHC treated with losartan, 50% displayed histologic improvement.[196] Lastly, an RCT of 109 patients with CHC treated with the PPARγ agonist farglitazar did not show any improvement in hepatic fibrosis at 18-month follow-up (Table 1).[197]

Other etiologies of hepatic fibrosis/cirrhosis

Interventions for various other etiologies of hepatic fibrosis and/or cirrhosis have been assessed for their potential to reverse fibrosis (Table 1). In alcohol-related cirrhosis, abstinence of alcohol was shown to have a survival benefit, although no fibrosis follow-up data were reported.[198] An RCT of 85 patients with alcohol-related cirrhosis treated with the ACE inhibitor candesartan showed a 33% improvement in fibrosis, compared to 12% with placebo.[199] Ursodeoxycholic acid (UDCA), a naturally occurring hydrophilic bile acid, has been used for treatment of primary biliary cholangitis (PBC) and other cholestatic liver disorders. UDCA works to protect cholangiocytes and hepatocytes against the cytotoxicity of static bile acids, as well as by stimulation of hepatobiliary secretion and subsequent anticholestatic effects.[200] An RCT including 146 patients with PBC treated with UDCA showed slowing of fibrosis

progression but no improvement in stage,[201] while 103 PBC patients treated with UDCA in a separate study also showed a lower rate of fibrogenesis.[202] The use of immunosuppression in autoimmune hepatitis (AIH) has also been evaluated for improvement in fibrosis. In a study of 87 patients with AIH treated with corticosteroids, 53% showed improvement in histologic fibrosis scores and there was no further progression in 26% of the patients. In addition, the frequency of cirrhosis decreased from 16% to 11% over a mean treatment period of 62 months.[203] A cohort of 19 patients with AIH treated with cyclosporine A versus prednisolone for 6 months, and then maintained on azathioprine, showed a significant decrease in fibrosis stage in paired liver biopsies, as well as a decrease in inflammatory grade and non-invasive biochemical markers of fibrosis.[204] There has been one recent study of 36 patients with hereditary hemochromatosis and severe fibrosis or cirrhosis (METAVIR scores F3 or F4), in which liver fibrosis or cirrhosis was assayed histologically following phlebotomy in C282Y homozygotes. Fibrosis regressed at least 2 METAVIR units in 69% of the patients with stage F3 fibrosis at baseline, and in 35% of the patients with stage F4 cirrhosis at baseline.[205]

There are currently no FDA-approved treatments for non-alcoholic fatty liver disease (NAFLD) or for non-alcoholic steatohepatitis (NASH), other than addressing risk factors, including weight loss through lifestyle modification. The NAFLD Activity Score (NAS) is a scoring

system to histologically assess the features observed; typically, a score equal to or greater than 5 is used for a histologic diagnosis of NASH.[206] An RCT assessing weight loss in 31 patients with NASH over a 48-week period found significant decreases in NAS after an average weight loss of 9.3% but did not show improvement in fibrosis.[207] Various other agents are under active investigation for treatment of NASH (Table 1). An RCT of 74 patients with NASH treated with the PPARγ agonist pioglitazone versus placebo for 12 months found that fibrosis and hepatocellular injury were significantly reduced in the treatment arm.[208] An RCT comparing pioglitazone versus vitamin E versus placebo in 247 patients with NASH found that vitamin E was superior to placebo in decreasing NAS, but showed no improvement in fibrosis. Pioglitazone therapy did not demonstrate any advantage over placebo in the primary outcome (improved NAS), though there was benefit in several secondary outcomes.[113] Another PPARγ agonist, rosiglitazone, was not found to have any effect on improvement of fibrosis in an RCT of 53 patients with NASH.[209] The phosphodiesterase inhibitor pentoxifylline was compared to placebo in an RCT of 55 patients with NASH showing a significant decrease in NAS, as well as a significant improvement in fibrosis.[210] In a much anticipated study, the farsenoid X receptor agonist obeticholic acid showed a statistically significant improvement in fibrosis (45% vs 21%, P=0.002) at 24-weeks versus placebo in 141 patients with NASH.[116]

Biologic and small molecule therapeutics under investigation to reverse fibrosis

There are no FDA-approved therapies for fibrosis reversal, though many promising agents are being actively investigated (Table 1). Hepatic fibrosis can be achieved via a variety of mechanisms, including suppression of HSC activation, reversal or transdifferentiation of activated HSCs, immune clearance of HSCs, induction of HSC apoptosis and promotion of HSC senescence. A few biologic therapies have also been investigated. A human monoclonal antibody targeting CTGF, pamrevlumab (FG-3019) was initially tested for hepatic fibrosis and is currently on fast-track approval by the FDA for treatment of idiopathic pulmonary fibrosis.[211] Targeting ECM genes such as lysyl oxidase-like 2 (LOXL2) to down-modulate fibrogenesis has been found to be an attractive target. Two phase IIb RCTs of simtuzumab, a humanized monoclonal

antibody to LOXL2, failed to decrease hepatic collagen content or hepatic venous pressure gradient.[212]

In addition to the molecules described in various areas earlier, in vivo inhibition of TGFβ1 activation via integrin αvβ6 antagonism with EMD527040 in a BDL model of cirrhosis was found to significantly decrease bile duct proliferation and peribiliary collagen deposition by 40–50%.[213] Cenicriviroc is an inhibitor to both CCR2 and CCR5 that leads to decreased recruitment of inflammatory monocytes and macrophages via CCR2 and decreased recruitment of HSCs and lymphocytes via CCR5. Cenicriviroc demonstrated significant improvement in NAS after 1 year in a phase IIb RCT of 289 patients with NASH[214] and is currently being studied in a phase III clinical trial (AURORA trial, ClinicalTrials.gov identifier NCT03028740). An RCT of 86 cirrhotic patients treated with emricasan, a potent irreversible pan-caspase inhibitor, found significant reductions in mean MELD and Child-Pugh scores after 3 months of treatment, an effect that was sustained or improved at 6 months.[215] Elafibranor, a PPARα and PPARδ agonist, was evaluated in a phase IIb RCT of 276 patients with NASH and found to significantly reduce fibrosis.[114] Inhibition of apoptosis signal-regulating kinase 1 (ASK1) by selonsertib with and without simtuzumab led to improvement in liver fibrosis, measured both directly and indirectly, in a phase II RCT of 72 patients with NASH.[216] These promising results are encouraging signs that effective therapies for NASH and potentially other hepatic fibrosis etiologies are on the horizon (Table 1).

Mesenchymal stem cell transplantation

Lastly, there have been efforts at utilizing mesenchymal stem cells (MSCs) derived from umbilical cord and from bone marrow to treat liver fibrosis. However, such efforts have been hampered by the inability to monitor the cells in the transplant recipient and by a lack of standardized protocols governing timing, dose and route of administration. Additionally, concerns have been raised regarding potential tumorigenicity in vivo, while other data suggested that there is an antitumor effect and down-modulation of signaling pathways associated with tumor growth and cell division. Nevertheless, the small case series thus far showed a clear trend toward improved MELD and liver function; and there is only one study that showed histologic improvement of fibrosis.[217] Several reports using intravenous administration of umbilical cord-derived MSCs showed various improvement in biochemical markers, including improvement in

MELD and liver function,[218,219] reduced ascites,[218,220] and decreased serum alkaline phosphatase and γ-glutamyltransferase levels.[220] Six studies of autologous bone marrow-derived MSCs administered via various routes showed improvements in MELD score,[221–224] Child-Pugh score,[217] liver function[222,224] and improvement in various liver function tests.[217,223,225] It should be noted that none of these studies have been performed in the US, most likely given the paucity of data regarding tumorigenicity; hence, additional in vitro and in vivo studies are required to better characterize the risk potential of autologous MSC transplantation.

Conclusion

The wealth of knowledge that has accumulated over the past 15 years illuminating the multifaceted intricacies of HSC activation, apoptosis, immune recruitment and senescence have paved the way for translational therapeutics with the potential to reverse fibrosis. As we continue to discover and elucidate the relevant cellular pathways, it is hoped that other therapeutic targets will be identified to develop new modalities of treatment for this all-too-common and increasing ailment. Promising agents currently underway in clinical trials will help to design and implement additional novel approaches with improved efficacy. Lastly, a greater understanding of the fundamental mechanisms of fibrosis and its reversal could and will lead to therapeutic agents for other organs, such as kidney, lung and heart.

Disclosure

The authors report no conflicts of interest in this work.

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