Therapeutic Potential of MicroRNAs and Their Nanoparticle-based Delivery in the Treatment of Liver Fibrosis

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Abstract Chronic liver disease is a global health problem owing to its high morbidity and the limited available treatment options. Liver fibrosis, the most common feature of chronic liver disease, is characterized by excessive accumulation of extracellular matrix (ECM) in the liver, eventually leading to cirrhosis. Hepatic stellate cells (HSCs), the major contributors to hepatic fibrosis, undergo transdifferentiation from a quiescent to an activated/myofibroblastic state, resulting in the accumulation of ECM. MicroRNAs (miRNAs) are small noncoding RNAs that are involved in the regulation of gene expression at the post-transcriptional level. Because miRNAs mediate a broad range of biological functions, dysregulation of miRNAs is strongly associated with various diseases, including liver fibrosis. Therefore, modulation of miRNAs by supplementing or inhibiting them represents a novel therapeutic strategy for liver fibrosis. With recent advances in our understanding of nanomedicines, nanoparticles are regarded as promising candidates for efficient delivery methods for miRNAs because of their biological and technical advantages. In this chapter, we review the pathogenesis of liver fibrosis, the roles of miRNAs in liver fibrosis, the therapeutic potential of miRNAs and their nanoparticle-based delivery for liver fibrosis, and the development of novel miRNA-based therapeutics for liver diseases.

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1 Introduction

Liver is the largest internal human organ in adults and has vital roles in maintaining homeostasis by regulating metabolism, bile production, and detoxification [1]. The liver has the greatest regenerative capacity of any organ in the body. Even with 70% surgical removal (partial hepatectomy) of the liver mass, the remnant tissue has the ability to grow into the original mass and to recover its functions [2, 3]. Because of this outstanding capacity, the liver can self-repair and restore itself after mild injury. However, when the liver damage is repetitive and/or severe, the regenerative capacity is impaired owing to massive death of hepatocytes, which triggers the proliferation of nonparenchymal cells, including hepatic stellate cells (HSCs), and replaces the damaged liver tissue [4, 5].

In the normal liver, HSCs are quiescent and function as the major storage facility for vitamin A metabolites known as retinoids [6]. However, when the liver is injured, HSCs undergo transdifferentiation from quiescent HSCs to activated/myofibroblastic HSCs, which are the principal cell types that produce extracellular matrix (ECM) proteins in the liver [7]. Excessive deposition of fibrous ECM components replaces the parenchyma with fibrotic tissue, which causes severe structural and functional alterations, leads to liver dysfunction, and eventually develops into liver fibrosis and cirrhosis [4, 5]. Cirrhosis, an end-stage disease, results in liver failure in many patients, leading to high mortality worldwide [8, 9]. The global incidences of cirrhosis and other chronic liver disease has been estimated at 1.5 billion and accounts for two million deaths per year [9]. Hence, many researchers have focused on the clearance, deactivation, or inactivation of HSCs as a therapy because of the essential role that HSCs play in pathogenesis [10]. Nevertheless, the therapies available for liver fibrosis are still limited [11]. Therefore, further investigation and development into new therapeutic strategies for liver fibrosis/cirrhosis are urgently needed.

MicroRNAs (miRNAs) are a class of short (approximately 18–24 nucleotides) endogenous noncoding RNA molecules that regulate gene expression during post-translation [12]. Since the discovery of miRNAs by Victor Ambros and his group in 1993 [13], their biological importance has rapidly emerged in recent decades. Usually, miRNAs bind to the 3′ untranslated region (UTR) of their target mRNAs and result in the translational inhibition, degradation, or cleavage of miRNAs depending on the degree of complementarity [14]. As a single miRNA can target hundreds of messenger RNAs and a single messenger RNA can also be targeted by numerous miRNAs, miRNAs influence complex networks of signaling pathways associated with almost all biological/cellular processes, including cell growth, differentiation, immune response, tissue remodeling, and cancer development [15–18]. Accumulating evidence has demonstrated that alterations in miRNA expression are intimately associated with the initiation and progression of human diseases, including chronic liver disease [19–21]. Consequently, modulating miRNA expression could be a key strategy for developing novel therapies for liver fibrosis/cirrhosis [22]. The systemic dosage of therapeutic miRNAs, unfortunately, has short half-lives in circulation and can induce toxicity; therefore, designing a delivery platform

that protects the therapeutic miRNAs, efficiently transporting therapeutic miRNAs to the liver and modulating the levels of specific miRNAs in vivo within activated HSCs, remains a challenge [23].

To address these obstacles, researchers are increasingly applying techniques developed in nanomedicines to deliver miRNA as a therapy for chronic liver disease [24, 25]. Our research group has developed a 'pseudo' poly[amino acid] polymer and has engineering L-tyrosine polyurethane (LTU) into biodegradable nanoparticles (NPs) as a delivery system for miRNA. The biological and technical advantages of these NPs include protection of miRNAs from enzymatic activity, absence of cellular toxicity by the NPs' degradation products, surface decoration of the NP with polyethylene glycol (PEG) to minimize the immune response, optimization of size distribution for endocytosis, and the ability to induce the proton sponge effect so that NPs can escape from endosomes and release nucleic acids into the cell's cytoplasm [26–28]. These features (Fig. 1) make LTU NPs an ideal delivery system for miRNA therapy and should be explored further as a therapeutic option for liver diseases [27, 29]. In this chapter, we summarize the general pathogenesis of liver

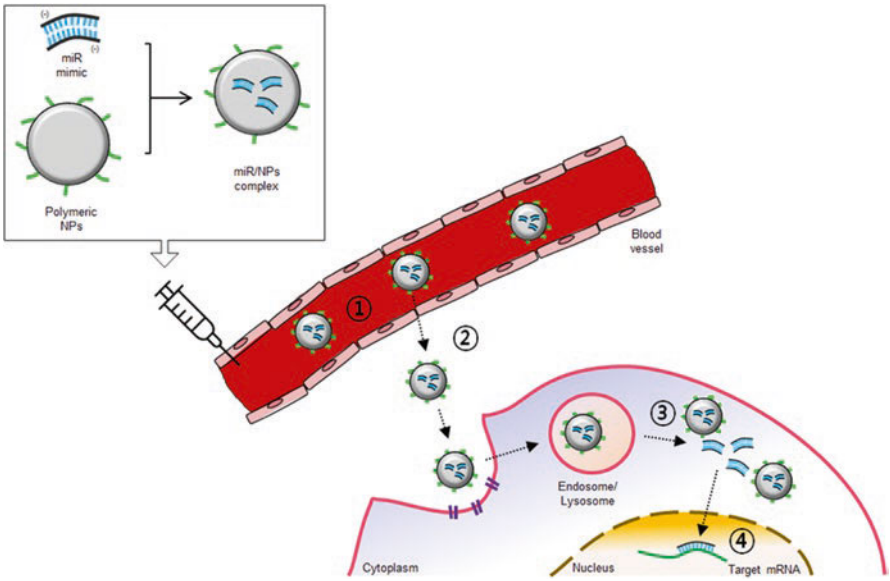

Fig. 1 A simplified model of nanoparticle (NP)-based delivery of microRNAs to target cells. Polymeric NPs are used as carriers of (miRNA) mimics and have substantial advantages over the administration of naked miRNA mimics. When miRNA is encapsulated into polymeric NPs and systemically administered to the body, the encapsulation prevents miRNA degradation by serum nucleases and results in a long period of circulation in the blood (①). Injury to tissues facilitates blood vessel dysfunction and gives NPs an opportunity to leak into diseased tissue and become endocytosed by hepatic stellate cells (②). Incorporating linear polyethylenimine into NPs can induce the proton sponge effect and allow NPs to escape from the endosomal/lysosomal pathway, and the NPs can be degraded and release miRNA in the cell's cytoplasm (③) so that it can enter the nucleus to induce gene knockdown (④)

fibrosis/cirrhosis, review the current roles of miRNAs in HSC activation and liver fibrosis, and evaluate the therapeutic potential of miRNAs encapsulated in LTU NPs as a treatment for liver fibrosis.

2 The Role of Hepatic Stellate Cells in Liver Fibrosis

Liver fibrosis is a wound-healing process in response to liver injury [4]. To date, substantial progress has been made in understanding the process of hepatic fibrosis, such as characterization of ECM components in fibrotic liver, identification of HSCs as the major source of ECM in liver fibrosis, and characterization of key signaling pathways in liver fibrosis (Fig. 2) [4, 10]. Of them, the identification and establishment of HSCs as the key cellular source of ECM in the liver have been a major advancement in elucidating liver fibrosis. The main fibrogenic cell type in the liver is activated or myofibroblastic HSCs [7, 10]. In the liver, HSCs reside in the

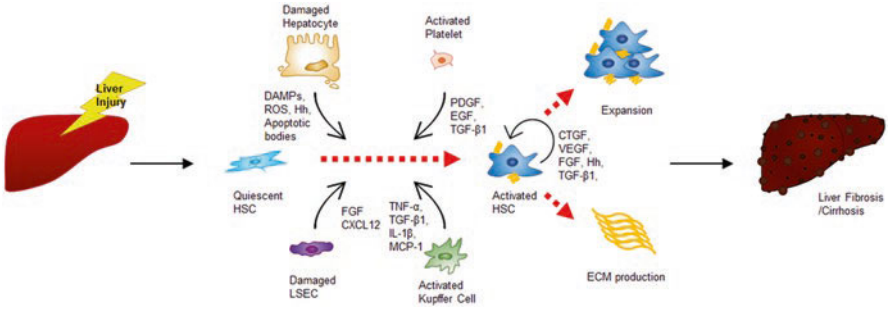

Fig. 2 A schematic summary of hepatic stellate cell (HSC) activation. Liver injury initiates the transdifferentiation/activation of quiescent HSCs to activated/myofibroblastic HSCs. Activation of HSCs consists of two phases: initiation and perpetuation. During the initiation phase, neighboring hepatic cells, including hepatocytes, Kupffer cells, liver sinusoidal endothelial cells (LSECs), and platelets, promote HSC activation by cytokines and other signaling molecules. Injured hepatocytes induce HSC activation by releasing multiple mediators, including damage-associated proteins (DAMPs), reactive oxygen species (ROS), hedgehog (Hh) ligands, and apoptotic bodies. Platelets are an important cellular source of platelet-derived growth factor (PDGF), epidermal growth factor (EGF), and TGF-β1, and these factors activate HSCs and promote liver fibrosis. Damaged LSECs produce fibroblast growth factor (FGF) and C-X-C motif chemokine ligand 12 (CXCL12), which are paracrine stimuli of HSCs. Activated Kupffer cells produce cytokines and chemokines, such as tumor necrosis factor alpha (TNF-α), transforming growth factor beta 1 (TGF-β1), interleukin-1 beta (IL-1β), and monocyte chemoattractant protein-1 (MCP-1), which directly influence HSC activation. During the perpetuation phase, autocrine and paracrine stimulations maintain the activated HSC phenotype and promote the production of fibrotic extracellular matrix (ECM). In addition to paracrine stimulation, activated HSCs produce and secrete connective tissue growth factor (CTGF), vascular endothelial growth factor (VEGF), FGF, Hh ligands, and TGF-β1 in an autocrine manner, which are known to promote the activation, maintenance, and expansion of HSCs and ECM production. These diverse paracrine/autocrine signals that converge upon HSCs promote HSC activation, leading to liver fibrosis and cirrhosis

subendothelial space of Disse between hepatocytes and sinusoidal endothelial cells and represent approximately 5–8% of the total number of resident cells [6]. In a normal healthy liver, HSCs exist in a quiescent state and serve as the principal storage site for retinoids by storing retinyl esters within lipid droplets present in the cytoplasm of HSCs. Following liver injury of any etiology, HSCs undergo an activation process, which involves cell transdifferentiation from quiescent cells into fibrogenic myofibroblasts [10]. This change is characterized by the loss of lipid droplets, increased proliferative and migratory activities, and accumulation of contractile filaments, including α-smooth muscle actin (α-SMA) [10]. Quiescent HSCs are also known to lose epithelial markers, such as E-cadherin, and gain mesenchymal markers, such as Snail1, thus undergoing an epithelial-to-mesenchymal transition (EMT)-like process to acquire myofibroblastic features during HSC activation [30]. Although other cell types, such as portal fibroblasts, also contribute to hepatic fibrogenesis, fate-tracing studies have confirmed that activated HSCs are the major source of ECM in chronically injured livers [31, 32].

Activation of HSCs consists of two phases: initiation and perpetuation [10]. During the initiation phase, paracrine stimulation from neighboring cells, including platelets, endothelial cells, and Kupffer cells, causes alterations in the gene expression and phenotype of HSCs that render them more responsive to other profibrogenic cytokines and stimuli [7, 10]. In addition, injured/dying hepatocytes release paracrine factors, such as damage-associated molecular patterns (DAMPs) and hedgehog (Hh) ligands, which promote the activation of HSCs [33]. Autocrine and paracrine stimulations during the perpetuation phase maintain the activated/myofibroblastic HSC phenotype and promote the production of fibrotic ECM components such as collagen, glycoprotein, and proteoglycans [7, 10]. Initiation is largely due to paracrine stimulation, whereas perpetuation involves autocrine and paracrine loops. Various growth factors, including platelet-derived growth factor (PDGF), epidermal growth factor, fibroblast growth factor, connective tissue growth factor (CTGF), and vascular endothelial growth factor), are known to promote the expansion and activation of HSCs [10]. A simplified illustration of the complex inter- and intracellular events in HSC activation and liver fibrosis is depicted in Fig. 1.

The intracellular processes by which HSCs regulate the initiation and perpetuation of fibrosis are complex and multifactorial involving various signal transduction pathways, such as the transforming growth factor-β (TGF-β), phosphatidylinositol 3-kinase (PI3K)/protein kinase B (AKT), Wnt/β-catenin and Hh pathways [10]. TGF-β, a well-known profibrotic cytokine, is produced by several cell types, including activated HSCs, platelets, and Kupffer cells, and promotes HSC activation through the mitogen-activated protein kinase and c-Jun N-terminal kinase pathways [34–36]. The PI3K/AKT pathway is activated and is required for the survival and proliferation of HSCs [37]. The Hh pathway, a well-characterized signal transduction pathway, is implicated in HSC activation and liver fibrosis. Hh signaling is critically involved in the proliferation and activation of HSCs, leading to liver fibrosis [33]. Inhibition of this pathway leads to decreased HSC activation and reduced hepatic fibrosis [38]. Fibrosis, if not treated, eventually progresses to advanced fibrosis and cirrhosis, which are the major causes of liver-related morbidity and

mortality; therefore, the development of antifibrotic treatments that prevent and/or reverse liver fibrosis is urgently needed [11]. An effective method of preventing or halting liver fibrosis is to attenuate the activation of HSCs in response to chronic hepatic injuries [10]. There are several predominant strategies that contribute to the clearance of activated HSCs and resolution of fibrosis, such as induction of HSC apoptosis or senescence and reversion of HSCs to an inactivated state [39–41]. Although the antifibrotic activities of many drugs or compounds have been demonstrated in vitro and in vivo, none has been clinically validated or commercialized as a therapy for liver fibrosis [11]. Therefore, further research is required to develop novel antifibrotic therapies in the treatment of chronic liver disease.

3 The Roles of MicroRNAs in Liver Fibrosis

As the liver has essential functions in the human body that require highly orchestrated and regulated processes, hepatic physiology is tightly controlled by a complex maze of regulatory networks. Hence, disruption of these regulatory networks is associated with the progression of liver diseases [11]. MiRNAs are known to be involved in the regulation of liver homeostasis, development, regeneration, and metabolic functions by modulating the gene expression of their targets [19]. Increasing evidence suggests that alterations of intrahepatic miRNA levels have been associated with almost every aspect of liver disease, including liver fibrosis/cirrhosis [19, 20]. Given that miRNAs are involved in regulating cell homeostasis and functions and that specific expression of miRNAs reflects the current state of cells, expression changes in miRNAs in liver tissues are closely associated with the progression of liver fibrosis. To date, more than 2500 miRNAs have been identified in humans [42]. MiRNA expression signatures are known to be highly tissue specific in human tissues, and approximately 300 miRNAs have been reported to be present in the human liver [43]. MiR-122 is the best-studied miRNA in hepatic miRNA pools. Most importantly, miR-122 is liver specific, and it is one of the most abundant miRNAs in the normal liver, making up 70% and 52% of the whole miRNA pools in adult mice and humans respectively [44–46]. MiR-122 is specifically expressed by healthy hepatocytes, which are the major parenchymal cells in the liver [44, 47] and primarily involved in normal hepatocyte functions to maintain liver homeostasis [48]. The level of miR-122 decreases in the injured liver and is related to the development of liver diseases [49–51]. MiR-122 was strongly decreased in the fibrotic livers of human patients with non-alcoholic steatohepatitis and liver fibrosis and in the hepatotoxin fibrosis model, where mice are injected with carbon tetrachloride (CCl_4) [52, 53]. In experimental animal models, knockout (KO) of miR-122 in mice promotes liver fibrosis and these responses are alleviated and reversed by the restoration of miR-122 levels in these mice [48, 54]. The molecular targets of miR-122 include profibrogenic factors, such as Krüppel-like factor (Klf6), TGF-β receptor, and Wnt-1 [48, 55–58]. These data suggest that miR-122 might have antifibrotic properties; however, Schueller et al. demonstrated that

miR-122 expression is neither regulated nor relevant to HSC activation [59]. As detectable levels of miRNAs in liver tissue vary significantly depending on the conditions of the liver, the relative abundance or scarcity of miRNAs is directly influenced by variations in the cell populations. Given that activated HSCs are highly proliferative after liver injury and produce large amounts of ECM proteins, relating expression changes of miRNAs to the activation status of HSCs could be critical to understanding and treating pathological conditions of the liver.

Many reports have profiled the differences in miRNA expression between quiescent and activated HSCs or between healthy and fibrotic liver tissues to identify miRNAs closely associated with HSC activation and liver fibrosis [60–64]. Examples of miRNAs that have been implicated in the development of liver fibrosis, activation of HSC, and deposition ECM are given Table 1. These miRNAs can be broadly categorized into either profibrotic or antifibrotic, which are either upregulated or downregulated, respectively, during fibrogenesis [114]. However, inconsistencies in dysregulated miRNAs have been reported because of the different methods that have used to analyze microarray results, to activate HSCs, and to induce hepatic injuries using different animal models. Nevertheless, several key miRNAs have been clearly demonstrated to play either profibrotic or antifibrotic roles in the regulation of HSCs and liver fibrosis, and the targets of miRNAs have been identified and confirmed by multiple studies [19, 20, 114]. In this section, we focus on miRNAs that have been well documented to have a close association with HSC activation and present their therapeutic potential in liver fibrosis.

3.1 Potential Antifibrotic miRNAs

MiR-29 family members, including miR-29a, miR-29b, and miR-29c, are one of the best-studied miRNAs for HSCs and have been shown to be antifibrogenic [114, 115]. The downregulation of miR-29s have been linked to human cirrhotic livers and rodent models of liver fibrosis induced by carbon tetrachloride (CCl_4) (Fig. 3b) and by bile duct ligation [64]. MiR-29s are highly expressed in primary quiescent HSCs isolated from rodents but downregulated in activated HSCs by in vitro culture-induced activation and in vivo activation by CCl_4 injection [64, 116]. Furthermore, restoration of miR-29b by the administration of miR-29b mimic or miR-29b-expressing adeno-associated virus suppresses HSC activation and liver fibrosis in CCl_4-treated mice [76, 117]. Wang et al. showed that miR-29b inhibits HSC proliferation by arresting the cell cycle in the G1 phase and induces HSC apoptosis by inhibiting the PI3K/AKT pathway [118]. Zhang et al. reported that miR-29b suppresses heat shock protein 47 (HSP47) and lysyl oxidase (LOX), which are necessary for ECM maturation, and inhibits the maturation and production of collagens by HSCs [119]. In addition, various target genes of miR-29s have been identified, and the majority of them are involved in HSC activation, such as Col1α1, Col4α5, Col5α3, elastin, TGF-β, PI3K receptor 1, AKT3, PDGF-C, insulin-like growth factor I (IGF-I), and histone deacetylase 4 (HDAC4) [76, 77, 115, 118, 120, 121].

Table 1 List of microRNAs associated with liver fibrosis

Name	Pro- or anti-fibrotic	Target(s) in liver fibrosis	References
let-7/Lin28	Anti	HMGA2	[65]
miR-15b	Anti	LOXL1	[66]
miR-16	Anti	LOXL1, Gα 12	[66, 67]
miR-19b	Anti	CCR2, CTGF, TGFβRII	[68, 69]
miR-21	Pro	HNF4α, PDCD4, SMAD7, SPRY2	[70–73]
miR-25	Anti	ADAM17, FKBP14	[74]
miR-27	Pro	LXRα, SREBP1c	[75]
miR-29	Anti	CD36, COL1α1, HDAC4, PDGFC, SMAD3	[64, 76–79]
miR-30	Anti	BECLIN1, KLF11, SNAI1	[80–82]
miR-34	Pro	ACSL1, PPARγ	[83, 84]
miR-101	Anti	KLF6, TGFβRI	[85, 86]
miR-122	Anti	CTGF, PACT, P4HA1	[51, 87, 88]
miR-125b	Anti	SMO	[89]
miR-130a	Anti	TGFβRI, TGFβRII	[90]
miR-133a	Anti	COL1α1	[91]
miR-142	Anti	TGFβRI	[92]
miR-145	Anti	ZEB2	[93]
miR-146a	Anti	IRAK1, TRAF6, WNT1, WNT5A	[94, 95]
miR-185	Anti	RHEB, RICTOR	[96]
miR-193	Anti	CAPRIN1, TGFβ2	[97]
miR-195	Pro	SMAD7	[98]
miR-199	Pro	KGF	[99]
miR-200a	Anti	GLI2, GLI3, SIRT1	[100–102]
miR-200c	Pro	FOG2	[103]
miR-214	Pro	MIG6, SUFU	[104, 105]
miR-222	Pro	CDKN1B, PPP2R2A, TIMP3	[106–108]
miR-378a	Anti	GLI3, PRKAG2	[62, 109]
miR-486	Anti	SMO	[110]
miR-542	Pro	BMP7	[111]
miR-942	Pro	BAMBI, PPARγ	[112, 113]

ACSL1 Acyl-CoA Synthetase Long Chain Family Member 1, *ADAM17* ADAM Metallopeptidase Domain 17, *BAMBI* BMP and Activin Membrane Bound Inhibitor, *BMP7* Bone Morphogenetic Protein 7, *CAPRIN1* Cell Cycle-Associated Protein 1, *CCR2* C-C Motif Chemokine Receptor 2, *CDKN1B* Cyclin-Dependent Kinase Inhibitor 1B, *COL1α1* Collagen Type I Alpha 1 Chain, *CTGF* Connective Tissue Growth Factor, *FKBP14* FKBP Prolyl Isomerase 14, *FOG2* Friend Of GATA 2, *Gα 12* Guanine nucleotide-binding a-subunit 12, *GLI* GLI-Krüppel Family Member, *HDAC4* Histone Deacetylase 4, *HMGA2* High Mobility Group AT-Hook 2, *HNF4α* Hepatocyte Nuclear Factor 4 Alpha, *IRAK1* Interleukin 1 Receptor-Associated Kinase 1, *KLF* Krüppel-Like Factor, *LOXL1* Lysyl Oxidase-Like 1, *LXRα* Liver X Receptor-Alpha, *miR* microRNA, *MIG-6* Mitogen-Inducible Gene 6, *PACT* PKR-Activating Protein, *PDCD4* Programmed Cell Death 4, *PDGFC* Platelet-Derived Growth Factor C, *PPARγ* Peroxisome Proliferator-Activated Receptor Gamma, *PPP2R2A* Protein Phosphatase 2 Regulatory Subunit Alpha, *PRKAG2* Protein Kinase AMP-Activated Noncatalytic Subunit Gamma 2, *P4HA1* Prolyl 4-Hydroxylase Subunit Alpha 1, *RHEB* Ras Homolog Enriched In Brain, *RICTOR* RPTOR Independent Companion Of MTOR Complex 2, *SMAD* SMAD Family Member, *SIRT1* Sirtuin 1, *SMO* Smoothened, *SNAI1* Snail Family Transcriptional Repressor 1, *SPRY2* Sprouty RTK Signaling Antagonist 2, *SREBP1c* Sterol Regulatory Element Binding Transcription Factor 1, *SUFU* Suppressor of Fused Homolog, *TGFβ* Transforming Growth Factor Beta, *TGFβR* TGFβ Receptor, *TIMP3* Tissue Inhibitor of Metalloproteinases 3, *TRAF6* TNF Receptor-Associated Factor 6, *WNT* Wnt Family Member, *ZEB2* Zinc Finger E-Box Binding Homeobox 2

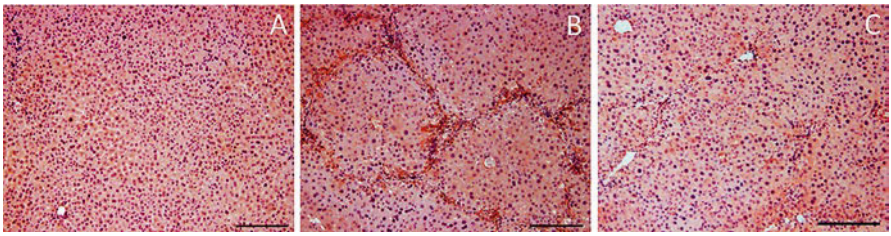

Fig. 3 Immunohistochemistry of liver sections for (**a**) normal, (**b**) carbon tetrachloride treatment, (**c**) L-tyrosine polyurethane (LTU) nanoparticle (NP) treatment for 3 weeks. Positive staining for α-smooth muscle actin can be observed for mice treated with carbon tetrachloride. LTU NP treatment prevented the matrix protein deposition. These images originally appeared in *Nature Communications* [62]

Other antifibrogenic miRNAs in the liver are miR-30 family members including miR-30a, miR-30b, miR-30c-1, miR-30c-2, miR-30d, and miR-30e. Abundant miR-30 levels in healthy liver are also reduced in fibrotic livers in human patients and in experimental mice [80, 122]. In primary HSCs isolated from mice, the expression of miR-30 decreases during in vivo and in vitro activation of HSCs [80]. Re-establishment of miR-30s by administration of miR-30-expressing lentivirus inhibits HSC activation and prevents CCl_4-induced liver fibrosis in rodents [80]. Direct targets of miR-30 include the following: CTGF, which is a profibrogenic cytokine; KLF11, which is a mediator of TGF-β signaling; and Snail1, which is a well-known EMT-stimulating transcription factor [80, 81, 123].

Our research group also reported that three members of the miR-378 family, mi-378a-3p, miR-378b, and miR-378d, are downregulated in both activated HSCs and cirrhotic livers of CCl_4-treated mice compared with quiescent HSCs and livers from corn oil-treated mice [62]. Among the three miR-378 family members, miR-378a-3p directly inhibits GLI-Krüppel family member 3 (Gli3), which is a transcriptional activator of the Hh pathway, and suppresses the activity of this pathway, leading to inactivation of HSCs [62]. In addition, restoration of miR-378a-3p in vivo by administration of biodegradable NPs releasing miR-378a-3p mimic attenuates CCl_4-induced liver fibrosis by downregulating Gli3 expression and suppressing HSC activation [62]. Therefore, these findings clearly demonstrate the inhibitory role of miRNA in HSC activation and liver fibrosis, suggesting them as potential therapeutics for treating liver fibrosis.

3.2 Potential Profibrotic miRNAs

Upregulation of miRNA during HSC activation usually plays a profibrotic role in liver fibrosis. MiR-222 and its paralog miR-221 are known to have oncogenic functions in the liver [124] and are also associated with liver fibrosis [106–108]. Ogawa et al. first showed that miR-221/222 expression is upregulated in patients with HCV

infection and non-alcoholic steatohepatitis (NASH) with liver fibrosis, and miR-221/222 expressions correlate positively with the messenger RNA expression of col1α1 and α-SMA [108]. Increased expression of miR-221/222 is also confirmed in a thioacetamide (TAA)-induced mouse model of liver fibrosis [108]. In addition, miR-221/222 is upregulated during HSC activation and regulates the expression of cyclin-dependent kinase inhibitor 1B (CDKN1B) [108]. Recently, Jiang et al. demonstrated that liver-specific miR-221/222 KO mice treated with CCl_4 exhibit a significant reduction in liver fibrosis compared with CCl_4-treated wild type mice [107]. In contrast, the reinduction of miR-221/222 by adenovirus infection worsened liver fibrosis in the CCl_4-treated miR-221/222 KO mice, suggesting the profibrotic potential of miR-221/222 [107]. However, these researchers employed the albumin-cre/LoxP system to produce liver-specific miR-221/222 KO mice, and miR-221/222 was abolished in only albumin-expressing cells, such as hepatocytes, but not in activated HSCs. Given that activated HSCs express miR-221/222 in mice with fibrotic liver, these cell-specific miR-221/222 KO mice should be further investigated. The direct targets of miR-221/222 have been identified as protein phosphatase 2A subunit B (PPP2R2A) and tissue inhibitor of metalloproteinase-3 (TIMP-3) [106, 107].

Another profibrogenic messenger RNA is miR-214. Fibrotic liver shows increased expression of miR-214 in human patients and CCl_4-injected mice, demonstrating a positive correlation with the degree of liver fibrosis [104, 125]. The level of miR-214 is elevated during both in vitro and in vivo activation of primary murine HSCs [104, 105, 125]. Furthermore, inhibition of miR-214 by antagomir-214 suppresses the proliferation and activation of HSCs in vitro and has ameliorated CCl_4-induced liver fibrosis in mice [104]. Ma et al. demonstrated that miR-214 directly inhibits the expression of suppressor-of-fused homolog (Sufu), a negative regulator of the Hh signaling pathway, and the knockdown of miR-214 expression in vivo enhances the expression of Sufu, which alleviates hepatic fibrogenesis [104].

4 Nanoparticle-based Delivery of MiRNA for Liver Fibrosis Therapy

Given the importance of miRNAs in modulating HSC activation and their implications in liver fibrosis, major efforts have been made to develop miRNA-based therapeutics to prevent and/or cure liver fibrosis [19–21]. As abnormal expression of miRNA is intimately associated with pathogenesis of liver fibrosis/cirrhosis, many researchers have reported that modulation of miRNA levels through restoration of antifibrotic miRNAs or suppression of profibrotic miRNAs leads to the recovery of liver fibrosis in various experimental animal models [19, 20]. Despite the therapeutic potential of miRNAs, the progress of miRNA-based therapeutics is hampered by an inability to effectively deliver miRNAs in vivo [29, 126, 127]. The major limitation of miRNA delivery includes its lack of stability in a circulatory system,

difficulty in reaching the target tissues, and immunotoxicity [128]. Naked miRNAs are degraded within seconds by an abundance of serum nucleases in the blood, or they are cleared rapidly via renal excretion, resulting in a short half-life in systemic circulation [127, 128]. The presence of naked miRNAs in the circulation also triggers secretion of inflammatory cytokines and type I interferons through Toll-like receptors, which provoke an inflammatory response and may cause systemic immune toxicity [29, 127, 128]. Even if miRNAs reach their target tissues, the negative charge of miRNA limits their ability to cross the cell membranes, [127] and any miRNAs that are endocytosed become trapped in endosomes and can be degraded by lysosomes [128, 129].

Viral vectors are frequently used as carriers to deliver miRNAs because high infection rates can be achieved [130]. Viruses have an innate ability to protect gene material within their capsids, recognize specific cells, transport their genetic material across cellular nuclear membranes, and escape from endosomes. These characteristics are attractive for miRNA delivery [130, 131]. However, these vectors are associated with diseases and raise significant medical concerns, and the process of generating recombinant viruses does not reduce their potential for immunogenicity or inducing cancer for retroviruses [27, 130].

In contrast, nonviral approaches are becoming attractive alternatives because many of the beneficial viral functions can be artificially replicated in the design of NPs without having the possibilities of inducing the diseases associated with viruses. Depending upon the materials and design, NPs could have low toxicity, low immune responses, surface decoration for targeting cellular receptors, biodegradability, and cost-efficient production [29, 131]. Although many types of polymer are available, the degradation rate and toxicity of the degradation products are critical to the success of NPs for gene therapy. Our research group has developed a polymer by modifying L-tyrosine [26, 28, 132], as amino acids are the building blocks of proteins. Although amino acids can be polymerized using peptide bonds and folded within cells into secondary and tertiary structures, folding limits the amount of nucleic acids that can be encapsulated, which also limits manufacturing on a large scale. These limitations have been overcome by chemically modifying the structure of L-tyrosine with two linkages (Fig. 4). Desaminotyrosine, an L-Dopa analog, is linked to the amide functional group using a peptide bond and connects to aromatic functional group on the L-tyrosine through polyurethane polymerization. The carboxyl terminal of L-tyrosine has been protected to prevent unwanted branching and undesired byproducts [132]. The polymerization results in LTU with a molecular weight of approximately 115 kDa [132] and is classified as a 'pseudo' poly [amino acid]. LTU is soluble in chloroform and can be easily processed into NPs using standard techniques. Previous studies show that LTU films degrade [133] through hydrolytic and enzymatic linkages in LTU's backbone. The degradation rate makes LTU an ideal candidate for the treatment of liver as it provides continuous release of nucleic acids for approximately 1 month. LTU and its degradation products also have been shown to be noncytotoxic. Human dermal fibroblasts incubated with 800 mg/ml of degradation products of LTU for 24 h show no significant reduction in cell viability compared with cells incubated with cell culture media [28].

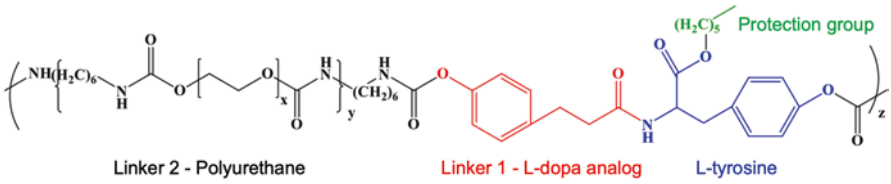

Fig. 4 Chemical structure of L-tyrosine polyurethane. L-tyrosine is modified with L-dopa analog and polyurethane linkages

Nanoparticles made with LTU are encapsulated with either FITC (LTU-FITC NPs, Fig. 5a) or miRNA-378a-3p (LTU-miRNA NP) against Gli3 messenger RNA using water-in-oil-in-water emulsion technique. Prior to the formation of these emulsions, nucleic acids are complexed with linear polyethylenimine (LPEI, MW 25 KDa) at a ratio of 1:1 and 5:1 respectively, which minimizes shear degradation when exposed to high mixing conditions. PEG-PLA, an amphiphilic copolymer that accumulates at the oil–water interfaces, is added to decorate the surface of NPs with PEG. After solvent evaporation, the NPs are washed and lyophilized. The resulting NPs are spherical with heterogeneous size distributions with a mean diameter of 1647 and 340 nm, respectively. As the size of the NPs is appropriate, the mechanism of uptake could be through endocytosis, and fluorescence microscopy shows LTU-FITC NPs were taken up by human hepatic stellate (LX2) cells (Fig. 5b and c).

Prior to the formation of an emulsion, nucleic acids are complexed to LEPI to prevent their degradation from exposure to high levels of shear stress during NP formation [26]. The analysis of the release studies (Fig. 5d) shows an initial burst release of mRNA; the release rate is initially slow (0.13 μg of mRNA per mg of NPs) between days 4 and 21. Afterward, the release rate rises sharply (1.2 μg of mRNA per mg of NPs) from week 3 to week 5 and reaches a steady state after week 5. Overall, a biphasic release of mRNA is observed. Thus, a sustained release of bioactive mRNA has been observed, and these NPs should be able to provide continuous release of miRNA for 5 weeks.

Liver fibrosis has been induced in a mouse model (male C57Bl/6) by intraperitoneal (IP) injections of carbon tetrachloride (CCl_4) at a concentration of 0.4 ml/kg. Figure 6a shows the injection schedule of both CCl_4 and LTU-miRNA NPs. Alanine transaminase (ALT), which is a clinical marker for liver disease, increased by 270% compared with control (no CCl_4 injections) after the first week of CCl_4 injections and remained high for the third week (230%, Fig. 6b). Animals injected with LTU-miRNA NPs show attenuation of ALT levels (23% higher than normal at week 3, Fig. 6b). The results for aspartate transaminase (AST), also a clinical marker for liver disease, mirrored the ALT results (increased by 26 and 46% for weeks 2 and 3, respectively, for CCl_4-injected mice and levels returning to 4% for LTU-miRNA NP-treated mice at week 3, Fig. 6c). Mice treated with CCl_4 show downregulation of miRNA-378a-3p (−82%, −72%, and −62% for weeks 1, 2, and 3, respectively,

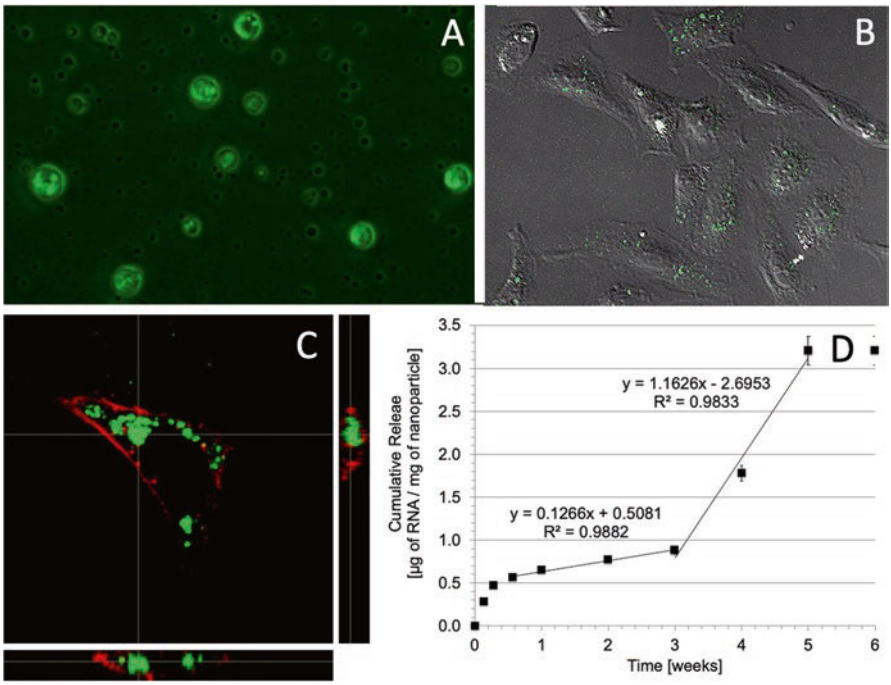

Fig. 5 Fluorescence microscopy and release profile of (**a**) L-tyrosine polyurethane fluorescein isothiocyanate LTU-FITC nanoparticles (NPs), (**b**) LX2 cells incubated with LTU-FITC NPs. (**c**) Confocal microscopy of LX2 cells with actin staining (red) incubated with LTU-FITC NPs, and (**d**) release profiles of LTU NPs loaded with miRNA. Images **a**–**c** originally appeared in *Nature Communications* [62]

compared with control tissues, Fig. 6d). In contrast, mice treated with LTU-miRNA NPs show −45% at week 1 (Fig. 6d). However, miRNA-378a-3p levels increase to 42% and 68% for weeks 2 and 3, respectively (Fig. 6d). These enzyme markers and miRNA-378a-3p levels correlated with increased matrix deposition (Fig. 3b) for CCl_4-treated mice. These results are confirmed by QRT-PCR results showing 35-, 19-, 12-, and 67-fold increases for gli3, collagen, α-smooth muscle actin, vimentin, and collagen, respectively. In contrast, mice treated with LTU-miRNA NPs show only 11-, 5-, 5-, and 18-fold increases, respectively. Together, exposure to CCl_4 initiates inflammation and fibrosis, but a single treatment of LTU NPs continuously releasing miRNA-378a-3p over a period of 3 weeks offsets the negative effects of CCl_4, prevents the deposition of matrix proteins (Fig. 3c), and normalizes the liver function (Fig. 6).

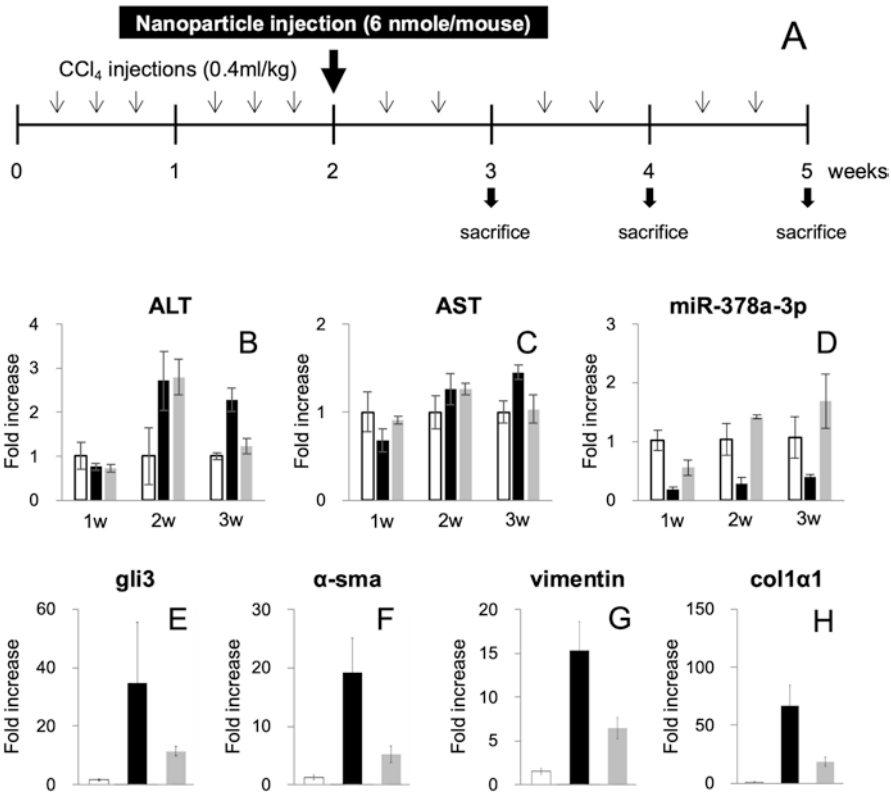

Fig. 6 Injection protocol and results. (**a**) Injection schedule and mRNA levels of (**b**) ALT, (**c**) AST, (**d**) miR-378-3p, (**e**) gli3, (**f**) α-SMA, (**g**) vimentin, and (**h**) collage for control (white), CCl_4 injected (black), and LTU-miRNA NP-treated animals (gray). These images originally appeared in *Nature Communications* [62]

5 Conclusions

Liver fibrosis is a common pathological feature for most chronic liver diseases regardless of the etiology and molecular progression to liver cirrhosis, which has high global morbidity and mortality [4]. A cure currently does not exist for liver fibrosis/cirrhosis, which was once thought to be irreversible, but recent medical evidence suggests that liver fibrosis might be reversed if treated properly. The diagnosis and management of liver diseases are also difficult because patients are asymptomatic until the advanced stages. As a substantial amount of research has linked a broad array of miRNAs to the pathogenesis of liver fibrosis, they also represent potential targets for the diagnosis and therapeutics of liver fibrosis [19, 22], and several clinical trials using miRNA-based treatment are in progress. However, limitations of the in vivo application of miRNAs include unwanted side effects

owing to their target multiplicity and redundancy and the development of a suitable delivery system [127, 128].

It has been shown that hepatic levels of miR-378a-3p correlate inversely with gli3 expression, and fibric liver can be rescued by the delivery of this molecule in an animal model using biodegradable NPs. A broad array of miRNAs are closely associated with the pathogenesis of liver fibrosis and represent potential targets for diagnosis and treatment of this disease [19, 22]. For example, Hu et al. used PLGA-based NPs to deliver miR-449b-5p to a rat model of ischemia/reperfusion (I/R) hepatic injury [134]. Although the I/R model is different from the liver fibrosis model, miR-449b-5p-loaded NPs inhibit the expression of high-mobility group box 1 (HMGB1), which is a target of miR-449b-5p, and mitigates I/R-induced hepatic injuries in rats [134]. The results using LTU-miRNA NP are also promising and underscore the valuable role of a delivery system with an intelligent design, but liver fibrosis has been caused by an artificial factor and treated with only one miRNA. Thus, directly translating our results for humans could be problematic because liver fibrosis is a chronic disease with multifactorial causes. A comprehensive strategy that delivers multiple miRNAs should be considered to advance novel therapies for fibrotic liver diseases, as many factors are involved in liver pathogenesis. A panel of miRNAs could even be identified for a specific patient and encapsulated into a cocktail of NPs, as technologies in high-throughput screening and personalized medicine have also shown recent advancements. The miRNAs for these types of therapies can be encapsulated into various formulations of NPs designed for multiple release kinetics to ensure the bioavailability of specific miRNA at the desired times. Table 1 gives a short list of potential targets and the corresponding miRNAs could be used for comprehensive miRNA delivery. Therefore, miRNAs are attractive for medical therapeutics because of their ability to regulate numerous pathways, molecular targets can be easily changed by altering the miRNA's genetic sequence, and a delivery system can be designed for safe, effective, and targeted delivery that minimizes unwanted side effects.

References

1. Boyer, T. D., & Lindor, K. D. (2016). *Zakim and Boyer's hepatology: A textbook of liver disease e-book*. Elsevier Health Sciences.
2. Higgins, G. (1931). Experimental pathology of the liver. *Archives of Pathology, 12*, 186–202.
3. Michalopoulos, G. K., & DeFrances, M. C. (1997). Liver regeneration. *Science, 276*(5309), 60–66.
4. Bataller, R., & Brenner, D. A. (2005). Liver fibrosis. *The Journal of Clinical Investigation, 115*(2), 209–218.
5. Schuppan, D., & Afdhal, N. H. (2008). Liver cirrhosis. *Lancet, 371*(9615), 838–851.
6. Friedman, S. L. (2008a). Hepatic stellate cells: Protean, multifunctional, and enigmatic cells of the liver. *Physiological Reviews, 88*(1), 125–172.
7. Friedman, S. L. (2008b). Mechanisms of hepatic fibrogenesis. *Gastroenterology, 134*(6), 1655–1669.

8. Bosetti, C., Levi, F., Lucchini, F., Zatonski, W. A., Negri, E., & La Vecchia, C. (2007). Worldwide mortality from cirrhosis: An update to 2002. *Journal of Hepatology, 46*(5), 827–839.
9. Moon, A. M., Singal, A. G., & Tapper, E. B. (2020). Contemporary epidemiology of chronic liver disease and cirrhosis. *Clinical Gastroenterology and Hepatology, 18*(12), 2650–2666.
10. Tsuchida, T., & Friedman, S. L. (2017). Mechanisms of hepatic stellate cell activation. *Nature Reviews. Gastroenterology & Hepatology, 14*(7), 397–411.
11. Trautwein, C., Friedman, S. L., Schuppan, D., & Pinzani, M. (2015). Hepatic fibrosis: Concept to treatment. *Journal of Hepatology, 62*(1 Suppl), S15–S24.
12. Kim, V. N. (2005). MicroRNA biogenesis: Coordinated cropping and dicing. *Nature Reviews. Molecular Cell Biology, 6*(5), 376–385.
13. Lee, R. C., Feinbaum, R. L., & Ambros, V. (1993). The C. elegans heterochronic gene lin-4 encodes small RNAs with antisense complementarity to lin-14. *Cell, 75*(5), 843–854.
14. Bartel, D. P. (2009). MicroRNAs: Target recognition and regulatory functions. *Cell, 136*(2), 215–233.
15. Bartel, D. P. (2004). MicroRNAs: Genomics, biogenesis, mechanism, and function. *Cell, 116*(2), 281–297.
16. Lee, H. M., Nguyen, D. T., & Lu, L. F. (2014). Progress and challenge of microRNA research in immunity. *Frontiers in Genetics, 5*, 178.
17. Rupaimoole, R., & Slack, F. J. (2017). MicroRNA therapeutics: Towards a new era for the management of cancer and other diseases. *Nature Reviews. Drug Discovery, 16*(3), 203–222.
18. Shenoy, A., & Blelloch, R. H. (2014). Regulation of microRNA function in somatic stem cell proliferation and differentiation. *Nature Reviews. Molecular Cell Biology, 15*(9), 565–576.
19. Murakami, Y., & Kawada, N. (2017). MicroRNAs in hepatic pathophysiology. *Hepatology Research, 47*(1), 60–69.
20. Szabo, G., & Bala, S. (2013). MicroRNAs in liver disease. *Nature Reviews. Gastroenterology & Hepatology, 10*(9), 542–552.
21. Wang, X. W., Heegaard, N. H., & Orum, H. (2012). MicroRNAs in liver disease. *Gastroenterology, 142*(7), 1431–1443.
22. Mahgoub, A., & Steer, C. J. (2016). MicroRNAs in the evaluation and potential treatment of liver diseases. *Journal of Clinical Medicine, 5*(5), 52.
23. Yu, B., Zhao, X., Lee, L. J., & Lee, R. J. (2009). Targeted delivery systems for oligonucleotide therapeutics. *The AAPS Journal, 11*(1), 195–203.
24. Giannitrapani, L., Soresi, M., Bondì, M. L., Montalto, G., & Cervello, M. (2014). Nanotechnology applications for the therapy of liver fibrosis. *World Journal of Gastroenterology, 20*(23), 7242–7251.
25. Poilil Surendran, S., George Thomas, R., Moon, M. J., & Jeong, Y. Y. (2017). Nanoparticles for the treatment of liver fibrosis. *International Journal of Nanomedicine, 12*, 6997–7006.
26. Ditto, A. J., Shah, P. N., Gump, L. R., & Yun, Y. H. (2009a). Nanospheres formulated from L-tyrosine polyphosphate exhibiting sustained release of polyplexes and in vitro controlled transfection properties. *Molecular Pharmaceutics, 6*(3), 986–995.
27. Ditto, A. J., Shah, P. N., & Yun, Y. H. (2009b). Non-viral gene delivery using nanoparticles. *Expert Opinion on Drug Delivery, 6*(11), 1149–1160.
28. Shah, P. N., & Yun, Y. H. (2013). Cellular interactions with biodegradable polyurethanes formulated from L-tyrosine. *Journal of Biomaterials Applications, 27*(8), 1017–1031.
29. Muthiah, M., Park, I. K., & Cho, C. S. (2013). Nanoparticle-mediated delivery of therapeutic genes: Focus on miRNA therapeutics. *Expert Opinion on Drug Delivery, 10*(9), 1259–1273.
30. Choi, S. S., Omenetti, A., Witek, R. P., Moylan, C. A., Syn, W. K., Jung, Y., Yang, L., Sudan, D. L., Sicklick, J. K., Michelotti, G. A., Rojkind, M., & Diehl, A. M. (2009). Hedgehog pathway activation and epithelial-to-mesenchymal transitions during myofibroblastic transformation of rat hepatic cells in culture and cirrhosis. *American Journal of Physiology. Gastrointestinal and Liver Physiology, 297*(6), G1093–G1106.

31. Iwaisako, K., Brenner, D. A., & Kisseleva, T. (2012). What's new in liver fibrosis? The origin of myofibroblasts in liver fibrosis. *J Gastroenterol Hepatol, 27 Suppl 2*(Suppl 2), 65–68.
32. Mederacke, I., Hsu, C. C., Troeger, J. S., Huebener, P., Mu, X., Dapito, D. H., Pradere, J. P., & Schwabe, R. F. (2013). Fate tracing reveals hepatic stellate cells as dominant contributors to liver fibrosis independent of its aetiology. *Nature Communications, 4*, 2823.
33. Omenetti, A., Choi, S., Michelotti, G., & Diehl, A. M. (2011). Hedgehog signaling in the liver. *Journal of Hepatology, 54*(2), 366–373.
34. Engel, M. E., McDonnell, M. A., Law, B. K., & Moses, H. L. (1999). Interdependent SMAD and JNK signaling in transforming growth factor-beta-mediated transcription. *The Journal of Biological Chemistry, 274*(52), 37413–37420.
35. Hanafusa, H., Ninomiya-Tsuji, J., Masuyama, N., Nishita, M., Fujisawa, J., Shibuya, H., Matsumoto, K., & Nishida, E. (1999). Involvement of the p38 mitogen-activated protein kinase pathway in transforming growth factor-beta-induced gene expression. *The Journal of Biological Chemistry, 274*(38), 27161–27167.
36. Hellerbrand, C., Stefanovic, B., Giordano, F., Burchardt, E. R., & Brenner, D. A. (1999). The role of TGFbeta1 in initiating hepatic stellate cell activation in vivo. *Journal of Hepatology, 30*(1), 77–87.
37. Reif, S., Lang, A., Lindquist, J. N., Yata, Y., Gabele, E., Scanga, A., Brenner, D. A., & Rippe, R. A. (2003). The role of focal adhesion kinase-phosphatidylinositol 3-kinase-akt signaling in hepatic stellate cell proliferation and type I collagen expression. *The Journal of Biological Chemistry, 278*(10), 8083–8090.
38. Michelotti, G. A., Xie, G., Swiderska, M., Choi, S. S., Karaca, G., Krüger, L., Premont, R., Yang, L., Syn, W. K., Metzger, D., & Diehl, A. M. (2013). Smoothened is a master regulator of adult liver repair. *The Journal of Clinical Investigation, 123*(6), 2380–2394.
39. Kendall, T. J., Hennedige, S., Aucott, R. L., Hartland, S. N., Vernon, M. A., Benyon, R. C., & Iredale, J. P. (2009). p75 Neurotrophin receptor signaling regulates hepatic myofibroblast proliferation and apoptosis in recovery from rodent liver fibrosis. *Hepatology, 49*(3), 901–910.
40. Krizhanovsky, V., Yon, M., Dickins, R. A., Hearn, S., Simon, J., Miething, C., Yee, H., Zender, L., & Lowe, S. W. (2008). Senescence of activated stellate cells limits liver fibrosis. *Cell, 134*(4), 657–667.
41. Oh, Y., Park, O., Swierczewska, M., Hamilton, J. P., Park, J. S., Kim, T. H., et al. (2016). Systemic PEGylated TRAIL treatment ameliorates liver cirrhosis in rats by eliminating activated hepatic stellate cells. *Hepatology, 64*(1), 209–223.
42. Bartel, D. P. (2018). Metazoan microRNAs. *Cell, 173*(1), 20–51.
43. Gamazon, E. R., Innocenti, F., Wei, R., Wang, L., Zhang, M., Mirkov, S., Ramírez, J., Huang, R. S., Cox, N. J., Ratain, M. J., & Liu, W. (2013). A genome-wide integrative study of microRNAs in human liver. *BMC Genomics, 14*, 395.
44. Chang, J., Guo, J. T., Jiang, D., Guo, H., Taylor, J. M., & Block, T. M. (2008). Liver-specific microRNA miR-122 enhances the replication of hepatitis C virus in nonhepatic cells. *Journal of Virology, 82*(16), 8215–8223.
45. Chang, J., Nicolas, E., Marks, D., Sander, C., Lerro, A., Buendia, M. A., Xu, C., Mason, W. S., Moloshok, T., Bort, R., Zaret, K. S., & Taylor, J. M. (2004). miR-122, a mammalian liver-specific microRNA, is processed from hcr mRNA and may downregulate the high affinity cationic amino acid transporter CAT-1. *RNA Biology, 1*(2), 106–113.
46. Lagos-Quintana, M., Rauhut, R., Yalcin, A., Meyer, J., Lendeckel, W., & Tuschl, T. (2002). Identification of tissue-specific microRNAs from mouse. *Current Biology, 12*(9), 735–739.
47. Jopling, C. L., Yi, M., Lancaster, A. M., Lemon, S. M., & Sarnow, P. (2005). Modulation of hepatitis C virus RNA abundance by a liver-specific MicroRNA. *Science, 309*(5740), 1577–1581.
48. Tsai, W. C., Hsu, S. D., Hsu, C. S., Lai, T. C., Chen, S. J., Shen, R., Huang, Y., Chen, H. C., Lee, C. H., Tsai, T. F., Hsu, M. T., Wu, J. C., Huang, H. D., Shiao, M. S., Hsiao, M., & Tsou,

A. P. (2012). MicroRNA-122 plays a critical role in liver homeostasis and hepatocarcinogenesis. *The Journal of Clinical Investigation, 122*(8), 2884–2897.
49. Cheung, O., Puri, P., Eicken, C., Contos, M. J., Mirshahi, F., Maher, J. W., Kellum, J. M., Min, H., Luketic, V. A., & Sanyal, A. J. (2008). Nonalcoholic steatohepatitis is associated with altered hepatic MicroRNA expression. *Hepatology, 48*(6), 1810–1820.
50. Halász, T., Horváth, G., Pár, G., Werling, K., Kiss, A., Schaff, Z., & Lendvai, G. (2015). miR-122 negatively correlates with liver fibrosis as detected by histology and FibroScan. *World Journal of Gastroenterology, 21*(25), 7814–7823.
51. Li, J., Ghazwani, M., Zhang, Y., Lu, J., Li, J., Fan, J., Gandhi, C. R., & Li, S. (2013). miR-122 regulates collagen production via targeting hepatic stellate cells and suppressing P4HA1 expression. *Journal of Hepatology, 58*(3), 522–528.
52. Shi, J., Aisaki, K., Ikawa, Y., & Wake, K. (1998). Evidence of hepatocyte apoptosis in rat liver after the administration of carbon tetrachloride. *The American Journal of Pathology, 153*(2), 515–525.
53. Williams, A. T., & Burk, R. F. (1990). Carbon tetrachloride hepatotoxicity: An example of free radical-mediated injury. *Seminars in Liver Disease, 10*(4), 279–284.
54. Hsu, S. H., Wang, B., Kota, J., Yu, J., Costinean, S., Kutay, H., Yu, L., Bai, S., La Perle, K., Chivukula, R. R., Mao, H., Wei, M., Clark, K. R., Mendell, J. R., Caligiuri, M. A., Jacob, S. T., Mendell, J. T., & Ghoshal, K. (2012). Essential metabolic, anti-inflammatory, and anti-tumorigenic functions of miR-122 in liver. *The Journal of Clinical Investigation, 122*(8), 2871–2883.
55. Ahsani, Z., Mohammadi-Yeganeh, S., Kia, V., Karimkhanloo, H., Zarghami, N., & Paryan, M. (2017). WNT1 gene from WNT signaling pathway is a direct target of miR-122 in hepatocellular carcinoma. *Applied Biochemistry and Biotechnology, 181*(3), 884–897.
56. Cao, F., & Yin, L. X. (2019). miR-122 enhances sensitivity of hepatocellular carcinoma to oxaliplatin via inhibiting MDR1 by targeting Wnt/β-catenin pathway. *Experimental and Molecular Pathology, 106*, 34–43.
57. Sun, Y., Wang, H., Li, Y., Liu, S., Chen, J., & Ying, H. (2018). miR-24 and miR-122 negatively regulate the transforming growth factor-β/Smad signaling pathway in skeletal muscle fibrosis. *Mol Ther Nucleic Acids, 11*, 528–537.
58. Yin, S., Fan, Y., Zhang, H., Zhao, Z., Hao, Y., Li, J., Sun, C., Yang, J., Yang, Z., Yang, X., Lu, J., & Xi, J. J. (2016). Differential TGFβ pathway targeting by miR-122 in humans and mice affects liver cancer metastasis. *Nature Communications, 7*, 11012.
59. Schueller, F., Roy, S., Trautwein, C., Luedde, T., & Roderburg, C. (2016). miR-122 expression is not regulated during activation of hepatic stellate cells. *Journal of Hepatology, 65*(4), 865–867.
60. Guo, C. J., Pan, Q., Cheng, T., Jiang, B., Chen, G. Y., & Li, D. G. (2009). Changes in microRNAs associated with hepatic stellate cell activation status identify signaling pathways. *The FEBS Journal, 276*(18), 5163–5176.
61. Hyun, J., Park, J., Wang, S., Kim, J., Lee, H. H., Seo, Y. S., & Jung, Y. (2016a). MicroRNA expression profiling in CCl_4-induced liver fibrosis of Mus musculus. *International Journal of Molecular Sciences, 17*(6), 691.
62. Hyun, J., Wang, S., Kim, J., Rao, K. M., Park, S. Y., Chung, I., Ha, C. S., Kim, S. W., Yun, Y. H., & Jung, Y. (2016b). MicroRNA-378 limits activation of hepatic stellate cells and liver fibrosis by suppressing Gli3 expression. *Nature Communications, 7*, 10993.
63. Lakner, A. M., Steuerwald, N. M., Walling, T. L., Ghosh, S., Li, T., McKillop, I. H., Russo, M. W., Bonkovsky, H. L., & Schrum, L. W. (2012). Inhibitory effects of microRNA 19b in hepatic stellate cell-mediated fibrogenesis. *Hepatology, 56*(1), 300–310.
64. Roderburg, C., Urban, G. W., Bettermann, K., Vucur, M., Zimmermann, H., Schmidt, S., Janssen, J., Koppe, C., Knolle, P., Castoldi, M., Tacke, F., Trautwein, C., & Luedde, T. (2011). Micro-RNA profiling reveals a role for miR-29 in human and murine liver fibrosis. *Hepatology, 53*(1), 209–218.

65. McDaniel, K., Huang, L., Sato, K., Wu, N., Annable, T., Zhou, T., Ramos-Lorenzo, S., Wan, Y., Huang, Q., Francis, H., Glaser, S., Tsukamoto, H., Alpini, G., & Meng, F. (2017). The let-7/Lin28 axis regulates activation of hepatic stellate cells in alcoholic liver injury. *The Journal of Biological Chemistry, 292*(27), 11336–11347.
66. Ma, L., Liu, J., Xiao, E., Ning, H., Li, K., Shang, J., & Kang, Y. (2021). MiR-15b and miR-16 suppress TGF-β1-induced proliferation and fibrogenesis by regulating LOXL1 in hepatic stellate cells. *Life Sciences, 270*, 119144.
67. Kim, K. M., Han, C. Y., Kim, J. Y., Cho, S. S., Kim, Y. S., Koo, J. H., Lee, J. M., Lim, S. C., Kang, K. W., Kim, J. S., Hwang, S. J., Ki, S. H., & Kim, S. G. (2018). Gα(12) overexpression induced by miR-16 dysregulation contributes to liver fibrosis by promoting autophagy in hepatic stellate cells. *Journal of Hepatology, 68*(3), 493–504.
68. Lan, T., Li, C., Yang, G., Sun, Y., Zhuang, L., Ou, Y., Li, H., Wang, G., Kisseleva, T., Brenner, D., & Guo, J. (2018). Sphingosine kinase 1 promotes liver fibrosis by preventing miR-19b-3p-mediated inhibition of CCR2. *Hepatology, 68*(3), 1070–1086.
69. Brandon-Warner, E., Benbow, J. H., Swet, J. H., Feilen, N. A., Culberson, C. R., McKillop, I. H., deLemos, A. S., Russo, M. W., & Schrum, L. W. (2018). Adeno-associated virus serotype 2 vector-mediated reintroduction of microRNA-19b attenuates hepatic fibrosis. *Human Gene Therapy, 29*(6), 674–686.
70. Zhang, Z., Zha, Y., Hu, W., Huang, Z., Gao, Z., Zang, Y., Chen, J., Dong, L., & Zhang, J. (2013). The autoregulatory feedback loop of microRNA-21/programmed cell death protein 4/activation protein-1 (MiR-21/PDCD4/AP-1) as a driving force for hepatic fibrosis development. *The Journal of Biological Chemistry, 288*(52), 37082–37093.
71. Ning, B. F., Ding, J., Liu, J., Yin, C., Xu, W. P., Cong, W. M., Zhang, Q., Chen, F., Han, T., Deng, X., Wang, P. Q., Jiang, C. F., Zhang, J. P., Zhang, X., Wang, H. Y., & Xie, W. F. (2014). Hepatocyte nuclear factor 4α-nuclear factor-κB feedback circuit modulates liver cancer progression. *Hepatology, 60*(5), 1607–1619.
72. Kennedy, L. L., Meng, F., Venter, J. K., Zhou, T., Karstens, W. A., Hargrove, L. A., Wu, N., Kyritsi, K., Greene, J., Invernizzi, P., Bernuzzi, F., Glaser, S. S., Francis, H. L., & Alpini, G. (2016). Knockout of microRNA-21 reduces biliary hyperplasia and liver fibrosis in cholestatic bile duct ligated mice. *Laboratory Investigation, 96*(12), 1256–1267.
73. Wu, K., Ye, C., Lin, L., Chu, Y., Ji, M., Dai, W., Zeng, X., & Lin, Y. (2016). Inhibiting miR-21 attenuates experimental hepatic fibrosis by suppressing both the ERK1 pathway in HSC and hepatocyte EMT. *Clinical Science (London, England), 130*(16), 1469–1480.
74. Genz, B., Coleman, M. A., Irvine, K. M., Kutasovic, J. R., Miranda, M., Gratte, F. D., Tirnitz-Parker, J. E. E., Olynyk, J. K., Calvopina, D. A., Weis, A., Cloonan, N., Robinson, H., Hill, M. M., Al-Ejeh, F., & Ramm, G. A. (2019). Overexpression of miRNA-25-3p inhibits Notch1 signaling and TGF-β-induced collagen expression in hepatic stellate cells. *Scientific Reports, 9*(1), 8541.
75. Li, Z., Ji, L., Su, S., Zhu, X., Cheng, F., Jia, X., Zhou, Q., & Zhou, Y. (2018b). Leptin upregulates microRNA-27a/b-3p level in hepatic stellate cells. *Experimental Cell Research, 366*(1), 63–70.
76. Matsumoto, Y., Itami, S., Kuroda, M., Yoshizato, K., Kawada, N., & Murakami, Y. (2016). MiR-29a assists in preventing the activation of human stellate cells and promotes recovery from liver fibrosis in mice. *Molecular Therapy, 24*(10), 1848–1859.
77. Huang, Y. H., Tiao, M. M., Huang, L. T., Chuang, J. H., Kuo, K. C., Yang, Y. L., & Wang, F. S. (2015). Activation of Mir-29a in activated hepatic stellate cells modulates its profibrogenic phenotype through inhibition of histone deacetylases 4. *PLoS One, 10*(8), e0136453.
78. Liang, C., Bu, S., & Fan, X. (2016). Suppressive effect of microRNA-29b on hepatic stellate cell activation and its crosstalk with TGF-β1/Smad3. *Cell Biochemistry and Function, 34*(5), 326–333.
79. Lin, H. Y., Wang, F. S., Yang, Y. L., & Huang, Y. H. (2019). MicroRNA-29a suppresses CD36 to ameliorate high fat diet-induced steatohepatitis and liver fibrosis in mice. *Cell, 8*(10), 1298.

80. Zheng, J., Wang, W., Yu, F., Dong, P., Chen, B., & Zhou, M. T. (2018). MicroRNA-30a suppresses the activation of hepatic stellate cells by inhibiting epithelial-to-mesenchymal transition. *Cellular Physiology and Biochemistry, 46*(1), 82–92.
81. Tu, X., Zheng, X., Li, H., Cao, Z., Chang, H., Luan, S., Zhu, J., Chen, J., Zang, Y., & Zhang, J. (2015). MicroRNA-30 protects against carbon tetrachloride-induced liver fibrosis by attenuating transforming growth factor beta signaling in hepatic stellate cells. *Toxicological Sciences, 146*(1), 157–169.
82. Chen, J., Yu, Y., Li, S., Liu, Y., Zhou, S., Cao, S., Yin, J., & Li, G. (2017). MicroRNA-30a ameliorates hepatic fibrosis by inhibiting Beclin1-mediated autophagy. *Journal of Cellular and Molecular Medicine, 21*(12), 3679–3692.
83. Li, X., Chen, Y., Wu, S., He, J., Lou, L., Ye, W., & Wang, J. (2015). microRNA-34a and microRNA-34c promote the activation of human hepatic stellate cells by targeting peroxisome proliferator-activated receptor γ. *Molecular Medicine Reports, 11*(2), 1017–1024.
84. Li, W. Q., Chen, C., Xu, M. D., Guo, J., Li, Y. M., Xia, Q. M., Liu, H. M., He, J., Yu, H. Y., & Zhu, L. (2011). The rno-miR-34 family is upregulated and targets ACSL1 in dimethylnitrosamine-induced hepatic fibrosis in rats. *The FEBS Journal, 278*(9), 1522–1532.
85. Lei, Y., Wang, Q. L., Shen, L., Tao, Y. Y., & Liu, C. H. (2019). MicroRNA-101 suppresses liver fibrosis by downregulating PI3K/Akt/mTOR signaling pathway. *Clinics and Research in Hepatology and Gastroenterology, 43*(5), 575–584.
86. Tu, X., Zhang, H., Zhang, J., Zhao, S., Zheng, X., Zhang, Z., Zhu, J., Chen, J., Dong, L., Zang, Y., & Zhang, J. (2014). MicroRNA-101 suppresses liver fibrosis by targeting the TGFβ signalling pathway. *The Journal of Pathology, 234*(1), 46–59.
87. Nakamura, M., Kanda, T., Sasaki, R., Haga, Y., Jiang, X., Wu, S., Nakamoto, S., & Yokosuka, O. (2015). MicroRNA-122 inhibits the production of inflammatory cytokines by targeting the PKR activator PACT in human hepatic stellate cells. *PLoS One, 10*(12), e0144295.
88. Teng, K. Y., Barajas, J. M., Hu, P., Jacob, S. T., & Ghoshal, K. (2020). Role of B cell lymphoma 2 in the regulation of liver fibrosis in miR-122 knockout mice. *Biology (Basel), 9*(7), 157.
89. Hyun, J., Wang, S., Kim, J., Kim, G. J., & Jung, Y. (2015). MicroRNA125b-mediated Hedgehog signaling influences liver regeneration by chorionic plate-derived mesenchymal stem cells. *Scientific Reports, 5*, 14135.
90. Wang, Y., Du, J., Niu, X., Fu, N., Wang, R., Zhang, Y., Zhao, S., Sun, D., & Nan, Y. (2017). MiR-130a-3p attenuates activation and induces apoptosis of hepatic stellate cells in nonalcoholic fibrosing steatohepatitis by directly targeting TGFBR1 and TGFBR2. *Cell Death & Disease, 8*(5), e2792.
91. Roderburg, C., Luedde, M., Vargas Cardenas, D., Vucur, M., Mollnow, T., Zimmermann, H. W., Koch, A., Hellerbrand, C., Weiskirchen, R., Frey, N., Tacke, F., Trautwein, C., & Luedde, T. (2013). miR-133a mediates TGF-β-dependent derepression of collagen synthesis in hepatic stellate cells during liver fibrosis. *Journal of Hepatology, 58*(4), 736–742.
92. Yang, X., Dan, X., Men, R., Ma, L., Wen, M., Peng, Y., & Yang, L. (2017b). MiR-142-3p blocks TGF-β-induced activation of hepatic stellate cells through targeting TGFβRI. *Life Sciences, 187*, 22–30.
93. Yang, J., Lu, Y., Yang, P., Chen, Q., Wang, Y., Ding, Q., Xu, T., Li, X., Li, C., Huang, C., Meng, X., Li, J., Zhang, L., & Wang, X. (2019). MicroRNA-145 induces the senescence of activated hepatic stellate cells through the activation of p53 pathway by ZEB2. *Journal of Cellular Physiology, 234*(5), 7587–7599.
94. Du, J., Niu, X., Wang, Y., Kong, L., Wang, R., Zhang, Y., Zhao, S., & Nan, Y. (2015). MiR-146a-5p suppresses activation and proliferation of hepatic stellate cells in nonalcoholic fibrosing steatohepatitis through directly targeting Wnt1 and Wnt5a. *Scientific Reports, 5*, 16163.
95. Zou, Y., Cai, Y., Lu, D., Zhou, Y., Yao, Q., & Zhang, S. (2017). MicroRNA-146a-5p attenuates liver fibrosis by suppressing profibrogenic effects of TGFβ1 and lipopolysaccharide. *Cellular Signalling, 39*, 1–8.

96. Zhou, L., Liu, S., Han, M., Ma, Y., Feng, S., Zhao, J., Lu, H., Yuan, X., & Cheng, J. (2018). miR-185 inhibits fibrogenic activation of hepatic stellate cells and prevents liver fibrosis. *Mol Ther Nucleic Acids, 10*, 91–102.
97. Ju, B., Nie, Y., Yang, X., Wang, X., Li, F., Wang, M., Wang, C., & Zhang, H. (2019). miR-193a/b-3p relieves hepatic fibrosis and restrains proliferation and activation of hepatic stellate cells. *Journal of Cellular and Molecular Medicine, 23*(6), 3824–3832.
98. Song, L. Y., Ma, Y. T., Wu, C. F., Wang, C. J., Fang, W. J., & Liu, S. K. (2017). MicroRNA-195 activates hepatic stellate cells in vitro by targeting Smad7. *BioMed Research International, 2017*, 1945631.
99. Bi, Z. M., Zhou, Q. F., Geng, Y., & Zhang, H. M. (2016). Human umbilical cord mesenchymal stem cells ameliorate experimental cirrhosis through activation of keratinocyte growth factor by suppressing microRNA-199. *European Review for Medical and Pharmacological Sciences, 20*(23), 4905–4912.
100. Li, L., Ran, J., Li, L., Chen, G., Zhang, S., & Wang, Y. (2020). Gli3 is a novel downstream target of miR-200a with an anti-fibrotic role for progression of liver fibrosis in vivo and in vitro. *Molecular Medicine Reports, 21*(4), 1861–1871.
101. Yang, J. J., Tao, H., Liu, L. P., Hu, W., Deng, Z. Y., & Li, J. (2017a). miR-200a controls hepatic stellate cell activation and fibrosis via SIRT1/Notch1 signal pathway. *Inflammation Research, 66*(4), 341–352.
102. Yu, F., Zheng, Y., Hong, W., Chen, B., Dong, P., & Zheng, J. (2015). MicroRNA-200a suppresses epithelial-to-mesenchymal transition in rat hepatic stellate cells via GLI family zinc finger 2. *Molecular Medicine Reports, 12*(6), 8121–8128.
103. Ma, T., Cai, X., Wang, Z., Huang, L., Wang, C., Jiang, S., & Hua, Y. (2017). Liu Q (2017) miR-200c accelerates hepatic stellate cell-induced liver fibrosis via targeting the FOG2/PI3K pathway. *BioMed Research International*, 2670658. https://doi.org/10.1155/2017/2670658
104. Ma, L., Yang, X., Wei, R., Ye, T., Zhou, J. K., Wen, M., Men, R., Li, P., Dong, B., Liu, L., Fu, X., Xu, H., Aqeilan, R. I., Wei, Y. Q., Yang, L., & Peng, Y. (2018). MicroRNA-214 promotes hepatic stellate cell activation and liver fibrosis by suppressing Sufu expression. *Cell Death & Disease, 9*(7), 718.
105. Okada, H., Honda, M., Campbell, J. S., Takegoshi, K., Sakai, Y., Yamashita, T., Shirasaki, T., Takabatake, R., Nakamura, M., Tanaka, T., & Kaneko, S. (2015). Inhibition of microRNA-214 ameliorates hepatic fibrosis and tumor incidence in platelet-derived growth factor C transgenic mice. *Cancer Science, 106*(9), 1143–1152.
106. Dong, R., Zheng, Y., Chen, G., Zhao, R., Zhou, Z., & Zheng, S. (2015). miR-222 overexpression may contribute to liver fibrosis in biliary atresia by targeting PPP2R2A. *Journal of Pediatric Gastroenterology and Nutrition, 60*(1), 84–90.
107. Jiang, X., Jiang, L., Shan, A., Su, Y., Cheng, Y., Song, D., Ji, H., Ning, G., Wang, W., & Cao, Y. (2018). Targeting hepatic miR-221/222 for therapeutic intervention of nonalcoholic steatohepatitis in mice. *eBioMedicine, 37*, 307–321.
108. Ogawa, T., Enomoto, M., Fujii, H., Sekiya, Y., Yoshizato, K., Ikeda, K., & Kawada, N. (2012). MicroRNA-221/222 upregulation indicates the activation of stellate cells and the progression of liver fibrosis. *Gut, 61*(11), 1600–1609.
109. Yu, F., Fan, X., Chen, B., Dong, P., & Zheng, J. (2016). Activation of hepatic stellate cells is inhibited by microRNA-378a-3p via Wnt10a. *Cellular Physiology and Biochemistry, 39*(6), 2409–2420.
110. Kim, J., Lee, C., Shin, Y., Wang, S., Han, J., Kim, M., Kim, J. M., Shin, S. C., Lee, B. J., Kim, T. J., & Jung, Y. (2020). sEVs from tonsil-derived mesenchymal stromal cells alleviate activation of hepatic stellate cells and liver fibrosis through miR-486-5p. *Molecular Therapy, 29*(4), 1471–1486.Published online December 19. https://doi.org/10.1016/j.ymthe.2020.12.025
111. Ji, F., Wang, K., Zhang, Y., Mao, X. L., Huang, Q., Wang, J., Ye, L., & Li, Y. (2019). MiR-542-3p controls hepatic stellate cell activation and fibrosis via targeting BMP-7. *Journal of Cellular Biochemistry, 120*(3), 4573–4581.

112. Tao, L., Xue, D., Shen, D., Ma, W., Zhang, J., Wang, X., Zhang, W., Wu, L., Pan, K., Yang, Y., Nwosu, Z. C., Dooley, S., Seki, E., & Liu, C. (2018). MicroRNA-942 mediates hepatic stellate cell activation by regulating BAMBI expression in human liver fibrosis. *Archives of Toxicology, 92*(9), 2935–2946.
113. Tao, L., Wu, L., Zhang, W., Ma, W. T., Yang, G. Y., Zhang, J., Xue, D. Y., Chen, B., & Liu, C. (2020). Peroxisome proliferator-activated receptor γ inhibits hepatic stellate cell activation regulated by miR-942 in chronic hepatitis B liver fibrosis. *Life Sciences, 253*, 117572.
114. Noetel, A., Kwiecinski, M., Elfimova, N., Huang, J., & Odenthal, M. (2012). microRNA are central players in anti- and profibrotic gene regulation during liver fibrosis. *Front Physiol, 3*, 49.
115. Huang, Y. H., Yang, Y. L., & Wang, F. S. (2018). The role of miR-29a in the regulation, function, and signaling of liver fibrosis. *International Journal of Molecular Sciences, 19*(7), 1889.
116. Sekiya, Y., Ogawa, T., Yoshizato, K., Ikeda, K., & Kawada, N. (2011). Suppression of hepatic stellate cell activation by microRNA-29b. *Biochemical and Biophysical Research Communications, 412*(1), 74–79.
117. Knabel, M. K., Ramachandran, K., Karhadkar, S., Hwang, H. W., Creamer, T. J., Chivukula, R. R., Sheikh, F., Clark, K. R., Torbenson, M., Montgomery, R. A., Cameron, A. M., Mendell, J. T., & Warren, D. S. (2015). Systemic delivery of scAAV8-encoded MiR-29a ameliorates hepatic fibrosis in carbon tetrachloride-treated mice. *PLoS One, 10*(4), e0124411.
118. Wang, J., Chu, E. S., Chen, H. Y., Man, K., Go, M. Y., Huang, X. R., Lan, H. Y., Sung, J. J., & Yu, J. (2015). microRNA-29b prevents liver fibrosis by attenuating hepatic stellate cell activation and inducing apoptosis through targeting PI3K/AKT pathway. *Oncotarget, 6*(9), 7325–7338.
119. Zhang, Y., Ghazwani, M., Li, J., Sun, M., Stolz, D. B., He, F., Fan, J., Xie, W., & Li, S. (2014). MiR-29b inhibits collagen maturation in hepatic stellate cells through down-regulating the expression of HSP47 and lysyl oxidase. *Biochemical and Biophysical Research Communications, 446*(4), 940–944.
120. Kwiecinski, M., Elfimova, N., Noetel, A., Töx, U., Steffen, H. M., Hacker, U., Nischt, R., Dienes, H. P., & Odenthal, M. (2012). Expression of platelet-derived growth factor-C and insulin-like growth factor I in hepatic stellate cells is inhibited by miR-29. *Laboratory Investigation, 92*(7), 978–987.
121. Kwiecinski, M., Noetel, A., Elfimova, N., Trebicka, J., Schievenbusch, S., Strack, I., Molnar, L., von Brandenstein, M., Töx, U., Nischt, R., Coutelle, O., Dienes, H. P., & Odenthal, M. (2011). Hepatocyte growth factor (HGF) inhibits collagen I and IV synthesis in hepatic stellate cells by miRNA-29 induction. *PLoS One, 6*(9), e24568.
122. Roy, S., Benz, F., Vargas Cardenas, D., Vucur, M., Gautheron, J., Schneider, A., Hellerbrand, C., Pottier, N., Alder, J., Tacke, F., Trautwein, C., Roderburg, C., & Luedde, T. (2015). miR-30c and miR-193 are a part of the TGF-β-dependent regulatory network controlling extracellular matrix genes in liver fibrosis. *Journal of Digestive Diseases, 16*(9), 513–524.
123. Duisters, R. F., Tijsen, A. J., Schroen, B., Leenders, J. J., Lentink, V., van der Made, I., Herias, V., van Leeuwen, R. E., Schellings, M. W., Barenbrug, P., Maessen, J. G., Heymans, S., Pinto, Y. M., & Creemers, E. E. (2009). miR-133 and miR-30 regulate connective tissue growth factor: Implications for a role of microRNAs in myocardial matrix remodeling. *Circulation Research, 104*(2), 170–178.
124. Fornari, F., Gramantieri, L., Ferracin, M., Veronese, A., Sabbioni, S., Calin, G. A., Grazi, G. L., Giovannini, C., Croce, C. M., Bolondi, L., & Negrini, M. (2008). MiR-221 controls CDKN1C/p57 and CDKN1B/p27 expression in human hepatocellular carcinoma. *Oncogene, 27*(43), 5651–5661.
125. Iizuka, M., Ogawa, T., Enomoto, M., Motoyama, H., Yoshizato, K., Ikeda, K., & Kawada, N. (2012). Induction of microRNA-214-5p in human and rodent liver fibrosis. *Fibrogenesis & Tissue Repair, 5*(1), 12.

126. Chen, X., Mangala, L. S., Rodriguez-Aguayo, C., Kong, X., Lopez-Berestein, G., & Sood, A. K. (2018). RNA interference-based therapy and its delivery systems. *Cancer Metastasis Reviews, 37*(1), 107–124.
127. Dowdy, S. F. (2017). Overcoming cellular barriers for RNA therapeutics. *Nature Biotechnology, 35*(3), 222–229.
128. Lee, S. W. L., Paoletti, C., Campisi, M., Osaki, T., Adriani, G., Kamm, R. D., Mattu, C., & Chiono, V. (2019). MicroRNA delivery through nanoparticles. *Journal of Controlled Release, 313*, 80–95.
129. Raemdonck, K., Vandenbroucke, R. E., Demeester, J., Sanders, N. N., & De Smedt, S. C. (2008). Maintaining the silence: Reflections on long-term RNAi. *Drug Discovery Today, 13*(21–22), 917–931.
130. Giacca, M., & Zacchigna, S. (2012). Virus-mediated gene delivery for human gene therapy. *Journal of Controlled Release, 161*(2), 377–388.
131. Kasuya, T., & Kuroda, S. (2009). Nanoparticles for human liver-specific drug and gene delivery systems: In vitro and in vivo advances. *Expert Opinion on Drug Delivery, 6*(1), 39–52.
132. Sen Gupta, A., & Lopina, S. T. (2004). Synthesis and characterization of l-tyrosine based novel polyphosphates for potential biomaterial applications. *Polymer, 45*(14), 4653–4662.
133. Sen Gupta, A., & Lopina, S. T. (2005). Properties of l-tyrosine based polyphosphates pertinent to potential biomaterial applications. *Polymer, 46*(7), 2133–2140.
134. Hu, F., Yang, D., Qian, B., Fan, S., Zhu, Q., Ren, H., Li, X., & Zhai, B. (2019). The exogenous delivery of microRNA-449b-5p using spermidine-PLGA nanoparticles efficiently decreases hepatic injury. *RSC Advances, 9*(60), 35135–35144.