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REVIEW ARTICLE

Antifibrotics in liver disease: are we getting closer to clinical use?

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Abstract
The process of wound healing in response to chronic liver injury leads to the development of liver fibrosis. Regardless of etiology, the profound impact of the degree of liver fibrosis on the prognosis of chronic liver diseases has been well demonstrated. While disease-specific therapy, such as treatments for viral hepatitis, has been shown to reverse liver fibrosis and cirrhosis in both clinical trials and real-life practice, subsets of patients do not demonstrate fibrosis regression. Moreover, where disease-specific therapies are not available, the need for antifibrotics exists. Increased understanding into the pathogenesis of liver fibrosis sets the stage to focus on antifibrotic therapies attempting to: (1) Minimize liver injury and inflammation; (2) Inhibit liver fibrogenesis by enhancing or inhibiting target receptor–ligand interactions or intracellular signaling pathways; and (3) Promote fibrosis resolution. While no antifibrotic therapies are currently available, a number are now being evaluated in clinical trials, and their use is becoming closer to reality for select subsets of patients.

Keywords Antifibrotic agents · Liver fibrosis · Pathogenesis

Introduction

Compelling evidence following antiviral therapy for hepatitis B or C supports the notion that hepatic fibrogenesis is a dynamic process and liver fibrosis is even reversible. However, many underlying liver diseases, including non-alcoholic fatty liver disease (NAFLD) and primary sclerosing cholangitis, still lack specific treatments to inhibit fibrosis progression. Moreover, significant number of patients cured from underlying liver disease do not demonstrate fibrosis reversal and remain at risk of developing a cirrhotic complication and hepatocellular carcinoma (HCC). Therefore, there remains a need to establish direct specific therapeutic targets for liver fibrosis. Our detailed understanding of hepatic fibrosis pathogenesis and the body's innate mechanisms to resorb scar is critical to designing antifibrotic agents. This review will focus on the mechanisms of liver fibrogenesis and potential novel therapies at various stages of development.

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Pathogenesis of hepatic fibrosis

Cellular sources and alteration of extracellular matrix

In the context of chronic liver injury, the excessive extracellular matrix is primarily produced by liver myofibroblasts. While hepatic stellate cells (HSCs) are the primary source of liver myofibroblasts, other hepatic mesenchymal cells including portal fibroblasts, bone marrow-derived fibrocytes, and hepatocytes or cholangiocytes in a process referred to as epithelial–mesenchymal transition may also contribute to the population of myofibroblasts, (Fig. 1), to varying degrees, depending on the etiology of the liver disease. Of these other potential sources, portal myofibroblasts have the most data supporting their role in biliary fibrosis. They play a role in cross-talk with cholangiocytes, influencing both their polarity and proliferation, and appear to be "first responders" in biliary fibrosis. While they contribute to 70% of the myofibroblast pool at 5 days post bile duct ligation, they become quantitatively less with progressive fibrosis, suggesting a recruitment/amplifying

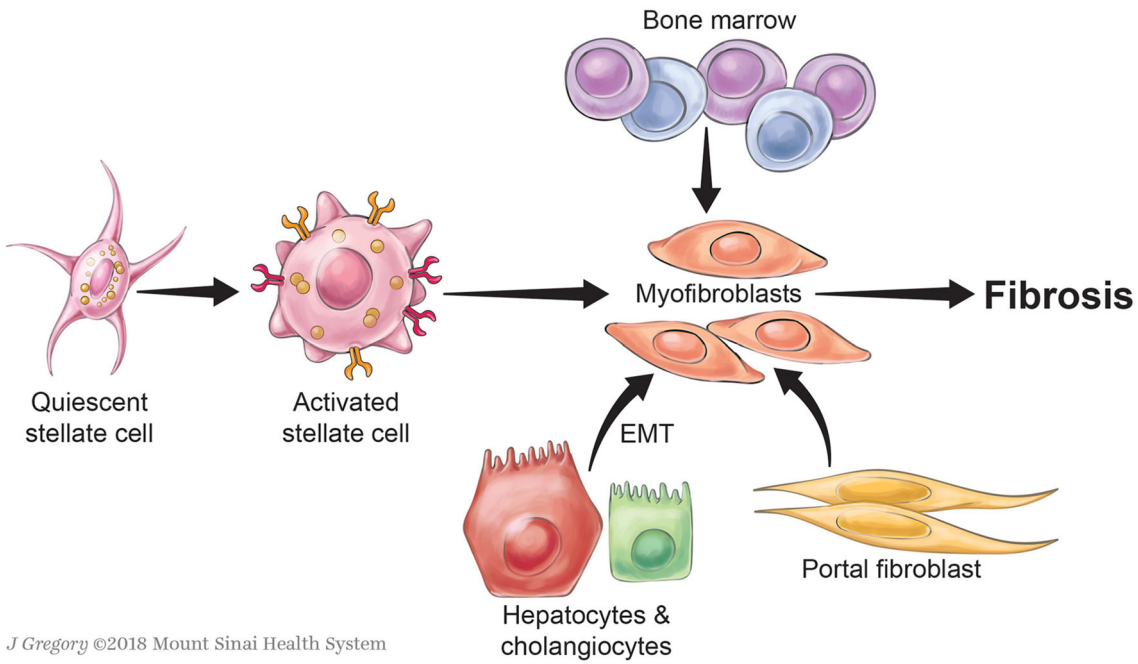

Fig. 1 Hepatic myofibroblast are originated from heterogenous types of fibrogenic cell. Activated hepatic stellate cells are considered to be a source of liver fibrogenic cells. Portal fibroblasts have also been demonstrated as a source of myofibroblast especially in cholestatic liver disease. Furthermore, reported minor proportion of liver fibrogenic cells includes bone marrow-derived cells and epithelial–mesenchymal transition (EMT)

role possibly via IL-13 [1] early in the initiation of the fibrotic process. Based on lineage tracing studies, HSCs, not portal myofibroblasts, contribute about 85% of collagen in experimental murine cholestatic injury [2]. Whether this translates to human cholestatic liver disease is not entirely clear. Therefore, hepatic stellate cells remain the primary target for antifibrotic therapies irrespective of the etiology of the liver disease.

While in the normal liver, quiescent stellate cells produce predominantly type IV collagen; with liver injury, activated myofibroblasts begin to produce predominantly collagen type I, III and other proteins including fibronectin, elastin, laminin, hyaluronan, and proteoglycans [3, 4]. The combination of progressive accumulation of ECM proteins and a change in matrix composition from collagen type IV and heparan sulfate proteoglycan to collagen type I and III results in an increase in ECM density and stiffness. Increased liver stiffness can serve as a mechanical stimulus to activate HSCs and, thus, forming a perpetuating positive feedback loop [5]. In addition, the expanded ECM has an affinity to bind growth factors involved in HSC proliferation such as platelet-derived growth factor (PDGF), vascular endothelial growth factor (VEGF) and fibroblast growth factor (FGF) and can, thus, serve as a reservoir [6].

Fibrotic matrix stabilization by collagen crosslinking is a crucial process for fibrosis progression and can limit reversibility of liver fibrosis. The lysyl oxidase (LOX) family is crosslinking enzymes overexpressed in liver fibrosis [7]. Among the five isoforms of LOX, lysyl oxidase-like-2 (LOXL2) is overexpressed by activated HSCs in chronic liver disease [8]. Even once in the "cirrhotic" phase, collagen continues to be deposited and becomes increasingly acellular. This decreased solubility of collagen over time contributes to the irreversibility of cirrhosis at later stages.

Critical role of hepatic stellate cells in liver fibrogenesis

Given the primary role of the activated stellate cells in fibrogenesis, they remain the focus of antifibrotic therapy development. Liver injury triggers the transdifferentiation of quiescent HSCs to proliferative, migratory, and contractile myofibroblasts [activated hepatic stellate cell (aHSCs)]. Cellular responses in the activated stellate cell are characterized by specific phenotypic changes involving proliferation, contractility, fibrogenesis, alteration in matrix degradation, and release of chemotactic and inflammatory signals. All changes result in the accumulation of secreted ECM molecules in the space of Disse and progressive scar formation. In contrast, the clearance of aHSCs by apoptosis or reversion to an inactive phenotype could reverse liver fibrosis.

Mechanisms of stellate cell activation

Extracellular events that promote HSC activation

Initiation of stellate cell activation is largely dependent on paracrine factors together with extrahepatic factors in both an etiology-specific and etiology-independent manner. Injury to neighboring cells, factors present in the portal circulation due to gut injury, as well as infiltration of circulating cells of the immune system all contribute to this process. Once activated, a number of extracellular factors and intracellular HSC responses drive progression of fibrosis, unless the injury is resolved.

Epithelial cell injury

Chronic injury to liver parenchymal cells such as hepatocytes and cholangiocytes causes cell death through apoptotic and necrotic pathways. Damage-associated molecular patterns (DAMPs), released from the dead or dying epithelial cells, initiate and perpetuate sterile inflammatory responses resulting in the generation of mediators that promote HSC activation such as tumor necrosis factor (TNF), reactive oxygen species (ROS), interleukin 1 beta (IL-1β), interleukin-6 (IL-6), and hedgehog ligands.

Immune regulation

Both the innate and adaptive immune systems play crucial roles in liver fibrogenesis. Substances released from damaged hepatocytes including apoptotic bodies, ROS, cytokines, TNF-α, vascular endothelial growth factor (VEGF), insulin-like growth factor (IGF-1), and chemokines lead to the recruitment and activation of the inflammatory system resulting in the accentuation of hepatic inflammation, additional hepatocyte damage, and direct activation of HSCs.

Chemokines play a key role in the recruitment of circulating immune cells to the site of injury. They direct lymphocytes, eosinophils, NK cells, basophils, and mast cells into sites of inflammation by binding with specific chemokine receptors expressed on these inflammatory cells. In response to liver injury, monocyte chemoattractant protein-1 (MCP-1 or CCL2) expressed by hepatocytes, endothelial cells, KCs, and HSCs promotes monocyte infiltration by binding with C–C chemokine receptor type 2 (CCR2) [9]. C–C chemokine CCL5 (also known as RANTES) produced by various cell types, including platelets, macrophages, endothelial, and stellate cells is increased in a variety of chronic liver diseases, promotes lymphocyte recruitment [10] and can elicit stellate cell responses by binding to CCR5 [11].

While inflammatory cells can contribute to fibrosis progression, importantly, some of these cells play a key role in fibrosis regression, A subset of macrophages and natural killer cell (NK cell), in particular, have emerged as important mediators of fibrosis regression. During fibrosis regression, there is the presence of restorative phenotype macrophages which display increased expression of genes related to phagocytosis, growth factors and, matrix metalloproteinase (MMPs) capable of degrading ECM. Natural killer (NK) cells can kill activated HSCs directly and also release IFN gamma (IFNγ) which can induce HSC apoptosis [12, 13].

Intracellular responses

Transdifferentiation from quiescent HSCs to activated HSCs is accompanied by changes in the phenotypic and functional properties of HSCs including (1) enhanced proliferation; (2) cell contractility; (3) fibrogenesis; (4) release of chemotactic and inflammatory signaling molecules; (5) alteration of matrix degradation (Fig. 2). This process involves a complex network of autocrine/paracrine pathways. A multitude of in vitro studies and rodent injury models have led to the identification of numerous cell surface receptors, nuclear receptors, and intracellular signaling molecules that are affected during HSC activation. Moreover, epigenetic modifications also can regulate HSC phenotype by controlling gene expression. Complex dysregulation of molecular pathways involved in HSCs activation is summarized in Fig. 3.

While there are a number of pathways that have led to the development of potential therapeutic compounds, many are detailed below with additional potential pathways outlined in Table 1. Discussion of these pathways sets the stage for understanding the rationale for therapeutic compounds currently being tested as antifibrotics.

Transforming growth factor beta (TGF-β)

TGF-β1, the principal isoform of TGF-β, is implicated in the process of fibrosis in many organs including the liver [14]. As a consequence of liver injury, TGF-β1 from both paracrine and autocrine sources binds to serine/threonine receptor kinases type I and type II on the stellate cell surface resulting in the phosphorylation of its downstream effectors, SMAD2 and SMAD3. SMAD2 and SMAD3 are released into the cytosol, where they form a complex with SMAD4 which can translocate into the nucleus and directly regulate fibrogenic genes, such as collagen type I, fibronectin and tissue inhibitors of metalloproteinases (TIMPs) [15, 16].

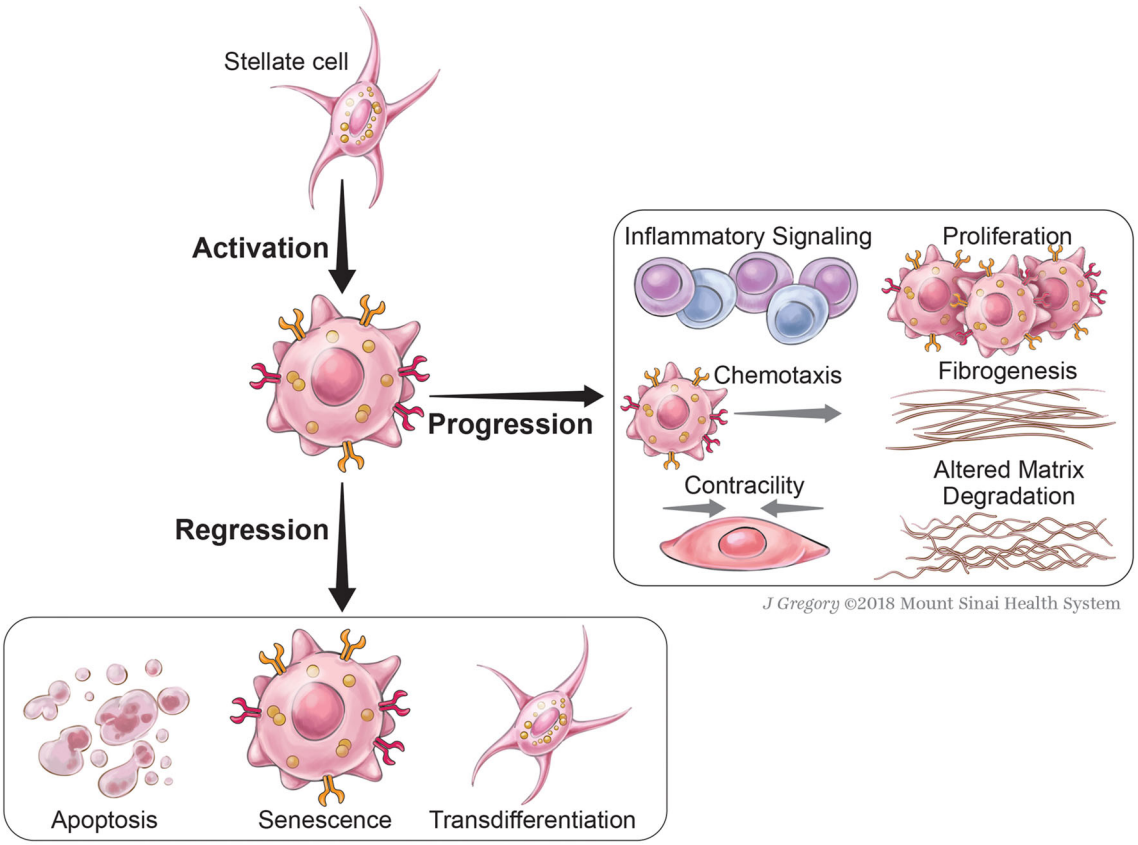

Fig. 2 Following liver damage, hepatic stellate cells transform from quiescent vitamin A-rich cells into fibrogenic myofibroblast. The major phenotypic changes after activation include proliferation, contractility, fibrogenesis, matrix degradation, chemotaxis and WBC chemoattraction. During resolution of liver injury, population of activated stellate cells may be reduced by transdifferentiating their phenotype back to a quiescent formed, cellular senescence and/or apoptosis

Tyrosine kinases

Many proliferative cytokines modulate liver fibrosis by signaling through tyrosine kinase receptors including fibroblast growth factor receptor (FGFR), vascular endothelial growth factor receptor (VEGFR), platelet-derived growth factor receptor (PDGFR), and epidermal growth factor receptor (EGFR). In addition, non-receptor TKs including c-ABL and Src kinases are also pro-fibrotic mediators by modulating TGF-β signaling pathways [17].

Rho kinases

Both Ras homolog gene family, member A (RhoA) and its downstream effector, Rho-kinase (ROCK), mediate HSC activities and motility. Contraction of aHSCs plays a significant role in the hepatic microcirculation contributing to intrahepatic vascular resistance and increased portal pressure in liver cirrhosis. Furthermore, the Rho/ROCK pathway can directly regulate the expression of genes involved in HSC proliferation [18].

Integrins

Integrins are transmembrane proteins which promote liver fibrosis by activating TGF-β. In patients with liver fibrosis secondary to a variety of etiologies, including viral hepatitis, primary biliary cholangitis, alcohol liver disease, integrin αvβ6 mRNA expression is increased. In the case of hepatitis C, a correlation between the level of integrin expression increases and fibrosis stage was appreciated [19]. Moreover, the critical importance of all αv integrin pathways in fibrosis suggests that it is a core pathway which may have clinical utility in a variety of fibrotic diseases [20].

Renin–angiotensin system

Activated HSCs can secrete angiotensin II that binds to angiotensin II type 1 receptor (AT1R) and promotes liver fibrosis by mediating Janus kinase 2 activation and activating transcription 3 (JAK2/STAT3) signaling pathways [21].

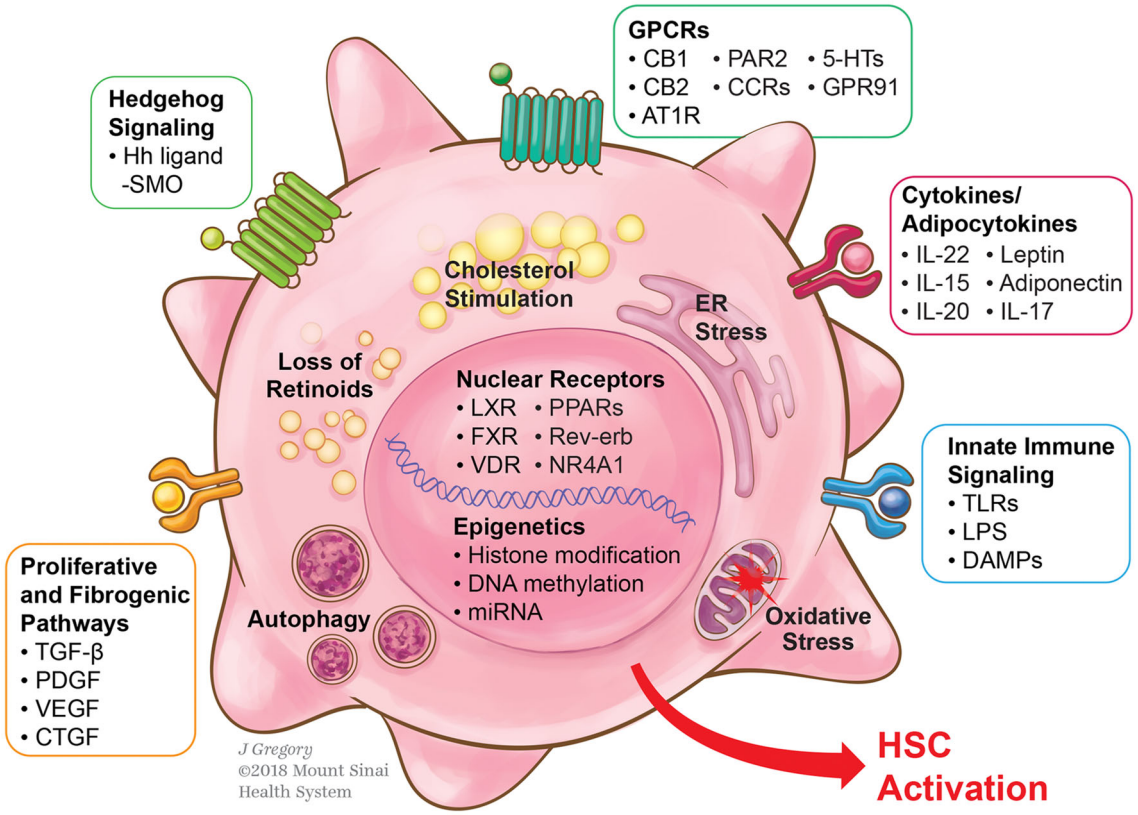

Fig. 3 Multiple signals and pathways involve in processes of HSC activation. Key fibrogenic and proliferative mediators contribute to fibrogenesis include tissue growth factor-β (TGF-β1), platelet-derived growth factor (PDGF), vascular endothelial growth factor (VEGF), and connective tissue growth factor (CTGF). Hedgehog (Hh) ligand and its receptor smoothened homolog (SMO). G protein-coupled receptors including cannabinoid receptor 1/2 receptors (CB1, CB2), 5-hydroxytryptamine receptors (5HTs), proteinase-activated receptor 2 (PAR2), C–C chemokine receptors (CCRs), succinate dehydrogenase-G protein-coupled receptor 91 (GPR91), type 1 angiotensin II receptor (AT1R) can affect HSC activation. Innate immune signaling induced by Toll-like receptors (TLRs), lipopolysaccharide, and damage-associated molecular patterns (DAMPs) has been implicated in stellate cell activation. Cytokines and chemokines mediate crosstalk between hepatic stellate cells and others non-parenchymal liver cells such as kupffer cell are also critical features in liver fibrogenesis. Endoplasmic reticulum (ER) stress and oxidative stress are features of chronic liver disease that activates HSCs. Alteration of nuclear receptors expression also involves in stellate cell activation both in positive and negative ways. Epigenetic signals including microRNAs (mi RNAs), DNA methylation and histone modification control both activation and inactivation of HSCs. FXR, farnesoid X receptor; LXR, liver X receptor; NR4A1, nuclear receptor subfamily 4 group A member 1; PPARs, peroxisome proliferator-activated receptors; VDR, vitamin D3 receptor; Rev-erb, nuclear receptor subfamily 1, group D, member 1

Table 1 Signaling pathways involved in stellate cell activation with potential development for therapeutics

Signaling pathways	Roles in stellate cell activation
Connective tissue growth factor (CTGF)	Has direct profibrotic activity with significant TGF-β1 crosstalk
Hedgehog (Hh) signaling	Promotes transition of quiescent HSC to fibrogenic myofibroblasts
Lysophospholipid pathway	Induces stellate cell and hepatocyte proliferation
Tumor necrosis factor-like weak inducer of apoptosis (TWEAK)	Enhances HSC proliferation via increased SIRT1 expression and inhibits HSC senescence
Growth arrest-specific gene 6 (*Gas6*)	Is required for full HSC activation
Wnt/β-catenin signaling	Promotes HSC activation and reduces apoptosis of HSC

Cannabinoid receptors

The cannabinoid system is involved in human neuro/immune-modulatory functions. Its two G protein-coupled receptors have opposing effects on fibrosis with the CB1 receptor promoting fibrosis, while the CB2 receptor is hepatoprotective. Antagonism of a serotonin receptor, 5HT2B on HSCs attenuates fibrosis and enhances liver regeneration [22].

Toll-like receptors (TLRs)

Toll-like receptors (TLRs) on both HSCs and KCs promote immune-related HSC activation. Stimulation of TLRs on HSCs by gut-derived microbial products comprising lipopolysaccharides (LPS), peptidoglycan, bacterial DNA and possibly viral or fungal particles results in the release of pro-inflammatory cytokines and an enhanced fibrogenic response by down-regulating a transmembrane suppressor of TGF-β1 (BAMBI) [23, 24]. Endogenous TLR ligands, released in the context of sterile cell injury such as high mobility group box 1 protein (HMGB1), can also promote HSC activation.

LPS-induced TLR4 activation also sensitizes HSCs to TGF-β activation by the MyD88-NF-κβ pathway. Bacterial DNA stimulates TLR9 to recruit macrophages which release the pro-inflammatory cytokine, IL1-β [25]. TLR2 stimulates the release of TNF-α in the intestine resulting in increased intestinal permeability and thus higher chance of bacterial translocation. Given the predominantly portal circulation of the liver and the low-pressure environment, increased bacterial translocation indirectly promotes fibrosis by facilitating intrahepatic TLR activation.

Adipokines

Leptin exerts a pro-fibrogenic effect on HSCs by increasing the release of TGF-β1 [26] and downregulating peroxisome proliferator-activated receptor-ϒ (PPAR-ϒ), one of the most significant anti-fibrogenic nuclear receptors on HSCs [27]. Leptin also reduces expression of sterol regulatory element of B-binding protein-1C (SREBP-1C) which can inhibit HSC activation [28]. Adiponectin diminishes liver fibrosis by increased production of nitric oxide [29], inhibits HSCs migration by promoting TIMP-1 secretion [30], and may sensitize aHSCs to apoptosis [31]. Finally, there is an interplay in the signaling cascades between leptin and adiponectin, wherein adiponectin can inhibit leptin signal transduction.

Beyond these two key adipokines, there are other adipocytokines such as plasminogen activator inhibitor-1 (PAI-1), apelin, and resistin that may drive the fibrogenic response in the liver [32].

Nuclear receptor signaling and transcriptional factors

Multiple nuclear receptors have been demonstrated to play a part in stellate cell activation. Farnesoid X receptor (FXR) signaling diminishes HSC contraction and fibrogenesis and reduces portal pressure in rodent models [33, 34]. PPARs (PPARα/γ/δ) also suppress angiogenic PDGFRβ signaling and TGF-β 1 production via the β-catenin pathway. Liver X receptor (LXR) activation suppresses HSC activation in murine HSCs [35]. Vitamin D receptor (VDR) ligands inhibit fibrosis by reducing TGF-β/SMAD3 target expression [36]. In HSCs, all-trans retinoic acid (ATRA) inhibits expression of procollagen I, III, IV, fibronectin, laminin, αSMA, TGF-β and IL-6 thereby inhibiting the fibrogenic activity of HSCs [37]. Up-regulation of rev-erb receptor in both the nucleus and cytoplasm of activated HSCs is associated with increased contractility and fibrogenic gene expression [38].

Beyond nuclear receptors, there are many transcription factors associated with stellate cell activation and are shown in Table 2.

Epigenetic transcriptional dysregulation

Epigenetic mechanisms including DNA methylation, histone modifications and non-coding RNAs (microRNAs) impact many aspects of liver fibrogenesis. Activated HSCs express a higher level of DNA methyl-binding proteins (MeCP2), which promote silencing of fibrotic gene expression. Histone acetylation plays a role in HSC activation. Histone methyltransferases can lead to increased transcription of collagen, TIMP-1, and TGF-β [39–41]. Among epigenetic signals, microRNAs (miRNAs) seem to play the most crucial roles in the regulatory control of stellate cell activation and can be sub-grouped into pro-fibrotic or antifibrotic miRNAs [42] (Table 3). While epigenetic regulation is critically important, the kinetics of these modifications during different stages of fibrosis, environmental factors, and the complex crosstalk among simultaneous events must be carefully considered when considering therapeutic options.

Pathways of fibrosis regression

Even in the advanced stages of fibrosis, fibrogenesis entails continual matrix deposition and matrix resorption. Understanding the ability of the liver to resorb scar and, thus, accelerating it may be exploited for antifibrotic therapies.

Table 2 Transcription factors associated with stellate cell activation

Transcription factors that can promote fibrosis	Transcription factors that can repress fibrosis
Sex-determining region Y-box 9 (SOX9)	Kruppel-like factors (KLFs)
GATA binding protein 4 (GATA4)	Aryl hydrocarbon receptor (AhR)
Myocardin-related transcription factor A (MRTF-A)	Embryonic stem cell-expressed RAS (ERAS)
Gα-interacting vesicle-associated protein (GIV)	Nuclear receptor subfamily 4 group A member 1/2 (NR4A1/2)
Yes associated protein (YAP)	

ECM degradation

The balance between MMPs which have a fibrolytic activity and their inhibitors, TIMPs, maintains a homeostatic extracellular matrix in the normal liver. With progressive liver fibrosis, enhancing the degradation of excess ECM by increasing the activity of MMPs or decreasing TIMP activity is an alternative approach for antifibrotic therapies. Collagen crosslinking through enzymes such as Lysol oxidase-like 2 (LoxL2) stabilizes the fibrotic matrix making it progressively more resistant to protease degradation, thus forming the basis for the study of LoxL2 monoclonal antibody for the treatment fibrosis [43].

Reduction of activated HSCs

Reduction in the number of aHSCs is another potential way to reverse liver fibrosis. Three major pathways that could eliminate aHSCs are: [1] Induction of HSC death; [2] Induction of HSC senescence; [3] Reversion or transdifferentiation to an inactivated phenotype.

Induction of HSC death or killing

Activation of the NF-κB survival pathway in activated HSCs results in its relative resistance to stimuli promoting cell death [44]. Therefore, targeting the NF-kB pathway either directly or indirectly promotes HSC apoptosis [45]. TNF-related apoptosis-inducing ligand (TRAIL) receptors up-regulate on aHSCs making them more sensitive to TRAIL-medicated cell death [46]. Additionally, several studies have shown that inhibition of cannabinoid receptors and 5-hydroxytryptamine (5HT) receptors could also induce HSC apoptosis [47].

Beyond directly promoting apoptosis of activated HSCs, activation of NK and NKT cells by interferon gamma (IFNγ) is another pathway to promote HSC death [48].

Induction of HSC senescence

Cellular senescence restricts cell division within a finite proliferative capacity. Therefore, the induction of HSC senescence might serve as an antifibrotic strategy. Phytochemicals such as curcumin can induce HSC senescence by activating PPARγ/p53 signaling [49]. Agonists of RAR/RXR and PPARγ may also induce HSC senescence [50].

Reversion or transdifferentiation

Activated HSCs have the potential to revert to a quiescent-like state if causative agents of liver damage have been removed, though reverted cells are more sensitized to reactivation into myofibroblasts after re-exposure to fibrogenic stimuli [51]. Recent studies in animal models demonstrated reprogramming of aHSCs into hepatocyte-like cells, called induced hepatocytes [52] though this phenomenon remains to be validated in humans.

Therapeutic perspectives

With our increased understanding of fibrogenesis and fibrosis resolution coupled with the numerous potential targets identified (Fig. 4), attention now must focus on converting compounds into therapies. Antifibrotic strategies aim to: (1) Minimize liver injury and inflammation; (2) Target receptor–ligand interactions and intracellular signaling to inhibit liver fibrogenesis; (3) Promote fibrosis resolution. The pipeline for new therapies includes drugs that are specifically designed to be antifibrotics, as well as available agents approved for other indications, but may have antifibrotic activity (Table 4). Here, we provide information regarding some therapies targeting the spectrum of the liver fibrosis trajectory.

Control/cure the primary disease or reduce tissue injury

Control primary disease

Removing the cause of liver injury is the most efficacious way to prevent progression of liver fibrosis. This strategy can also reverse fibrosis at all stages. However, the ability to reverse cirrhosis is not universal and can depend on the

Table 3 miRNA and stellate cell activation

Up-regulated miRNAs in activated hepatic stellate cells	Potential roles in HSCs			
	Proliferation	Migration	Activation	Fibrogenesis
MiR-9a-5p	+	+		
MiR-17-5p	+		+	
MiR-21			+	+
MiR-31	+	+	+	
MiR-33a			+	
MiR-34-a and-34-c			+	
MiR-126			+	+
MiR-130a and -130b	+		+	
MiR-181b	+			
MiR-214			+	
MiR-214-5p			+	
MiR-221 and -222				+

Down-regulated miRNAs in activated hepatic stellate cells	Potential roles in HSCs					
	Apoptosis	Proliferation	Contractility	Migration	Activation	Fibrogenesis
miR-16	+					
miR-19b		+				
miR-29		+				+
miR-29a					+	+
miR-29b						+
miR-30						+
miR-101		+		+		+
miR-122						+
miR-126		+	+			
miR-133a						+
miR-144					+	
miR-146a		+				
miR-150					+	+
miR-155					+	
miR-195		+				
miR-200a		+			+	
miR-214						+
miR-335				+		+
miR-370		+				
miR-454					+	
miR-483					+	

duration the patient has been in the cirrhotic stage, the degree of collagen cross-linking, and the cellularity of the scar. Data have established that clearance of hepatitis C can lead to remarkable improvement in liver histology and clinical outcomes [53, 54]. Similarly, sustained suppression of HBV with oral antiviral therapies has been associated with significant histologic improvement [55, 56]. Finally, with the growing use of bariatric surgery in morbid obesity, evidence of reduced fibrogenesis and improved fibrosis has begun to emerge [57].

Suppress hepatic inflammation or decrease hepatocyte injury/apoptosis

Galectin inhibitor

Galectins are secreted proteins that bind galactose residues on components of the ECM and on cell surface receptors. Galectin-3 is highly expressed on macrophages and plays an important role in cell adhesion and inflammation. Based on results in animal models with the galectin 3 inhibitor,

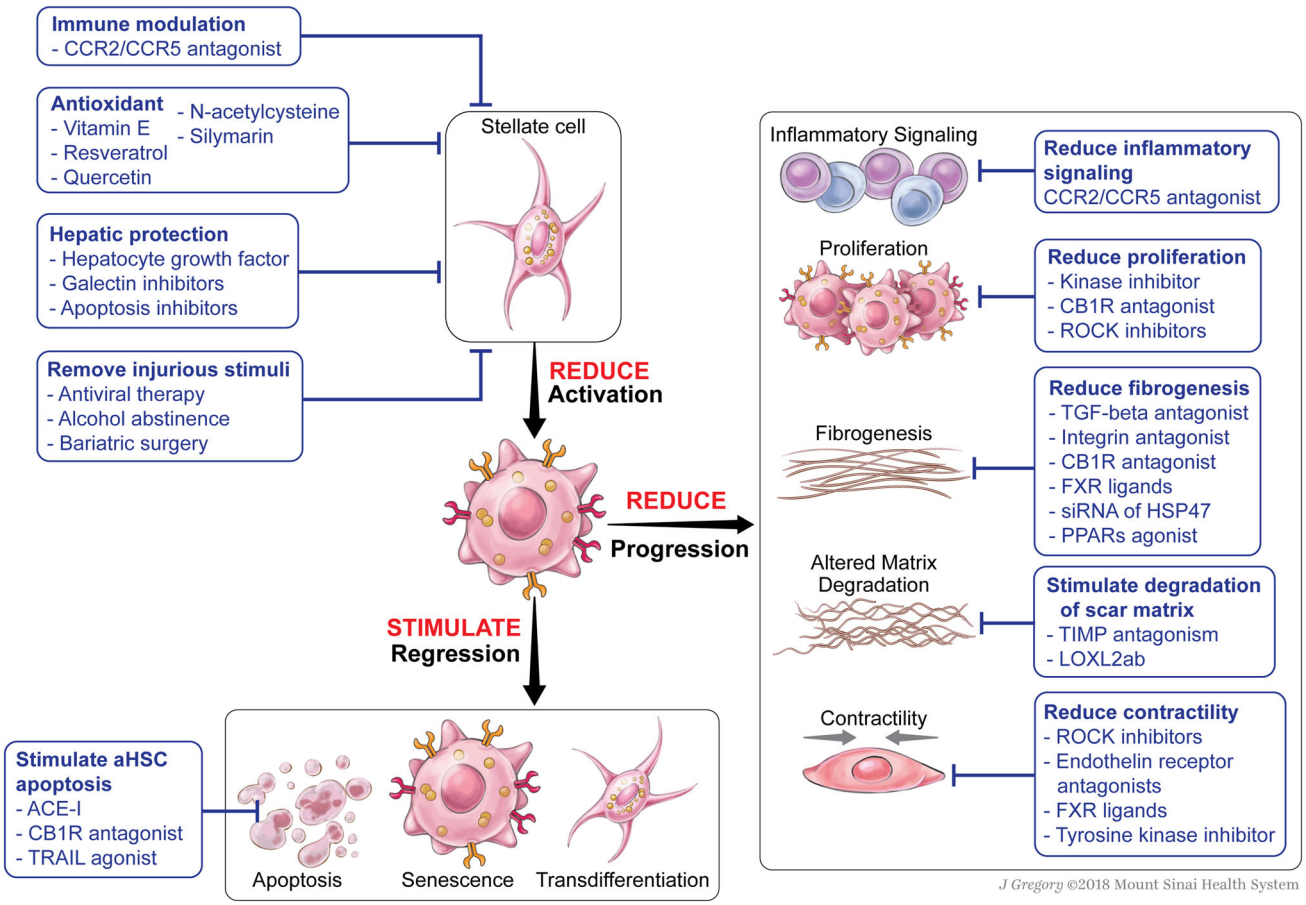

Fig. 4 Mechanisms by which antifibrotic therapies may lead to fibrosis regression. [1] Reduction of HSC activation by reducing hepatic inflammatory response including removed injurious stimuli, introduced antioxidant, introduced hepatic protection agents and applied immune modulation. [2] Inhibition of the most potent of the pro-fibrogenic pathways, for example, preventing expression of pro-fibrogenic mediators, blocking hepatic stellate cell proliferation and reducing contraction of hepatic stellate cells. [3] The resolution of fibrosis can be promoted by enhancing the apoptosis of activated hepatic stellate cells and by increasing the degradation of the extracellular matrix or preventing its cross-linking with antagonists to LOXL2. CCR2/CCR5, C–C chemokine receptor 2 and 5, FXR, farnesoid X receptor; PPAR, peroxisome proliferator-activated receptor; CB1, cannabinoid receptor type 1; ACE-I, angiotensin converting enzyme inhibitors; TGFβ, transforming growth factor β; siRNA of HSP47, small interfering RNA of heat shock protein 47; TIMP, tissue inhibitor of metalloproteinase; LOXL2, lysyl oxidase 2

GR-MD-02 (galactoarabino-rhamnogalaturonan), where liver fibrosis was attenuated and liver cirrhosis reversed [58], this agent is being investigated in a phase 2 clinical trial in NASH patients with cirrhosis and portal hypertension (NCT 02462967).

Apoptosis inhibitor

The pan-caspase inhibitor, Emricasan, is under investigation in clinical trials in the setting of post-transplant HCV reinfection after SVR and NASH patients (NCT02138253, NCT02686762, NCT02960204; EN- CORE trials).

Apoptosis signal-regulating kinase 1 inhibitor

Liver injury promotes a range of stress signals (ROS, ER stress, etc.) for which adaptive responses have evolved. ASK1 (apoptosis signal-regulating kinase 1) is a member of the large MAPK pathways that is activated in the setting of stress and can worsen hepatic inflammation, apoptosis, and fibrosis. In a murine NASH model, selonsertib (formerly GS-4997), a selective inhibitor of ASK1, showed improvements in both metabolic parameters associated with NASH and histologic grading of NASH (steatosis, inflammation, and fibrosis) [59]. In a recently published multicenter phase II clinical trial exploring the safety and efficacy of treatment with selonsertib alone or in combination with simtuzumab versus simtuzumab alone in patients with NASH with stage 2 or 3 liver fibrosis, the

Table 4 Summary of the ongoing clinical trials with fibrosis reversal endpoints (Clinicaltrials.gov)

Drugs/class	Condition	Target methods	Phase	NCT
Cenicriviroc CCR2-CCR5 antogonist	NASH with fibrosis roll over from CENTAUR study	Immune modulation	2	NCT03059446
Cenicriviroc CCR2-CCR5 antagonist	NASH with fibrosis	Immune modulation	3	NCT03028740
GR-MD-02 Galectin 3 inhibitor	NASH with cirrhosis	Suppression of hepatic inflammation	2	NCT02462967
Emricasan Caspase inhibitor	NASH" with fibrosis (excluding cirrhosis)	Reduction of hepatic apoptosis	2	NCT02686762
Selosertib ASK1 inhibitor	NASH F3	Reduction of hepatic inflammation and apoptosis	3	NCT03053050
Selosertib ASK1 inhibitor	Compensated cirrhosis due to NASH	Reduction of hepatic inflammation and apoptosis	3	NCT03053063
Selonsertib, GS-0976(Acetyl-CoA carboxylase), and GS-9674 (FXR agonist)	NASH with different stages of fibrosis	Reduction of hepatic inflammation and apoptosis, reduction of de novo lipogenesis, reduction of hepatic fibrogenesis	2	NCT02781584
BMS-986,036 Pegylated analog of human fibroblast growth factor 21	NASH and stage 3 liver fibrosis	Regulation of hepatic lipid metabolism	2	NCT03486899
Tropifexor (LJN452) and Cenicriviroc Non-bile Acid FXR Agonist + CCR2-CCR5 antogonist	NASH with fibrosis stage F2/F3	Reduction of fibrogenesis, reduction of HSCs contractility, Immune modulation	2	NCT03517540
Erlotinib Tyrosine kinase inhibitor	Compensated cirrhosis	Reduction HSCs proliferation and contractility		NCT02273362
Obeticholic acid FXR agonist	NASH subjects with stage 2 or 3 fibrosis	Reduction of fibrogenesis, reduction of HSCs contractility	3	NCT02781584
Obeticholic acid FXR agonist	Primary biliary cholangitis	Reduction of fibrogenesis, reduction of HSCs contractility	4	NCT02308111
Saroglitazar Dual PPAR agonists, which include PPARα and PPARγ	NAFLD	Reduction of hepatic fibrogenesis	2	NCT03061721
BMS 986,263 A vitamin A-coupled lipid nanoparticle containing siRNA against HSP47	HCV post SVR	Stellate cell-specific targeting	2	NCT03420768
Hesperidin and Flaxseed	NASH (S2-3)		Dietary supplement	NCT03377140
Prebiotic fiber supplement	NAFLD		Dietary supplement	NCT02568605

selonsertib-treated arm had higher rates of fibrosis improvement and lower rates of fibrosis progression compared to patients treated with simtuzumab alone over a 24-week treatment period [60]. In this proof-of-concept trial, achievement of the primary outcome supported proceeding to a phase 3 clinical trial in adults with NASH with bridging (F3) fibrosis (STELLAR 3) (NCT03053050) and compensated cirrhosis due to NASH (STELLAR 4) (NCT03053063).

Immune modulation through cell receptor targeting

CC chemokine receptor antagonist

Cenicriviroc (CVC) is an oral dual CCR2/CCR5 inhibitor that demonstrated promising antifibrotic activity by reducing recruitment and migration of pro-inflammatory monocyte/macrophages to injured liver tissue in a

thioacetamide-induced rodent model [61]. It has been evaluated in a phase 2b study in 289 NASH patients who have evidence of significant liver fibrosis. While the primary endpoint of NASH resolution was not achieved, the fibrosis endpoint was met in significantly more subjects on CVC than placebo. This was the first study wherein reduction in fibrosis was not accompanied by a reduction in inflammation. This decoupling is interesting and may reflect a true direct antifibrotic effect. Interestingly, treatment benefits were clearly shown in patients who had higher disease activity and fibrosis stage at baseline [62] and, thus, the phase 3 study includes NASH patients with more advanced fibrosis (NCT 03028740).

Inhibit fibrogenesis

Cannabinoid antagonists

Rimonabant, a CB1 antagonist, had been approved as a weight reduction agent in 2006 but was withdrawn from the market and all clinical trials in 2008 due to a high incidence of severe depression that is a predictable consequence of central CB1 blockade. However, the development of peripheral CB1 receptor antagonists that cannot cross the blood–brain barrier has renewed enthusiasm for antagonizing CB1 signaling in the liver to inhibit fibrogenesis. However, thus far, no data with these more selective agents have been published.

Antioxidants

Oxidative stress is a well-known stimulus for stellate cell activation. This knowledge provides a rationale for the use of antioxidants such as vitamin E to suppress fibrogenesis. However, a large trial of vitamin E did not show a benefit on fibrosis reduction in non-diabetic patients with NASH [63]. Beyond vitamin E, other antioxidants such as resveratrol, quercetin, and *N*-acetylcysteine (NAC) have demonstrated efficacy on inhibition of stellate cell activation and prevention of liver injury in cell culture systems and animal models [64–66].

Silymarin

A natural component of milk thistle, Silymarin (*Silybum marianum*), is widely used as a nonprescription agent in patients with the chronic liver disease. While this agent has exhibited promising antifibrotic activity in preclinical data, the systematic review of 18 randomized clinical trials assessed milk thistle in 1088 chronic liver diseases patients found no clear evidence showing any benefit on reduction in mortality, improvement in liver histology, or biochemical markers of liver function [67]. Therefore, while likely to do no harm, it also has no proven benefit.

Farnesoid X receptor ligands

The anti-inflammatory and antifibrotic efficacy of obeticholic acid in primary biliary cholangitis (PBC) [68] and phase 2 results in NASH patients are also promising. Additionally, a novel role of FXR as an antifibrotic agent has been uncovered by the study that demonstrated a direct antifibrotic effect of the most active FXR endogenous ligand, chenodeoxycholic acid (CDCA), in a porcine serum-treated rat model [4]. In the meantime, at least 2 FXR agonists are being tested in NASH patients with some degree of liver fibrosis (NCT02781584, NCT02548351) [4, 69].

Angiotensin-converting enzyme inhibitors or angiotensin receptor blockade

Antifibrotic activity of angiotensin II antagonists has been demonstrated both in vitro and in animal studies [70]. Despite this compelling experimental evidence and the wide availability of ACEI or ARBs, large RCTs assessing the potential antifibrotic effects of these drugs in patients with liver fibrosis have not been conducted [71, 72].

TGF-β antagonists

Neutralizing of TGF-β by monoclonal antibodies and protease inhibitors to block TGF-β activation has shown benefit on fibrosis reduction in animal and culture studies [73–75]. Unfortunately, conducting a human trial on TGF-β antagonism has been limited by safety concerns. Theoretically, global inhibition of TGF-β may promote hepatocellular growth or reduced apoptosis and could increase the risk of tumor development especially in a patient population already at increased risk.

Integrins contribute to TGF-β activation and, thus, is another exploratory target of liver fibrosis. Antagonism of αVβ6 integrin has been proposed with good evidence of efficacy in fibrosis reduction in animal models [76]. This strategy seems especially attractive because integrin antagonism is already an established therapy for other indications such as Crohn's disease [77].

Endothelin receptor antagonists

Endothelin stimulates stellate cell contractility which might play a part in stellate cell activation. Blocking the endothelin receptor by bosentan demonstrated antifibrotic activity and reduced the degree of stellate cell activation in an experimental hepatic fibrosis model [78]. Despite initial

enthusiasm, evidence of hepatotoxicity limited further development of bosentan for this indication.

Multi-targeted kinase inhibitors

Preclinical animal models demonstrate significant benefits of multitargeted TK inhibitors in liver fibrosis [79]. In early clinical trials of sorafenib, a potent multikinase inhibitor for treating hepatocellular carcinoma, cirrhotic patients who received sorafenib therapy, had at least a 36% decrease in portal venous flow [80]. However, a pilot multi-center placebo-controlled randomized clinical trial of the effect of sorafenib on portal pressure in patients with cirrhosis did not confirm effects in reducing portal pressure [68]. Even though it showed no benefit in very advanced liver disease, it may slow down the progression of liver fibrosis in the context of mild–moderate liver fibrosis. Erlotinib reduced the number of aHSCs by depressing EGFR phosphorylation in HSCs [81]. An ongoing clinical trial (NCT02273362) is being conducted to evaluate the effects of erlotinib on fibrogenesis inhibition and HCC prevention.

ROCK inhibitor

The ROCK inhibitor Y-27632 has been shown to inhibit stellate cell activation and prevent the development of liver fibrosis in rodent disease models [82]. However, the clinical utility of systemic administration of Y-27632 is limited because it can induce severe hypotension [83]. Fasudil, a small molecule ROCK inhibitor, showed an excellent safety profile and was approved in Japan for other clinical indications. Interestingly, a pilot study in cirrhotic patients showed that fasudil lowers portal and systemic vascular resistance, resulting in both decreased portal venous pressure and arterial pressure [84].

Promote resolution of fibrosis

Increase matrix degradation

Simtuzumab is a humanized IgG4 monoclonal antibody that inhibits collagen crosslinking function of LOXL2 explicitly and might reduce the stability of fibrosis allowing it to be more accessible to proteases for fibrosis resolution. Results from small studies [85] as well as larger clinical trials conducted in patients with advanced liver fibrosis (NCT01672879) and cirrhosis (NCT01672866), however, failed to show any efficacy.

Promote quiescence and/or apoptosis of stellate cells

Selective eradication of aHSCs, with preservation of quiescent HSCs or other liver cells, is critical to developing therapeutic agents based on this strategy. Activated HSCs are tolerant to typical apoptotic stimuli including FasL and TNF-α. In contrast, aHSCs become more sensitive to TRAIL-induced cell death. Therefore, treatment with recombinant TRAIL could be an appealing strategy to ameliorate liver fibrosis. The proof of concept study in rats demonstrated direct induction of aHSCs apoptosis and reduction of fibrosis after intravenous injection of PEGylated TRAIL (TRAILPEG) [86]. However, new therapies promoting aHSCs apoptosis are still not available in clinical trials.

Stellate cell-specific targeting

A vitamin A-coupled liposome system has been designed as a selective approach to target stellate cells that could limit collateral injury to other liver cell types. Vitamin A-coupled liposomes are coupled with a small interfering RNA (siRNA) that controls collagen production. In the case of BMS986263, the siRNA is targeted against heat shock protein 47 (HSP 47) which showed efficacy in an animal model of liver fibrosis [87] and is being tested for safety and efficacy in a phase 2 trial in patients post-SVR for HCV (NCT03420768).

Discussion

Although the pipeline is rich with numerous drugs demonstrating antifibrotic efficacy in animal models, none thus far have been approved by the FDA for use in humans. With the advent of outstanding and well-tolerated drugs for HCV and the emergence of NASH as a major cause of liver disease, the time is ripe for translating these compounds into therapies. Several lessons have been learned in these early stages of development: (1) Murine models, in which many drugs are tested, do not accurately mimic human chronic liver disease, particularly due to differences in the immune–inflammatory axis; (2) Early clinical trial design may have included patients with too little fibrosis, limited duration of drug exposure, and insufficient surrogate endpoints. (3) Patients with more advanced disease are more appropriate for clinical trials; (4) Long study trial duration is needed but poses a challenge for recruitment; (5) While the liver biopsy remains the standard for endpoints, surrogate endpoints (serum or radiologic) need to be validated to promote recruitment, retention, and safety while also proving efficacy; (6) The vast majority of studies are being conducted in NASH and, therefore, applicability to other

chronic liver diseases will need to be assessed; (7) Given the complex nature of liver fibrosis with multiple pathways potentially involved, combination therapy may be promising with a number currently in phase 2 clinical trials (Table 4); (8) Genetic heterogeneity of fibrosis progression may require better selection of patients for clinical trials based on their likelihood of faster fibrosis progression. The learning curve associated with these early studies has led to improved refinement of study design with higher likelihood of identifying agents with both safety and efficacy.

Conclusion

Enhanced understanding of fibrogenesis and fibrosis resolution has revealed a number of potential antifibrotic targets. While numerous agents have been tested in preclinical models and many are currently in human clinical trials, none are currently FDA approved. Those furthest along in the pipeline include CCR2-CCR5 antagonist, galactin 3 inhibitor, caspase inhibitor, ASK1 inhibitor, FXR agonist and a vitamin A-coupled lipid nanoparticle containing siRNA. As we continue to refine the relevant endpoints coupled with better patient selection, antifibrotic therapies will likely be a reality in the next decade. Since most are being tested in NASH, applicability to other etiologies will also have to be determined.

Compliance with ethical standards

Conflict of interest Dr. Meena B. Bansal and Dr. Naichaya Chamroonkul have no potential conflict of interest.

Research involving human participants and/or animals This review article is not a part of any research that involves human participants and/or animals.

Informed consent There is no informed consent needed in this review.

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