Liver fibrosis is a wound-healing process induced by chronic liver injuries, such as nonalcoholic steatohepatitis, hepatitis, alcohol abuse, and metal poisoning. The accumulation of excessive extracellular matrix (ECM) in the liver is a key characteristic of liver fibrosis. Activated hepatic stellate cells (HSCs) are the major producers of ECM and therefore play irreplaceably important roles during the progression of liver fibrosis. Liver fibrogenesis is highly correlated with the activation of HSCs, which is regulated by numerous profibrotic cytokines. Using RNA interference to downregulate these cytokines in activated HSCs is a promising strategy to reverse liver fibrosis. Meanwhile, microRNAs (miRNAs) have also been exploited for the treatment of liver fibrosis. This review focuses on the current siRNA- and miRNA-based liver fibrosis treatment strategies by targeting activated HSCs in the liver. (Translational Research 2019; 000:1–13)
INTRODUCTION

Liver fibrosis is a wound-healing process induced by chronic liver injury caused by nonalcoholic steatohepatitis, hepatitis, alcohol abuse, and metal poisoning. The accumulation of excessive extracellular matrix (ECM) in the liver is a key characteristic of liver fibrosis.\(^1,2\) Although liver fibrosis is reversible, but if not treated, it develops to liver cirrhosis, which is a significant cause of mortality and morbidity. According to an epidemiology study of cirrhosis in the United States, approximately 0.27% of American adults are affected.\(^3\) To date, the only effective treatment for liver cirrhosis is liver transplantation, which is highly restricted by the availability and suitability of liver grafts.\(^4\) Therefore, development of effective treatments for liver fibrosis is critical in preventing the progression to liver cirrhosis. Several antifibrotic agents, such as candesartan (NCT03770936), cenicriviroc (NCT03028740), tropifexor (LJN452, NCT03517540), and pegbelfermin (BMS-986036, NCT02413372, NCT03486912) are currently in clinical trials.\(^5,7\)

In the process of liver fibrogenesis, hepatic stellate cells (HSCs) play an important role as the major producers of ECM, although HSCs only constitute 5%–8% of total liver cells.\(^8,9\) HSCs can be activated by numerous factors,\(^10\) and the activation process includes 2 stages: initiation and perpetuation, starting from changes in gene expression to the changes in proliferation and fibrogenesis.\(^11\) Cytokines including platelet-derived growth factor (PDGF) and tumor necrosis factor alpha (TNF-α) secreted from Kupffer cells and endothelial cells regulate the proliferation of HSCs in a paracrine manner and promote their survival when the liver is injured.\(^12,13\) Once transdifferentiated to an activated myofibroblast-like phenotype, HSCs exhibit more migration and proliferation and less apoptosis with high expressions of α-smooth muscle actin (α-SMA) and ECM.\(^10\) The expression of ECM increases more than 50-fold when quiescent HSCs are activated into myofibroblast-like cells.\(^1,14-16\) Based on the important role of activated HSCs during the progression of liver fibrosis, inhibiting the activation of HSCs either by reversing the activation phenotype to quiescent cells or driving activated HSCs to apoptosis can be an effective strategy for the resolution of liver fibrosis.\(^17\)

RNA interference (RNAi) is a relatively new technology to specifically knockdown target genes using small interfering RNAs (siRNAs) of 21–23 nucleotides.\(^11,18-22\) Once transfected into target cells, double-strand siRNAs are separated by an ATP-dependent helicase, and the functional antisense strands are further incorporated into the RNA-induced silencing complex (RISC) and subsequently guide the RISC to specifically bind and degrade target mRNAs.\(^19\) In early 2018, the Food and Drug Administration (FDA) approved a lipid nanoparticle-formulated siRNA (patisiran) as the first siRNA drug to treat hereditary transthyretin-mediated amyloidosis. A number of siRNA-based therapies are now in clinical trials, and one of them is the lipid nanoparticle containing HSP47 siRNA for the treatment of liver fibrosis (NCT02227459).\(^23\)

During the progression of liver fibrosis, numerous membrane receptor signaling pathways, nuclear receptor signaling pathways, and transcription factors are dysregulated in HSCs.\(^10\) Because of its high specificity and potency to downregulate genes that are associated with liver fibrogenesis, siRNA is a type of promising therapeutic agent to reverse liver fibrogenesis.\(^17,24\) Table I summarizes the siRNA-based antifibrotic therapeutics that have been investigated since the discovery of RNAi. However, the highly negative charge and large molecular weight (~14 kDa) of siRNA limit its therapeutic application in vivo.\(^25\) In previous decades, different siRNA delivery systems have been developed, including cationic liposomes, polymeric nanoparticles, and micelles, to carry antifibrotic siRNA to activated HSCs.\(^17\) Moreover, due to the reduced perisinusoidal space and flow exchange in the fibrotic liver, ligands that can specifically target activated HSCs have also been discovered to enhance the delivery of siRNA to fibrotic liver.\(^18\) Please refer to a recently published review for more details about HSC-specific ligands.\(^26\) For example, using a novel biopanning strategy, a high-affinity peptide (peptide-431) was discovered to specifically target the highly expressed insulin-like growth factor II receptor on activated HSCs.\(^27\) Using the peptide-431, Zhao et al developed a peptide-modified siRNA nanocomplex to specifically deliver an antifibrotic siRNA to fibrotic liver.\(^18\)
COLLAGENS

The excessively accumulated ECM caused by over-expression and reduced degradation is the key characteristic of liver fibrosis.\(^1\) The ECM consists of different types of collagens, glycoproteins, proteoglycans, and glycoaminoglycans.\(^{28}\) In activated HSCs, type I collagen is the most abundant component of ECM and is produced by the COL1A1 and COL1A2 genes.\(^{28}\) Two COL1A1 gene-generated procollagen \(\alpha1(I)\) chains combine with one COL1A2 gene-generated procollagen \(\alpha2(I)\) chain to form a rope-like, triple-stranded procollagen, which is secreted by HSCs and then enzymatically processed and cross-linked to form stable type I collagen fibers.\(^{28}\)

In normal liver, collagens engage in a constant flux of remolding via a balance between synthesis and degradation by matrix metalloproteinases (MMPs). In fibrotic liver, the expression of collagens is upregulated by the SMAD and p38 MAPK signaling pathways.\(^{28,29}\) Profibrogenic cytokines such as IL-4/IL-13 and transforming growth factor beta (TGF-\(\beta\)) upregulate the expression of the COL1A1 gene. Additionally, the activity of MMPs was inhibited by tissue inhibitors of MMPs (TIMPs), resulting in excessively accumulated ECM.\(^{17,28,29}\)

Using siRNA to directly regulate collagen expression in HSCs has been proven to resolve liver fibrosis in vivo and in vitro.\(^{30-32}\) Jimenez Calvente et al developed a lipid-like nanoparticle to deliver COL1A1 siRNA and found that type I collagen expression was significantly inhibited in human HSC cell line LX-2 and carbon tetrachloride (CCL\(_4\))-induced fibrotic liver in mice without inducing innate immunity responses.\(^{30}\)

Moreover, a vitamin A-coupled lipid-like nanoparticle was developed to carry COL1A1 siRNA, which significantly inhibited collagen production without any severe adverse effects at a 3-mg siRNA/kg dose.\(^{31}\) Kaps et al also developed a cationic nanohydrogel particle to deliver COL1A1 siRNA for the treatment of CCL\(_4\)-induced liver fibrosis in a mice model.\(^{32}\) These cationic nanohydrogel particles significantly knocked down 70% of the COL1A1 mRNA in fibroblasts after 48 hours of transfection in vitro and suppressed 50% of COL1A1 mRNA in the liver of fibrotic mice.\(^{32}\)

In addition to direct regulation of the COL1A1 gene, other proteins related to the expression of collagens, such as \(\alpha\)-complex protein 2 (\(\alpha\)CP2), transport and Golgi organization 1 (TANGO1) and heat shock protein 47 (HSP47) have also been investigated to resolve liver fibrosis.\(^{11,33,34}\) The abnormal accumulation of ECM during liver fibrosis is positively correlated with an increase in the half-life of the COL1A1 mRNA from 1.5 hours in quiescent HSCs to more than 24 hours in activated HSCs.\(^{35,36}\) The increase in the mRNA half-life is mediated by \(\alpha\)CP2 protein (encoded by the PCBP2 gene), which belongs to the \(\alpha\)CP family and specifically binds to the 3'-untranslated region (3'-UTR) of COL1A1 mRNA, leading to stabilization of the mRNA.\(^{37}\) Shukla et al recently discovered a PCBP2 siRNA and found that the expression of type I collagen in alcohol-activated HSCs was significantly reduced via PCBP2 gene knockdown (Fig 1).\(^{37}\) Moreover, downregulation of \(\alpha\)CP2 protein in HSCs reverses alcohol-, TGF-\(\beta\)-, PDGF-, and epidermal growth factor (EGF)-induced fibrogenesis in vitro.\(^{11}\) Jain et al further developed a PCBP2 siRNA/peptide

<p>| Table I. siRNA for the treatment of liver fibrosis in vivo |</p>
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nucleic acid hybrid nanocomplex and efficiently reversed CCl4-induced liver fibrosis in a rat model.\textsuperscript{38} HSP47 is a collagen-specific chaperone protein, which assists the proper triple-helix formulation of procollagen in the endoplasmic reticulum to facilitate the secretion of collagen.\textsuperscript{34,39} Sato et al developed a vitamin A-coupled liposome to deliver an siRNA targeting gp46, the rat homology of HSP47, to resolve liver cirrhosis in dimethylnitrosamine-treated rats.\textsuperscript{34} TANGO1 is another type I collagen secretion-related protein, which is upregulated by TGF-β in murine and human cirrhotic liver tissues.\textsuperscript{33} Specific knockdown of the TANGO1 gene with siRNA led to retention of type I procollagen in the endoplasmic reticulum, promoting unfolded protein response (UPR)-mediated HSC apoptosis. Silencing of the TANGO1 gene using siRNA reduces the deposition of type I collagen in primary human HSCs, suggesting that inhibition of TANGO1 is a promising antifibrotic therapy.\textsuperscript{33}

\textbf{TGF-β}

The TGF-β superfamily contains a large number of functional proteins, including activins, inhibins, bone morphogenetic proteins (BMPs), growth differentiation factors, and glial-derived neurotrophic factors.\textsuperscript{40} Among them, the TGF-β family members TGF-β1, TGF-β2, and TGF-β3 have been found to be related to fibrotic diseases, especially liver fibrosis.\textsuperscript{40–42} They were identified in all phases of the development of chronic liver injury including hepatocyte apoptosis and HSCs activation.\textsuperscript{43} TGF-βs produced in Kupffer cells, endothelial cells, and HSCs can bind to their receptors (TβR1 and TβR2) and regulate specific gene transcriptions in liver cells through the TGF-β/SMAD signaling pathway (canonical pathway)\textsuperscript{40,43} to induce HSC activation, proliferation, and migration; induce collagen expression; inhibit HSC apoptosis; and inhibit ECM degradation.\textsuperscript{40,44–47} These TGF-βs and activins induce the expression of different collagens, including COL1A1, COL3A1, COL5A2, COL6A1, COL6A3, and COL7A1 via mediation of SMAD-2/3 transcription factors.\textsuperscript{48,49} Meanwhile, BMPs act as antifibrotic mediators via SMAD-1/5/8 transcription factors to suppress TGF-β-induced fibrotic gene expression.\textsuperscript{50} TGF-β1 drives ECM-related gene expression via the SMAD-2/3 axis and is accumulated itself during liver fibrogenesis.\textsuperscript{48,51} Similar to TGF-β1, TGF-β2 displays fibrotic activity via the SMAD-2/3 axis.\textsuperscript{52} Activins promote
the proliferation and differentiation of fibroblasts and the accumulation of ECM. Antagonists of TGF-β1 and TGF-β2 could be potentially used as antifibrotic agents. Direct knockdown TGF-β1 using siRNA has been reported to significantly decrease α-SMA and type I collagen expression in HSC-T6 cells and exerted antifibrotic effects in CCL4-induced mice and rats. In addition, silencing TβR2 with a short-hairpin RNA (shRNA) also inhibited the activation of HSCs and reduced the expression of collagens.

Unlike the profibrotic function of the SMAD-2/3 pathway, the SMAD-1/5/8 axis exhibited an antifibrotic effect activated by BMPs (BMP canonical pathway). BMPs were first identified to induce bone and cartilage formation and further discovered to have multiple functions in the liver regenerative response. Among all the activators, BMP-7 counteracts the TGF-β/SMAD signaling pathway by increasing the phosphorylation of the SMAD-1/5/8 axis through the type I receptor (activin-like kinase 3), which is another negative fibrosis regulator. Due to the exhibited opposite effect between TGF-β and BMP-7 during the progression of liver fibrosis, induced BMP-7 expression through a recombinant adenovirus vector showed an antifibrotic effect in rat and human HSCs and in a thioacetamide-induced fibrotic rat model. Gremlin 1 is a target protein that is upregulated along with TGF-β1 expression, phosphorylates SMAD2/3 in TGF-β signaling and is an antagonist of BMP-7. Using siRNA to knockdown gremlin 1 can suppress HSC activation and attenuate liver fibrosis in a CCL4-induced rat model. In addition, the miR-23b/27b cluster can also downregulate the expression of gremlin 1 to suppress HSC activation.

BMP-9 is another member of the BMP family that has been found to be overexpressed in liver fibrosis patients. Gremlin 1 using siRNA or other gene knockout treatments can attenuate the process of liver fibrosis in CCL4-induced mice.

PDGF

The PDGF family contains 4 different polypeptide subunits, PDGF-A, PDGF-B, PDGF-C, and PDGF-D, which function as 5 homo-/heterogeneous dimers (PDGF-AA, AB, BB, CC, and DD). As secreted cytokines, PDGFs are primarily produced by platelets, Kupffer cells, and endothelial cells and act on neighboring mesenchymal cells in a paracrine manner. PDGFs regulate many physiological and pathophysiological processes and affect proliferation, migration and survival of mesenchymal and other cells. During the process of liver damage, the expression of the PDGF family, especially PDGF-A and -B, increases significantly in endothelial cells, macrophages and activated HSCs. In addition, PDGF-B was determined to be one of the most potent factors for the activation of HSCs. Matsumoto et al demonstrated that miR-29a can promote liver fibrosis recovery in CCL4- or thioacetamide-induced mice through regulation of the mRNA.
expression of COL1A1 and PDGF-C. The PDGF family exerts their functional actions by binding with 2 receptors: PDGFR-α and PDGFR-β, which belong to the receptor tyrosine kinase family and bind to PDGFs with 3 different dipolymers: PDGFR-αα, αβ, and ββ, which are generally located in Kupffer cells, vascular endothelial cells, and fibroblasts. Homogeneous PDGFR-αα binds to PDGF-AA, -AB, -BB, and -CC, while homogeneous PDGFR-ββ interacted with PDGF-BB and -DD. By contrast, heterogeneous dipolymer PDGFR-αβ can bind with all subunits except the -AA dimer, followed by receptor phosphorylation and signal transduction. PDGFRs remain at a very low level in normal liver cells but significantly upregulated in a fibrotic liver. Previous studies have demonstrated the biological effects of PDGF-BB binding with PDGFR-β for the activation of HSCs during chronic liver injury, and specifically blocking the PDGF-BB/PDGFR-ββ axis can be a potential therapeutic regimen for liver fibrosis treatment. Receptor tyrosine kinase inhibitors, including sorafenib, imatinib, and sunitinib, which can target PDGFR-β, have been used to treat activated HSCs. Compared to these nonselective PDGFR-β inhibitors, using gene therapy to selectively knockdown the PDGFR-β expression in activated HSCs can be another therapeutic option. Using PDGFR-β siRNA, the activation and proliferation of HSCs in vitro and the progression of liver fibrosis in vivo were significantly suppressed. Codelivery of miR-29b1 targeting several profibrogenic genes including PDGFR-β with a small molecule hedgehog inhibitor can decrease the expression of collagen and α-SMA and has synergistic effect in the treatment of CBDL-induced mouse liver fibrosis. In addition, PDGFR-α is another target for liver fibrosis treatment, not only in activated HSCs but also in injured liver hepatocytes. Lim et al reported that PDGFR-α knockout mice exhibited decreased activation and proliferation in HSCs and attenuated the thioacetamide-induced cirrhotic liver, and the activation of LX-2 cells was suppressed by coculture with PDGFR-α siRNA-transfected Hep3B cells.

**MMPS AND TIMPS**

In a healthy liver, MMPs belong to a family of zinc-dependent endopeptidases and play an important role in maintaining the composition of ECM. There are 25 different MMPs that have been identified as being associated with the degradation of ECM in the liver, and their functions were reviewed in a recent article. The balance of matrix degradation activity is regulated by MMPs and their inhibitors (TIMPs), which are both important for the progression and regression of liver fibrosis. When the liver is injured, activated HSCs produce abundant amounts of types I and III collagens and also increase the secretion of TIMPs to inhibit the degradation of ECM regulated by MMPs. The hepatic expression of MMPs has been identified as being responsible for fibrogenesis and liver regression. Among them, MMP-2 and MMP-14 were highly expressed in activated HSCs and promote the migration, proliferation, and activation of HSCs. A MMP-2-specific siRNA was delivered by a vitamin A-coupled liposome and significantly reduced the expression of type I collagen and α-SMA in HSC-T6 cells.

In addition, knockdown of the expression of TIMPs in activated HSCs can be another effective strategy for fibrotic liver resolution. Among the identified 4 TIM family members, TIMP-1 has been identified as the most relevant MMP inhibitor, which is upregulated by several cytokines during the progression of liver fibrosis. Studies have also demonstrated that TIMP-1 not only prevented ECM degradation from MMPs but also inhibited the apoptosis of activated HSCs. Moreover, combining siRNAs targeting TIMP-1 and connective tissue growth factor exhibited a superior efficacy in hepatic precancerous fibrotic rats. TIMP-2 is another member of the TIMP family that is upregulated during liver fibrosis. Hu et al used a modified synthetic siRNA targeting TIMP-2 to inhibit TIMP-2 expression in CCl4-induced rats. Moreover, the siRNA increased the expression of MMP-13, which further promoted the degradation of ECM and enhanced the hepatocyte regeneration.

**PHOSPHOINOSITIDE 3-KINASES**

Phosphoinositide 3-kinases (PI3Ks) constitute a complicated signaling pathway involved in various cell function regulation processes. Once phosphatidylinositol-3,4,5-bisphosphate (PIP2) is converted to phosphatidylinositol-3,4,5-trisphosphate (PIP3) through PI3K catalyzation, it will further activate phosphoinositide-dependent kinase 1 and the serine-threonine kinase Akt, which play critical roles in cell proliferation, survival, and glucose metabolism.
pathway is triggered by the activation and stimulation of HSCs and growth factors. Studies have shown that activation of the PI3K pathway facilitates the proliferation, migration, and type I collagen expression of HSCs. Significantly, liver fibrosis progression is related to the PI3K/Akt pathway with multiple regulation aspects. The depletion of thymosin β4 (Tβ4) remarkably facilitates the proliferation and migration of LX-2 cells and has been reported to be associated with the activation of LX-2 cells via activation of the PI3K/Akt signaling pathway. Chen et al transfected LX-2 cells with Tβ4 siRNA and observed upregulated proliferation and migration of the cells. Another mechanism for regulating PI3K/Akt signaling is through siRNA targeting embryonic liver fordin (ELF) and glucose glycolysis-related proteins in activated HSCs.

Vascular endothelial growth factor (VEGF) is associated with pathological angiogenesis, and the proliferation of chronic liver fibrosis has already been reported in previous studies. Placental growth factor (PIGF), a member of the VEGF family, was reported to play an important role in liver fibrosis and angiogenesis progression. PIGF was found to be highly expressed in activated HSCs, which contributed to liver cirrhosis. Li et al used PIGF siRNA to reduce PIGF overexpression on HSCs, and the silencing effect of siRNA not only alleviated inflammation and fibrosis but also inhibited the activation and proliferation of HSCs via activation of the PI3K/Akt signaling pathways. Additionally, PIGF siRNA was involved in the downregulation of hepatic microvesSEL formation density and angiogenic factors, which were induced by hypoxia-dependent factor-1α (HIF-1α), VEGF and VEGF receptor-1. Growth factor receptor-bound 2 (GRB2) regulates HSC proliferation and differentiation. The activation of GRB2 was mediated via high-mobility group protein box1 (HMGB1), which was released from hepatocytes and increased in concentration during liver fibrosis. To reduce the overexpression of GRB2 that stimulates the cell proliferation and HMGB1 through the activation of the PI3K/Akt pathway in HSCs, Ge et al used GRB2 siRNA to ablate the proliferation of HSCs induced by HMGB1 and upregulation of α-SMA and collagen. The result further demonstrated that the relationship among HMGB1, GRB2, and PI3K/Akt phosphorylation could be an effective target for treating liver fibrosis.

These results also show that the PI3K/Akt signaling pathway plays a critical role in liver fibrogenesis, and siRNA targeting the PI3K/Akt pathway could be a novel therapeutic approach for chronic liver disease.

Receptor-type tyrosine-protein phosphatase O (PTPRO) is another molecule that is involved in physiological and pathological processes in liver fibrosis. Zhang et al revealed that synthetic shRNA targeting PTPRO could offset the proliferation of HSCs induced by PDGF-BB and downregulate PI3K/Akt, ERK, and other pathways. These results indicated that PTPRO is implicated in liver fibrogenesis and provided a novel approach for the treatment of liver fibrosis. Many studies have provided evidence that miRNAs are involved in the induction or inhibition of fibrogenesis. It has been found that unusual expression of miR-200s is associated with hepatic fibrosis. Xiao et al found that miR-200b increased cell growth and migration of HSCs and enhanced the PI3K/Akt signaling pathway and the expression of PI3K/Akt signaling and the expression of MMP-2. The miR-200b is therefore a potential marker for liver fibrosis progression and HSC activation. Moreover, specific inhibitors of miR-200b could be possibly used to reverse liver fibrosis.

NUCLEAR FACTOR-KAPPA B

The Nuclear factor-kappa B (NF-κB) signaling pathway is involved in multiple biological functions, such as the immune system, inflammation, cell apoptosis, embryonic development, pathogenesis, and oncogenesis. NF-κB signaling is regulated by binding to the inhibitor κB (IκB) family of proteins, IκB proteins, and the IκB kinase complex. Previous studies have indicated that the receptor for advanced glycation end products (RAGE) activates the NF-κB pathway by interacting with the ligands that enhances receptor expression. It has been reported that RAGE is significantly increased in activated HSCs. Cai et al investigated the mechanism and silencing effect of RAGE-specific siRNA on liver fibrosis in a rat model. The results showed that RAGE-specific siRNA attenuates the progression of liver fibrogenesis and reduces the expression of multiple inflammatory activities via NF-κB signaling pathway regulation. Moreover, the expression levels of α-SMA and type I collagen were also decreased after silencing RAGE. NLRC5 has been found to play an essential role in the negative regulation of the NF-κB signaling pathway in liver fibrosis progression. Liu et al investigated the mechanism of NLRC5 in hepatic fibrogenesis and the reversal process bytransfecting HSCs with PEGFP-C2-NLRC5 or NLRC5-siRNA, respectively. The results showed that NLRC5 was overexpressed during liver fibrosis and increased the expression of α-SMA and Col1α in...
HSCs. However, as NLRC5 was downregulated or knocked down by NLRC5-siRNA, the fibrotic response was reduced via inhibition of TGF-β1/SMAD-induced proliferation. HSC apoptosis was increased, and the activation of NF-κB signaling pathways was facilitated.\textsuperscript{110} Lou et al reported that the fibroblast growth factor receptor 1 pathway regulates the activation of lipopolysaccharide-induced HSCs. Fibroblast growth factor receptor 1-targeted siRNA decreased the activation of the lipopolysaccharide-induced NF-κB signaling pathway, inflammation, fibrosis, and proliferation in activated HSCs.\textsuperscript{111}

miRNAs

In addition to siRNA-based treatments, miRNAs can be another type of therapeutics for the treatment of liver fibrosis.\textsuperscript{112} miRNAs are endogenous small non-coding RNAs that regulate RNA expression post-transcriptionally. miRNA genes are transcribed into primary miRNAs by RNA polymerase II and then cleaved by RNase type III endonucleases to form double-stranded miRNAs.\textsuperscript{113} Finally, the double-stranded miRNAs are separated, and one of the strands is incorporated into the RISC complex, triggering specific degradation of target mRNA.\textsuperscript{113,114}

It has been demonstrated that some miRNAs correlate with various liver diseases, including liver fibrosis.\textsuperscript{112} For example, a 144-participants-enrolled clinical trial (NCT03905746) is designed to study the 800 circulating miRNAs in patients with acutely decompensated cirrhosis. miRNAs play different roles during the progression of liver fibrosis (Table II).\textsuperscript{115,116} The relationship between miRNAs and HSCs during liver fibrogenesis was recently reviewed.\textsuperscript{117} miRNAs can be either upregulated or downregulated during liver fibrogenesis. Upregulated miRNAs can be reversed by miRNA sponges, anti-miRNA oligonucleotides, and miRNA masking. For example, antagomirs are a class of chemically modified oligonucleotides that specifically and efficiently block the functions of upregulated miRNA.\textsuperscript{118,119} Antagomirs are, therefore, used to treat diseases caused by upregulated miRNAs. For example, miravirsen (SPC3649) is a locked nucleic acid-DNA mixmer that effectively inhibits the function of miR-122 in a phase IIa study (NCT01200420) for hepatitis C virus infection.\textsuperscript{120,121} Downregulated miRNAs can be restored by miRNA mimics or plasmid expressing the miRNAs.\textsuperscript{22}

Numerous miRNAs have been found to be upregulated during liver fibrogenesis. For example, miR-542-3p was found to control the activation of HSCs and to be upregulated in CCl\textsubscript{4}-induced mice. It promotes liver fibrosis by downregulating BMP-7 expression.\textsuperscript{115} The overexpression of miR-199 and miR-200 families was also found to be positively correlated with the TGF-β/SMAD signaling pathway during liver fibrogenesis in both a fibrotic mouse model and human clinical samples.\textsuperscript{116} miR-21 is an important gene implicated in the development of liver fibrosis, with a 24-fold induction in activated HSCs via the SMAD-7 signaling pathway.\textsuperscript{122,123} miR-125b is another upregulated miRNA that promotes the activation of HSCs through the RhoA signaling pathway, and the inhibition of miR-125b attenuated liver fibrosis in vivo.\textsuperscript{124}

In contrast to these upregulated miRNAs in liver fibrosis, some miRNAs, which inhibit fibrogenesis, were found to be downregulated.\textsuperscript{125,126} Chen et al reported that miR-129-5p, which binds to the 3'-UTR of the COL1A1 mRNA, is decreased in osteopontin-induced fibrotic liver tissues. Transfection of miR-129-5p mimic reduced the expression of type I collagen in activated HSCs.\textsuperscript{125} miR-454 is another miRNA found to be downregulated in the TGF-β1-stimulated mouse liver.\textsuperscript{127} Transfection with the miRNA-454 mimic significantly inhibited the activation and proliferation of TGF-β1-treated HSC-T6 cells, reduced the expression of type I collagen and α-SMA, and further inhibited cirrhosis progression in vivo.\textsuperscript{126}

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<td>Human fibrotic liver, bile duct ligation, Angll/mice</td>
<td>122,123</td>
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<tr>
<td>miR-129-5p</td>
<td>Downregulation</td>
<td>Type I collagen</td>
<td>Human fibrotic liver</td>
<td>125</td>
</tr>
<tr>
<td>miRNA-454</td>
<td>Downregulation</td>
<td>Wnt110a</td>
<td>CCl\textsubscript{4}/fat</td>
<td>126,127</td>
</tr>
<tr>
<td>miR-378a-3p, miR-378b, miR-378d</td>
<td>Downregulation</td>
<td>Hh signal pathway/Gli3</td>
<td>CCl\textsubscript{4}/mice</td>
<td>129</td>
</tr>
<tr>
<td>miR-122</td>
<td>Downregulation</td>
<td>Type I collagen/P4HA1</td>
<td>CCl\textsubscript{4}/mice</td>
<td>132,133</td>
</tr>
</tbody>
</table>

Table II. miRNA for the treatment of liver fibrosis
The hedgehog (Hh) signaling pathway was reported to be involved in the progression of liver fibrosis by promoting the activation and proliferation of HSCs. When the liver is injured, the Hh ligands Sonic Hh, Indian Hh, and Desert Hh are secreted from hepatocytes and initiate the proliferation of HSCs. HSCs further produce Hh ligands and activate the Hh signaling pathway via autocrine and paracrine mechanisms. The Hh ligand/receptor axis releases Smoothened (Smo), which translocates the glioblastoma (Gli) family into the nucleus to function as transcriptional activators. The activated Hh signaling pathway activates HSCs from quiescent to activated status and promotes the progression of liver fibrosis. Several miRNAs have been discovered to be correlated with the Hh signaling pathway. Among them, miR-378a-3p, along with its family members (miR-378b and miR-378d), was found to be significantly downregulated in activated HSCs and CCl4-treated fibrotic mouse liver. Hyun et al reported that miR-378a-3p suppressed the activation of HSCs via suppressing Gli3 expression. Meanwhile, the expression of miR-378a-3p is inhibited by Smo through the p65 subunit of the NF-kB signaling pathway. Delivery of the miR-378a-3p mimic by nanoparticles reduced the expression of Gli3 and liver fibrosis in CCl4-treated mice. In another study, miR-125b from chorionic plate-derived mesenchymal stem cells was reported to suppress the activation of the Hh signaling pathway and subsequently attenuate liver fibrosis. miR-122 was found to be highly expressed in HSCs, but its expression is downregulated during liver fibrogenesis. Zeng et al intravenously injected lentivirus expressing miR-122 and inhibited liver fibrogenesis in a CCl4-induced mouse model. In another approach, miR-122-modified adipose tissue-derived mesenchymal stem cells (AMSC-122) were developed to reverse liver fibrosis in vivo by mediating the miR-122 communication through exosomes between AMSCs and HSCs.

As mentioned in a previous review, the methods to restore miRNA expression in targeted cells or tissues are more difficult than to block miRNA expression because designed miRNA mimics should be double stranded and not extensively modified to maintain their ability to incorporate into the RISC complex. Similar to siRNAs, the key challenges for miRNAs-based therapeutics is to overcome serum degradation and achieve targeted delivery. A variety of viral and nonviral delivery systems have been developed for miRNA mimics. Up to now, there are still no microRNA-based therapeutics enrolled in clinical trials for liver fibrosis.

In addition to therapeutic applications, abnormal expression of miRNAs can be used as biomarkers for liver fibrosis and hepatocellular carcinoma. Liver fibrosis and cirrhosis were found at high risk in patients who have chronic hepatitis B (CHB) or C (CHC). In an early 250 patients enrolled study, serum miR-122 were determined at a lower level in patients with hepatic decompensation compared to compensated liver disease group, indicating that the serum miR-122 can be a novel biomarker to predict survival in patients with liver cirrhosis. In another study of 280 patients with different stage liver fibrosis, the expression of 13 fibrosis-related miRNAs was analyzed using RT-qPCR, and the expression of hepatic miR-122 was reduced in both CHB and CHC patients with advanced fibrosis/cirrhosis than mild/moderate. In patients with CHB, the serum miR-122 was reduced in advanced fibrosis stage, but no difference was found in CHC patients. In addition, serum miR-122 in CHB patients is 28-fold higher compared to CHC patients. Nakamura et al found a similar result about the serum miR-122 in CHB and CHC patients and demonstrated that the level of serum miR-122 in HBV-infected patients was higher than patients without liver disease. Other miRNAs including miR-34a, miR-181, miR-214, miR-571, and miR-652 were also reported as potential biomarkers for liver fibrosis.

CONCLUSION

The siRNA- and miRNA-based therapeutics is now being translated from bench to bedside, and 1 siRNA was recently approved by the FDA. The major challenge for nucleic acid-based therapeutics including siRNA, shRNA, and miRNA is the specific delivery to target cells or tissues. Numerous viral and nonviral delivery systems have been developed for nucleic acid-based therapeutics in vitro and in vivo for different diseases including liver fibrosis.

Liver fibrogenesis is highly correlated with the activation of HSCs, which is regulated by numerous profibrotic cytokines. Using RNAi to downregulate these cytokines in activated HSCs is a promising strategy to reverse liver fibrosis. Meanwhile, expressions of certain miRNAs correlate with the development of liver fibrosis. Regulation of fibrogenesis-related miRNA is, therefore, another promising strategy to reverse liver fibrosis. This review focuses on the current siRNA- and miRNA-based liver fibrosis treatment strategies by targeting activated HSCs in the liver. There is an antifibrotic siRNA in clinical trial, but miRNAs are still in preclinical stages. With the approval of the first-ever siRNA drug and gene therapy by the FDA, nucleic acid-based therapeutics have gained a lot of commercial interest, which will definitely facilitate the
development of siRNA- and miRNA-based antifibrotic therapies.

ACKNOWLEDGMENTS

Conflicts of Interest: The authors have read the journal’s policy on disclosure of potential conflict of interest and declare no potential conflicts of interest.

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