Gene Expression, Vol. 16, pp. 155–162
Printed in the USA. All rights reserved.
Copyright © 2015 Cognizant Comm. Corp.

1052-2166/15 $90.00 + .00
DOI: http://dx.doi.org/10.3727/105221615X14399878166078
E-ISSN 1555-3884
www.cognizantcommunication.com

Review

Role of Noncoding RNAs as Biomarker and Therapeutic Targets for Liver Fibrosis

Kun-Yu Teng and Kalpana Ghoshal

Department of Pathology, Comprehensive Cancer Center, The Ohio State University, Columbus, OH, USA

Noncoding RNAs (ncRNAs) including microRNAs (miRNAs) regulate gene expression at the posttranscriptional level, whereas long coding RNAs (lncRNAs) modulate gene expression both at transcriptional and posttranscriptional levels in mammals. Accumulated evidence demonstrates the widespread aberrations in ncRNA expression associated with almost all types of liver disease. However, the role of ncRNAs in liver fibrosis is poorly understood. Liver fibrosis is the process of excessive accumulation of extracellular matrix (ECM) proteins in the liver that lead to organ dysfunction and tumorigenesis. In this review, we summarize the current knowledge on the role of ncRNAs in promoting or repressing liver fibrosis caused by nonviral agents, potential use of circulating miRNAs as biomarkers of liver fibrosis, and therapeutic approaches to treat liver fibrosis by targeting the dysregulated miRNAs.

Key words: Liver fibrosis; Noncoding RNAs (ncRNAs); MicroRNAs (miRNAs); Long noncoding RNAs (lncRNAs)

INTRODUCTION

The central dogma that DNA is transcribed into RNA that is translated into protein to mediate biological functions has been well established many decades ago (1). Surprisingly, sequencing of the genome and transcriptome has revealed that the majority of RNAs in the mammalian genome do not code for any protein and are, therefore, designated noncoding RNAs (ncRNAs) (2). ncRNAs are highly heterogeneous in size, function as microRNA (miRNA) and long noncoding RNA (lncRNA), and play crucial roles in the development of human diseases (3,4). miRNAs are short RNA molecules (~22 nucleotides), regulating gene expression via translational repression and mRNA degradation predominantly by binding to the 3′ untranslated region (3′UTR) of specific mRNA (5). miRNAs regulate diverse physiological and developmental processes. It is estimated that at least one third of human protein coding genes are regulated by miRNAs (6). lncRNAs are RNA molecules containing longer than 200 nucleotides in length, with limited or no protein-coding capacity (7,8). Unlike miRNAs that regulate gene expression at posttranscriptional level, lncRNAs participate in both transcriptional and posttranscriptional regulation, and some are shown to be associated with pathogenesis of human diseases (9–11).

Liver fibrosis is a precancerous stage characterized by excessive accumulation of extracellular matrix (ECM) proteins due to repeated wound healing response, which occurs in almost all types of the chronic liver diseases (12,13). Fibrosis, if not cured, eventually leads to significant organ dysfunction, cirrhosis, and cancer (14,15). It is widely accepted that hepatic stellate cell (HSC) activation is the key event during liver fibrosis, in which HSCs are transformed into myofibroblast-like cells to synthesize ECM proteins such as collagens that cause stiffness of the liver (16). HSC activation can be triggered by oxidative stresses, inflammatory responses, growth factors, and apoptotic bodies of hepatocytes caused by liver damage (12,13). Emerging evidences show that both miRNAs and lncRNAs are involved in regulating liver fibrogenesis (17,18).

HCV or HBV infection-induced liver pathogenesis has been summarized in many recent reviews (19). In this review, we focus on the current knowledge on the role of

Address correspondence to Kalpana Ghoshal, Department of Pathology, Comprehensive Cancer Center, The Ohio State University, College of Medicine 646C MRF, 420 W. 12th Avenue, Columbus, OH 43210, USA. Tel: 614-292-8865; E-mail: Kalpana.Ghoshal@osumc.edu

ncRNAs in liver fibrosis caused by nonviral agents. We summarize the roles of selected ncRNAs and their regulatory mechanisms that lead to liver fibrosis (Table 1). We also discuss the potential usage of miRNAs as noninvasive biomarkers and therapeutic targets for liver fibrosis.

PROFIBROTIC miRNAs IN LIVER

miR-21

miR-21, derived from an intron of a protein-coding gene *TMEM49*, is an oncogenic miRNA (oncomiR) that targets the well-known tumor-suppressor phosphatase and tensin homolog (PTEN) as well as other tumor suppressors in different types of cancer (20,21). Recent reports demonstrate that miR-21 is a profibrogenic miRNA (fibromiR) involved in renal, myocardial, pulmonary, and hepatic fibrosis by modulating transforming growth factor-β (TGF-β) pathway (22).

TGF-β is a critical cytokine that drives fibrosis by promoting hepatic stellate cell (HSC) proliferation and ECM production (12,13). The elevated expression of miR-21 during fibrosis is mainly regulated through TGF-β-Smad3-mediated transcriptional induction and Smad2/Drosha complex-enhanced miR-21 maturation (23,24). Furthermore, a recent study has demonstrated that the upregulation of miR-21 in mouse HSCs facilitates liver fibrosis (25).

A key mechanism of miR-21-mediated liver fibrosis is by the suppression of Smad7, an antagonist of TGF-β signaling pathway (22,25). Smad7 negatively regulates TGF-β pathway by blocking the signaling cascades involving TGF-β receptor type 1 (TGF-β-RI) and Smad proteins, and by facilitating E3 ubiquitin-protein ligase Smurf2-mediated ubiquitination and degradation of TGF-β-RI (26,27). Indeed, overexpression of miR-21 abolishes this Smad7-mediated suppression of TGF-β pathway and consequently promotes liver fibrosis. Thus, TGF-β pathway and miR-21 work cooperatively in driving HSC activation, which leads to massive liver fibrosis.

miR-221/222

miR-221 and its homolog, miR-222, obtained by the processing of the same ncRNA, are both oncomiRs and fibromiRs (28,29). miR-221/222 is highly upregulated in chronic hepatitis C (CHC) patients with liver fibrosis and in experimental liver fibrosis models under NF-κB transcriptional regulation (30). NF-κB is a strong fibrogenic factor that regulates hepatocyte injury, inflammatory signals, and fibrogenic responses in HSCs, all of which are crucial processes to promote liver fibrosis (14).

The increasing level of miR-221/222 suppresses cyclin-dependent kinase inhibitor 1B (CDKN1B, $p27^{Kip1}$) expression in HSCs by directly binding to its 3′UTR (29). CDKN1B, an inhibitor of cyclin-dependent kinases (CDKs), prevents binding of cyclin D and cyclin E to CDK2 and CDK4, respectively, which results in cell cycle arrest in G_1 phase and apoptosis. Hence, increase in miR-221/222 levels results in HSC proliferation (29,30). Further, ectopic expression of miR-222 in HSCs increases collagen type I (COL1A1) and matrix metalloproteinase-2 (MMP-2) expression, indicating that miR-221 and miR-222 can independently induce HSC activation (29). Collectively, the activation of NF-κB and upregulation of miR-221/222 promote liver fibrosis by (a) transcriptionally upregulating ECM proteins, (b) enhancing HSC proliferation, and (c) reducing HSC apoptosis.

miR-181b

miR-181a and miR-181b are located on chromosome 1 in human (31). miR-181b, induced by TGF-β signaling pathway, has been shown to promote hepatocarcinogenesis by suppressing tissue inhibitor of metalloproteinases-3 (TIMP-3). In contrast, inhibition

Table 1. A List of the Fibrotic or Antifibrotic ncRNAs

	Targets	Mechanism
Profibrotic		
miR-21	Smad7	Increase HSC proliferation, ECM production
miR-181b	p27(CDKN1B)	Promote HSC proliferation
miR-221/222	p27(CDKN1B)	Promote HSC proliferation, ECM production, reduce HSC apoptosis
Antifibrotic		
miR-29 family	Collagen, PDGF-C, IGF-1	Inhibit HSC proliferation and ECM production
miR-101	TGF-β-RI, KLF6	Suppress HSC proliferation and migration
miR-122	KLF6, P4HA1	Decrease HSC proliferation and ECM production
miR-214	CTGF	Decrease HSC proliferation , migration, and ECM production
lncRNA MEG3	p53	Induce HSC apoptosis, decrease HSC proliferation and ECM production

of miR-181b reduces hepatocellular carcinoma (HCC) growth in a xenograft mouse model (32). Interestingly, TGF-β also induces miR-181b expression in HSCs (33). Overexpression of miR-181b, but not miR-181a, promotes HSCs cell cycle progression by increasing S phase cell population, indicating that miR-181b facilitates HSCs proliferation. The alteration of cell cycle by ectopic expression of miR-181b is probably due to the decreasing level of CDKN1B ($p27^{Kip1}$), a common target of miR-181b and miR-221/222 (29,33).

ANTIFIBROTIC miRNAs AND lncRNAs IN LIVER

miR-29b

miR-29 family members are located at two different loci in human. miR-29b-1 and miR-29a are located on chromosome 7q32.3, while miR-29b-2 and miR-29c are on chromosome 1q32.2. miR-29b-1 and miR-29b-2 share identical mature sequences, called miR-29b (34). miR-29 members function as antifibrotic miRNAs in cardiac fibroblast by suppressing collagen synthesis (35). Following this observation, a series of studies have focused on understanding the role of this miRNA in fibrosis. miR-29b is downregulated particularly in HSCs in carbon tetrachloride (CCl_4)-induced and bile duct ligation (BDL)-induced liver fibrosis models (36). Studies have demonstrated that TGF-β and NF-κB transcriptionally repress miR-29b in HSCs, resulting in dramatic increase in ECM production (22,37,38).

miR-29b directly represses a variety of fibrogenic molecules including collagen types I, IV family, platelet-derived growth factor C (PDGF-C), and insulin-like growth factor-1 (IGF-1) in HSCs (37–39). Furthermore, overexpression of miR-29b attenuates TGF-β-induced collagen synthesis in HSCs, suggesting the antifibrotic role of miR-29b (37–39). In addition, miR-29b indirectly upregulates PTEN expression by targeting DNA methyltransferase 3b (DNMT3b) that methylates its promoter region (40). Thus, the decrease in DNMT3b levels results in hypomethylation of PTEN promoter, thereby increasing PTEN expression to promote HSC apoptosis and suppression of HSC proliferation (40). In summary, miR-29b inhibits liver fibrosis by (a) directly inhibiting production of ECM proteins, (b) blocking expression of fibrogenic signaling molecules, (c) increasing HSC apoptosis, and (4) attenuating HSC proliferation.

miR-101

miR-101 is transcribed from the eighth intron of RCL1 gene in humans (41). miR-101 has been identified as a tumor suppressor by directly targeting EZH2 and c-Myc in different types of cancer including HCC (42,43). miR-101 level is reduced both in HSCs and hepatocytes in CCl_4-induced liver fibrosis model. Loss of miR-101 causes elevation in the levels of TGF-β-RI and Kruppel-like factor 6 (KLF6) in HSCs and hepatocytes. Both TGF-β-RI and KLF6 are tightly associated with TGF-β signaling pathways. TGF-β-RI forms a heteromeric complex with TGFR-βII to transduce signals that results in activation of downstream *Smads*. KLF6 forms a complex with Smad3-AP1-KLF6 to transcriptionally regulate fibrogenic gene expression (44,45). These data support the notion that downregulation of miR-101 is probably one of the mechanisms underlying initiation and/or progression of liver fibrosis. In addition, overexpression of miR-101 inhibits TGF-β-induced proliferation/migration in HSCs and prevents TGF-β-induced apoptosis in hepatocytes (45). This observation further demonstrates the antifibrotic role of miR-101 in mediating liver fibrosis (45).

miR-122

miR-122, processed from an intergenic ncRNA, is the most abundant liver-specific miRNA. Depletion of miR-122 in mice leads to spontaneous hepatitis, hepatic fibrosis, and HCC with age (46,47). It has been shown that KLF6, a miR-122 direct target in hepatocytes, transactivates TGF-β to initiate the signaling cascades for the downstream activation of HSCs (47). Knocking down of KLF6 with short hairpin RNA (shRNA) in miR-122 knockout mouse livers successfully reduced liver fibrosis, further supporting the critical role of KLF6 in triggering liver fibrosis in these mice (47).

miR-122 is predominately expressed in hepatocytes and is undetectable in HSCs. Nevertheless, there are reports suggesting a potential role of miR-122 in suppressing HSC activation (48). miR-122 is downregulated in HSCs in CCl_4-induced liver fibrosis model due to suppression of the transcription factor C/EBPα. Reduced level of miR-122 in HSCs derepresses prolyl 4-hydroxylase, α polypeptide I (P4HA1), which facilitates collagen maturation (48). Furthermore, overexpression of miR-122 in HSCs moderately inhibits HSC proliferation. This phenomenon may be explained by downregulation of Bcl-w and IGFR-1, two reported miR-122 targets involved in cell survival and apoptosis, respectively (48).

miR-214-3p

miR-214 is located in intron 14 of the Dynamin-3 gene (DNM-3) in chromosomal 1q24.3 and is regulated by TGF-β in different types of cells including hepatocytes and HSCs (49). In contrast to miR-122, which is predominately expressed in hepatocytes, miR-214-3p is mainly expressed in HSCs (49). In experimental animal models, miR-214-3p exhibits antifibrotic properties by directly regulating connective tissue growth factor (CTGF, CCN2), a downstream mitogen of TGF-β pathway (50). CTGF, a strong fibrogenic factor, is produced in both HSCs and hepatocytes, and promotes HSC adhesion, proliferation,

migration, and collagen production in autocrine as well as paracrine manners (51,52).

The delivery of miR-214-3p from HSCs to hepatocytes has been shown to be mediated by exosomes (53). Exosomes are nanosized membrane vesicles of endocytic origin that are involved in transfer of miRNAs between different cell types. Exosomal miR-214-3p secreted from HSCs modulates CTGF expression in both HSCs and hepatocytes (50). Blocking exosomal delivery of miR-214-3p increases ECM protein production in HSCs, indicating that liver fibrosis is regulated not only by endogenous miRNAs but also by exosomal miRNAs (50).

Interestingly, while downregulation of miR-214-3p occurs in liver fibrosis, expression of miR-214-5p is a marker of fibrotic liver (49). miR-214-5p is also known to be upregulated in activated HSCs and in patients with liver fibrosis. Ectopic expression of miR-214-5p increases MMP-2, MMP-9, α-SMA, and TGF-β1 expression in HSC cell line (49).

lncRNA MEG3

lncRNA maternally expressed gene 3 (lncRNA MEG3) is a maternally imprinted gene located at chromosome 14q32.3 in humans. MEG3 has been established as a tumor suppressor in many types of cancer including HCC. A recent study has demonstrated that the methylation-dependent downregulation of MEG3 facilitates HCC development (54).

MEG3 was found to be downregulated in CCl_4-induced liver fibrosis model and fibrotic patients. TGF-β-mediated methylation of MEG3 promoter causes decrease in MEG3 expression, which supports previous findings that MEG3 expression could be epigenetically regulated (20,54). Although the mechanism by which MEG3 regulates liver fibrosis still remains unclear, MEG3 could induce p53-mediated apoptosis via modulating Bax/Bcl-2 and cytoplasmic cytochrome c expression (20). Besides, overexpression of MEG3 decreases HSC activation by suppressing cell proliferation (G_0/G_1 cell cycle arrest) and curtailing ECM protein synthesis (α-SMA and Cola1a1) (20).

miRNAs AS NONINVASIVE MARKERS FOR HEPATIC FIBROSIS

Recent studies have shown the presence of circulating miRNAs in various types of body fluids including plasma, serum, and bile (55). Most of the circulating miRNAs are protected by incorporation into lipid or lipoprotein complexes such as apoptotic bodies, exosomes, and microvesicles (55,56). Thus, these circulating miRNAs are highly stable despite the presence of ribonucleases in the circulation. Because of the remarkable stability of circulating miRNAs and the alteration in their levels in different disease, these small molecules are considered ideal biomarkers in liver disease (56).

CIRCULATING miRNAs miR-34a, miR-571, AND miR-513-3p ARE UPREGULATED IN LIVER FIBROSIS

Lipid accumulation and cellular apoptosis both play crucial roles during the progression of fibrogenesis. miR-34a functions as a fibromiR by targeting acyl-CoA synthetase long-chain family member 1 (ACSL1), resulting in alteration in lipid/fatty acid metabolism and cell survival in HSCs during liver fibrosis (57). In addition, the level of circulating miR-34a was significantly elevated in the sera of fibrotic patients, but not in healthy individuals (58,59). Further, the circulating miR-34a level correlates with the liver fibrosis score; it was about threefold higher in F3–F4 (Metavir fibrosis score) compared to early stage fibrosis (F0–F1) in CHC patients (58).

Although the potential role of miR-571 and miR-513-3p in liver fibrosis has not been fully established, their levels are significantly elevated in the sera of cirrhotic patients compared to normal populations (59). In one systematic miRNA profiling study, serum miR-571 and miR-513-3p levels were found to be nearly eightfold and fivefold upregulated, respectively, in patients with alcohol- or hepatitis C-induced liver cirrhosis. However, only the serum level of miR-571 reflected the disease severity in liver cirrhosis (59).

The level of miR-571 is elevated in sera and in the livers of cirrhotic patients compared to sera from healthy individuals. The upregulation of miR-571 most likely occurs in hepatic cells rather than the immune cells in the liver (59). Further evidence showed that the upregulation of miR-571 is probably triggered by TGF-β in human primary cultured HSCs. Overexpression of miR-571 in HSCs increases ECM protein synthesis and deposition, indicating that miR-571 is a profibrotic miRNA (59).

Downregulation of Circulating miRNAs miR-29a and miR-652

miR-29 exhibits strong antifibrotic activity, as described in the previous section. There was a significant inverse relationship between the serum level of miR-29a, but not miR-29b, and liver fibrosis progression in human HCC patients (38). A marked inverse correlation also exists between serum miR-29a level and Model for End-Stage Liver Disease (MELD) score, a scoring system indicative of the severity of chronic liver disease. These observations imply that miR-29a could serve as a noninvasive biomarker to monitor liver fibrosis progression in humans (38).

The serum level of miR-652 was also found to be downregulated in patients with alcohol- or HCV infection-induced liver fibrosis compared to healthy people (59). Unlike miR-29a, the downregulation of miR-652 in fibrotic patients' sera did not correlate with severity of fibrosis, probably due to its expression in circulating monocytes rather than in the parenchymal or other resident liver cells (59). It appears that the reduced miR-652

level in the serum may not be liver specific, but might reflect more general inflammatory response.

miRNAs AS POTENTIAL THERAPEUTIC TARGETS FOR HEPATIC FIBROSIS

miRNAs have been shown to play an important role in regulating gene expression. The deregulation of miRNAs is frequently found in almost all human diseases. Two laboratories including ours have independently observed that loss of miR-122 in liver leads to hepatitis, hepatic fibrosis, and HCC, while delivery of miR-122 reduces liver injuries and hepatocarcinogenesis by using knockout mouse models (46,47). Thus, manipulating the expression of miRNAs by miRNA inhibitors or miRNA mimic delivery might be an effective strategy to generate miRNA-directed therapeutic approaches for human diseases.

miRNA Therapy in Preclinical Models

miR-29 family has a strong antifibrotic ability against hepatic fibrosis by repressing ECM production, decreasing HSC proliferation, blocking fibrogenic signaling cascades, and inducing HSC apoptosis (36–40). It is therefore logical to treat liver fibrosis by increasing the expression of miR-29 in the liver. Indeed, there is significant evidence suggesting that miR-29 can be used as a therapeutic target due to suppression of fibrogenic characteristics following its overexpression in HSCs (36,37). Expression of miR-29a/b using adenovirus or estradiol (E_2)-inducible system successfully diminishes liver fibrosis in the CCl_4-induced liver fibrosis in mice (38). Further, significant reduction in tumor growth was observed in the HCC xenografts transfected with miR-29b in nude mice (60), further supporting the therapeutic role of miR-29 in different liver diseases.

miR-101 is another antifibrotic miRNA that could be therapeutically effective against liver fibrosis. miR-101 has been shown to suppress HSC proliferation and migration or hepatocyte apoptosis by mitigating TGF-β signaling in vitro (45). In addition, lentivirus-mediated expression of miR-101 exhibited pronounced antifibrotic effect in CCl_4-induced liver fibrosis model by repressing KLF6 and TGF-β-RI in both hepatocytes and HSCs (45).

Inhibition of fibromiRs is another attractive alternative to alleviate liver fibrosis. Expression of miR-21 has been shown to increase HSC activation and TGF-β signaling by repressing inhibitory Smad7 (22). Although miR-21 is not a liver-specific miRNA, it could still serve as an ideal target to treat liver fibrosis by using a liver-specific delivery system such as recombinant adeno-associated virus serotype 8 (rAAV8)(61). Indeed, a combination of rAAV8 and miR-21-Tough Decoy RNAs (TuDs), a stabilized stem-loop RNA with two microRNA binding sites to interfere miRNA functions, efficiently reduced miR-21 expression in liver and schistosomiasis-induced liver fibrosis (61).

Members of miR-17-92 cluster (e.g., miR-19a, -19b, -92a) have been shown to be significantly downregulated in primary HSCs activated in culture compared to quiescent HSCs (22,62). Studies with human patients also demonstrated a similar trend for miR-19b, whose level is significantly reduced in the liver and sera of fibrotic patients (62). Indeed, miR-19b has been reported to interfere with TGF-β signaling by targeting its receptor, TGF-β-RII, in HSCs, and overexpression of miR-19b reduced HSC differentiation to myofibroblasts and ECM production in primary cultured autonomous-activated rat HSCs (62).

CONCLUDING REMARKS

Regulatory Mechanisms of Liver Fibrosis

ncRNAs have been shown to be involved in various biological processes and human disease including hepatic fibrosis. Here we discussed two different types of ncRNAs (e.g., miRNAs and lncRNAs) and their regulatory roles in liver fibrosis (Fig. 1). It is well established that the central event in liver fibrogenesis is HSC activation, which is promoted by increased HSC proliferation, migration, differentiation, and reduced HSC apoptosis. Based on numerous systematic studies in cell culture-based experiments, animal models, and clinical human patients, it is clear that the process of HSC activation is, at least in part, regulated by ncRNAs. Thus, a better understanding of the regulatory mechanisms of ncRNAs in HSC activation will help us to gain the mechanistic insights into hepatic fibrogenesis and therapeutic strategies to inhibit liver fibrosis.

Advantages and Limitations for Using ncRNAs as Biomarkers

A good biomarker for detecting human diseases such as liver fibrosis should exhibit the following features: (a) general characteristics that differentiate normal healthy individuals from patients, (b) high stability in circulation, (c) ease of collecting through noninvasive method, and (d) reflects disease progression. Circulating miRNAs fit very well in all the categories. It is known that circulating RNAs are stable and their level can be easily quantified by quantitative RT-PCR or high throughput assays such as nanostring or miRNA microarrays. In addition, several miRNAs in circulation such as miR-571 and miR-34a have unique profiles that reflect different stages of liver fibrosis. More importantly, the accuracy in predicting liver fibrosis by profiling certain miRNAs rather than the conventional platelet count, prothrombin international normalized ratio (INR), or albumin appears to be highly significant (59). Thus, certain miRNA expression profiles in sera in combination with biochemical data may be better noninvasive diagnostic and prognostic markers for liver fibrosis. lncRNAs, albeit less stable than miRNAs, are also detectible in blood and have comparable half-lives to mRNAs (63,64). Additional studies are needed to

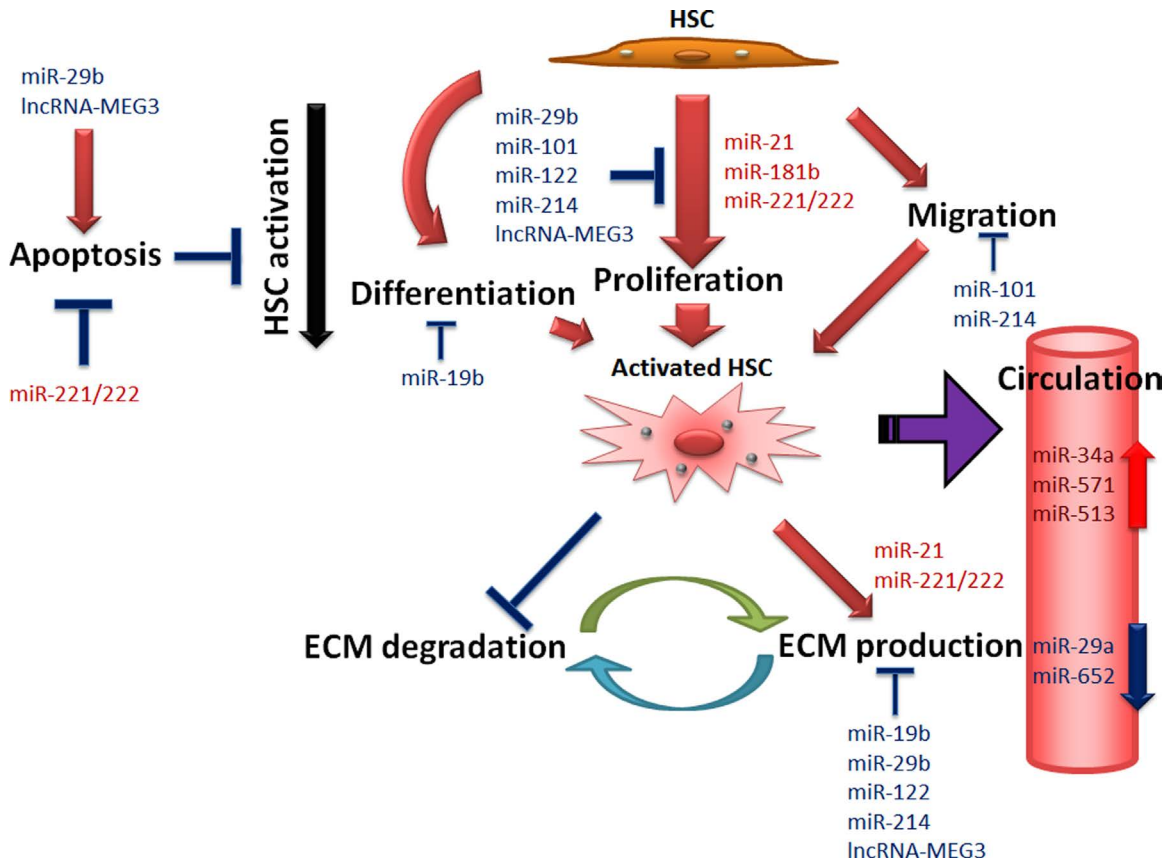

Figure 1. An overview of ncRNAs that modulate different stages of liver fibrosis.

ascertain the potential of lncRNAs as reliable biomarkers for liver fibrosis.

Although the serum levels of miRNAs may be utilized as biomarkers for detecting liver fibrosis, some critical issues need to be addressed before their translational applications. Lack of an established control to normalize circulating miRNAs has been a problem to quantify miRNA levels in the serum. Currently, adding the spiked-in RNAs into circulation is a preferred method to quantify circulating miRNAs. Although it may not reflect the absolute amount of miRNAs in circulation, this method has better consistency to act as a baseline for quantification. In addition to the quantification issue, larger patient cohorts with distinct hepatic fibrosis etiology and differential fibrosis stages should be analyzed to establish circulating miRNAs as biomarkers for liver fibrosis.

miRNA-Based Therapy for Liver Fibrosis

miRNAs are very attractive targets for novel therapeutic intervention due to their deregulation in human diseases and regulatory roles in multiple physiological processes. Overexpression of the downregulated miRNAs or depletion of the upregulated miRNAs in patients is the central concept to develop miRNA-based therapy. Several strategies to modulate miRNA expression in different liver diseases including fibrosis have been successfully implemented in preclinical models. It is indeed exciting that the first miRNA-based therapy for HCV infection (Miravirsen), an LNA-modified anti-miR-122 DNA–RNA hybrid oligonucleotide, is currently in phase II clinical investigation (65).

Although the therapeutic strategies involving manipulation of miRNA levels appear promising, some challenges still remain. Lack of proper in vivo delivery and off-target effects are two major problems for the miRNA-directed therapy. Fortunately, nanoparticle technology, providing high target specificity and low cytotoxicity in vivo, is being developed (66). miRNAs can be precisely delivered to their targets with very high efficiency using nanoparticles as carriers. Currently, studies of nanoparticle-based miRNA delivery are actively pursued in many laboratories (66,67). With more advanced knowledge and technology, miRNA-directed therapy to suppress liver fibrosis and hence hepatocarcinogenesis should be realized in the near future.

ACKNOWLEDGMENTS: We specially thank Dr. Samson Jacob for critically reading the review and providing constructive suggestions. This study is supported, in part, by grants R01CA086978 and R01CA193244 from NIH.

REFERENCES

1. Crick F. Central dogma of molecular biology. Nature 1970; 227(5258):561–563.
2. Cech TR, Steitz JA. The noncoding RNA revolution-trashing old rules to forge new ones. Cell 2014; 157(1): 77–94.
3. Moazed D. Small RNAs in transcriptional gene silencing and genome defence. Nature 2009; 457(7228):413–420.
4. Shi X, Sun M, Liu H, Yao Y, Song Y. Long non-coding RNAs: A new frontier in the study of human diseases. Cancer Lett 2013; 339(2):159–166.
5. Filipowicz W, Bhattacharyya SN, Sonenberg N. Mechanisms of post-transcriptional regulation by microRNAs: are the answers in sight? Nat Rev Genet 2008; 9(2):102–114.
6. He L, Hannon GJ. MicroRNAs: Small RNAs with a big role in gene regulation. Nat Rev Genet 2004; 5(7):522–531.
7. Batista PJ, Chang HY. Long noncoding RNAs: Cellular address codes in development and disease. Cell 2013; 152(6):1298–1307.
8. Yang L, Froberg JE, Lee JT. Long noncoding RNAs: Fresh perspectives into the RNA world. Trends Biochem Sci 2014; 39(1):35–43.
9. Kapranov P, Cheng J, Dike S, Nix DA, Duttagupta R, Willingham AT, et al. RNA maps reveal new RNA classes and a possible function for pervasive transcription. Science 2007; 316(5830):1484–1488.
10. Wapinski O, Chang HY. Long noncoding RNAs and human disease. Trends Cell Biol 2011; 21(6):354–361.
11. Mercer TR, Dinger ME, Mattick JS. Long non-coding RNAs: Insights into functions. Nat Rev Genet 2009; 10(3): 155–159.
12. Bataller R, Brenner DA. Liver fibrosis. J Clin Invest 2005; 115(2):209–218.
13. Friedman SL. Liver fibrosis—From bench to bedside. J Hepatol 2003; 38(Suppl 1):S38–53.
14. Luedde T, Schwabe RF. NF-kappaB in the liver--Linking injury, fibrosis and hepatocellular carcinoma. Nat Rev Gastroenterol Hepatol 2011; 8(2):108–118.
15. Wallace MC, Friedman SL. Hepatic fibrosis and the microenvironment: Fertile soil for hepatocellular carcinoma development. Gene Expr 2014; 16(2):77–84.
16. Friedman SL, Roll FJ, Boyles J, Bissell DM. Hepatic lipocytes: The principal collagen-producing cells of normal rat liver. Proc Natl Acad Sci USA 1985; 82(24):8681–8685.
17. He Y, Huang C, Zhang SP, Sun X, Long XR, Li J. The potential of microRNAs in liver fibrosis. Cell Signal 2012; 24(12):2268–2272.
18. He Y, Wu YT, Huang C, Meng XM, Ma TT, Wu BM, et al. Inhibitory effects of long noncoding RNA MEG3 on hepatic stellate cells activation and liver fibrogenesis. Biochim Biophys Acta 2014; 1842(11):2204–2215.
19. Rehermann B, Nascimbeni M. Immunology of hepatitis B virus and hepatitis C virus infection. Nat Rev Immunol 2005; 5(3):215–229.
20. Krichevsky AM, Gabriely G. miR-21: A small multi-faceted RNA. J Cell Mol Med 2009; 13(1):39–53.
21. Meng F, Henson R, Wehbe-Janek H, Ghoshal K, Jacob ST, Patel T. MicroRNA-21 regulates expression of the PTEN tumor suppressor gene in human hepatocellular cancer. Gastroenterology 2007; 133(2):647–658.
22. Noetel A, Kwiecinski M, Elfimova N, Huang J, Odenthal M. microRNA are central players in anti- and profibrotic gene regulation during liver fibrosis. Front Physiol 2012; 3:49.
23. Davis BN, Hilyard AC, Lagna G, Hata A. SMAD proteins control DROSHA-mediated microRNA maturation. Nature 2008; 454(7200):56–61.
24. Zhong X, Chung AC, Chen HY, Meng XM, Lan HY. Smad3-mediated upregulation of miR-21 promotes renal fibrosis. J Am Soc Nephrol 2011; 22(9):1668–1681.
25. Zhang Z, Zha Y, Hu W, Huang Z, Gao Z, Zang Y, et al. 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. J Biol Chem 2013; 288(52):37082–37093.
26. Kavsak P, Rasmussen RK, Causing CG, Bonni S, Zhu H, Thomsen GH, et al. Smad7 binds to Smurf2 to form an E3 ubiquitin ligase that targets the TGF beta receptor for degradation. Mol Cell 2000; 6(6):1365–1375.
27. Massague J, Chen YG. Controlling TGF-beta signaling. Genes Dev 2000; 14(6):627–644.
28. le Sage C, Nagel R, Egan DA, Schrier M, Mesman E, Mangiola A, et al. Regulation of the p27(Kip1) tumor suppressor by miR-221 and miR-222 promotes cancer cell proliferation. EMBO J 2007; 26(15):3699–3708.
29. Ogawa T, Enomoto M, Fujii H, Sekiya Y, Yoshizato K, Ikeda K, et al. MicroRNA-221/222 upregulation indicates the activation of stellate cells and the progression of liver fibrosis. Gut 2012; 61(11):1600–1609.
30. Polyak K, Lee MH, Erdjument-Bromage H, Koff A, Roberts JM, Tempst P, et al. Cloning of p27Kip1, a cyclin-dependent kinase inhibitor and a potential mediator of extracellular antimitogenic signals. Cell 1994; 78(1):59–66.
31. Ji J, Yamashita T, Wang XW. Wnt/beta-catenin signaling activates microRNA-181 expression in hepatocellular carcinoma. Cell Biosci 2011; 1(1):4.
32. Wang B, Hsu SH, Majumder S, Kutay H, Huang W, Jacob ST, et al. TGFbeta-mediated upregulation of hepatic miR-181b promotes hepatocarcinogenesis by targeting TIMP3. Oncogene 2010; 29(12):1787–1797.
33. Wang B, Li W, Guo K, Xiao Y, Wang Y, Fan J. miR-181b promotes hepatic stellate cells proliferation by targeting p27 and is elevated in the serum of cirrhosis patients. Biochem Biophys Res Commun 2012; 421(1):4–8.
34. Kriegel AJ, Liu Y, Fang Y, Ding X, Liang M. The miR-29 family: Genomics, cell biology, and relevance to renal and cardiovascular injury. Physiol Genomics 2012; 44(4): 237–244.
35. van Rooij E, Sutherland LB, Thatcher JE, DiMaio JM, Naseem RH, Marshall WS, et al. Dysregulation of microRNAs after myocardial infarction reveals a role of miR-29 in cardiac fibrosis. Proc Natl Acad Sci USA 2008; 105(35):13027–13032.
36. Roderburg C, Urban GW, Bettermann K, Vucur M, Zimmermann H, Schmidt S, et al. Micro-RNA profiling reveals a role for miR-29 in human and murine liver fibrosis. Hepatology 2011; 53(1):209–218.
37. Kwiecinski M, Noetel A, Elfimova N, Trebicka J, Schievenbusch S, Strack I, et al. Hepatocyte growth factor (HGF) inhibits collagen I and IV synthesis in hepatic stellate cells by miRNA-29 induction. PLoS One 2011; 6(9):e24568.
38. Zhang Y, Wu L, Wang Y, Zhang M, Li L, Zhu D, et al. Protective role of estrogen-induced miRNA-29 expression in carbon tetrachloride-induced mouse liver injury. J Biol Chem 2012; 287(18):14851–14862.
39. Kwiecinski M, Elfimova N, Noetel A, Tox U, Steffen HM, Hacker U, et al. Expression of platelet-derived growth

factor-C and insulin-like growth factor I in hepatic stellate cells is inhibited by miR-29. Lab Invest 2012; 92(7):978–987.
40. Zheng J, Wu C, Lin Z, Guo Y, Shi L, Dong P, et al. Curcumin up-regulates phosphatase and tensin homologue deleted on chromosome 10 through microRNA-mediated control of DNA methylation--A novel mechanism suppressing liver fibrosis. FEBS J 2014; 281(1):88–103.
41. Liu JJ, Lin XJ, Yang XJ, Zhou L, He S, Zhuang SM, et al. A novel AP-1/miR-101 regulatory feedback loop and its implication in the migration and invasion of hepatoma cells. Nucleic Acids Res 2014; 42(19):12041–12051.
42. Varambally S, Cao Q, Mani RS, Shankar S, Wang X, Ateeq B, et al. Genomic loss of microRNA-101 leads to overexpression of histone methyltransferase EZH2 in cancer. Science 2008; 322(5908):1695–1699.
43. Wang L, Zhang X, Jia LT, Hu SJ, Zhao J, Yang JD, et al. c-Myc-mediated epigenetic silencing of MicroRNA-101 contributes to dysregulation of multiple pathways in hepatocellular carcinoma. Hepatology 2014; 59(5): 1850–1863.
44. Botella LM, Sanz-Rodriguez F, Komi Y, Fernandez LA, Varela E, Garrido-Martin EM, et al. TGF-beta regulates the expression of transcription factor KLF6 and its splice variants and promotes co-operative transactivation of common target genes through a Smad3-Sp1-KLF6 interaction. Biochem J 2009; 419(2):485–495.
45. Tu X, Zhang H, Zhang J, Zhao S, Zheng X, Zhang Z, et al. MicroRNA-101 suppresses liver fibrosis by targeting the TGFbeta signalling pathway. J Pathol 2014; 234(1):46–59.
46. Hsu SH, Wang B, Kota J, Yu J, Costinean S, Kutay H, et al. Essential metabolic, anti-inflammatory, and anti-tumorigenic functions of miR-122 in liver. J Clin Invest 2012; 122(8):2871–2883.
47. Tsai WC, Hsu SD, Hsu CS, Lai TC, Chen SJ, Shen R, et al. MicroRNA-122 plays a critical role in liver homeostasis and hepatocarcinogenesis. J Clin Invest 2012; 122(8):2884–2897.
48. Li J, Ghazwani M, Zhang Y, Lu J, Li J, Fan J, et al. miR-122 regulates collagen production via targeting hepatic stellate cells and suppressing P4HA1 expression. J Hepatol 2013; 58(3):522–528.
49. Iizuka M, Ogawa T, Enomoto M, Motoyama H, Yoshizato K, Ikeda K, et al. Induction of microRNA-214-5p in human and rodent liver fibrosis. Fibrogenesis Tissue Repair 2012; 5(1):12.
50. Chen L, Charrier A, Zhou Y, Chen R, Yu B, Agarwal K, et al. Epigenetic regulation of connective tissue growth factor by MicroRNA-214 delivery in exosomes from mouse or human hepatic stellate cells. Hepatology 2014; 59(3):1118–1129.
51. Kodama T, Takehara T, Hikita H, Shimizu S, Shigekawa M, Tsunematsu H, et al. Increases in p53 expression induce CTGF synthesis by mouse and human hepatocytes and result in liver fibrosis in mice. J Clin Invest 2011; 121(8):3343–3356.
52. Rachfal AW, Brigstock DR. Connective tissue growth factor (CTGF/CCN2) in hepatic fibrosis. Hepatol Res 2003; 26(1):1–9.
53. Valadi H, Ekstrom K, Bossios A, Sjostrand M, Lee JJ, Lotvall JO. Exosome-mediated transfer of mRNAs and microRNAs is a novel mechanism of genetic exchange between cells. Nat Cell Biol 2007; 9(6):654–659.
54. Braconi C, Kogure T, Valeri N, Huang N, Nuovo G, Costinean S, et al. microRNA-29 can regulate expression of the long non-coding RNA gene MEG3 in hepatocellular cancer. Oncogene 2011; 30(47):4750–4756.
55. Szabo G, Bala S. MicroRNAs in liver disease. Nat Rev Gastroenterol Hepatol 2013; 10(9):542–552.
56. Kosaka N, Iguchi H, Ochiya T. Circulating microRNA in body fluid: A new potential biomarker for cancer diagnosis and prognosis. Cancer Sci 2010; 101(10):2087–2092.
57. Li WQ, Chen C, Xu MD, Guo J, Li YM, Xia QM, et al. The rno-miR-34 family is upregulated and targets ACSL1 in dimethylnitrosamine-induced hepatic fibrosis in rats. FEBS J 2011; 278(9):1522–1532.
58. Cermelli S, Ruggieri A, Marrero JA, Ioannou GN, Beretta L. Circulating microRNAs in patients with chronic hepatitis C and non-alcoholic fatty liver disease. PLoS One 2011; 6(8):e23937.
59. Roderburg C, Mollnow T, Bongaerts B, Elfimova N, Vargas Cardenas D, Berger K, et al. Micro-RNA profiling in human serum reveals compartment-specific roles of miR-571 and miR-652 in liver cirrhosis. PLoS One 2012; 7(3):e32999.
60. Xiong Y, Fang JH, Yun JP, Yang J, Zhang Y, Jia WH, et al. Effects of microRNA-29 on apoptosis, tumorigenicity, and prognosis of hepatocellular carcinoma. Hepatology 2010; 51(3):836–8345.
61. He X, Xie J, Zhang D, Su Q, Sai X, Bai R, et al. Recombinant adeno-associated virus-mediated inhibition of miRNA-21 protects mice against the lethal schistosome infection by repressing both IL-13 and transforming growth factor beta1 pathways. Hepatology 2015; 61(6):2008–2017.
62. Lakner AM, Steuerwald NM, Walling TL, Ghosh S, Li T, McKillop IH, et al. Inhibitory effects of microRNA 19b in hepatic stellate cell-mediated fibrogenesis. Hepatology 2012; 56(1):300–310.
63. Clark MB, Johnston RL, Inostroza-Ponta M, Fox AH, Fortini E, Moscato P, et al. Genome-wide analysis of long noncoding RNA stability. Genome Res 2012; 22(5):885–898.
64. Panzitt K, Tschernatsch MM, Guelly C, Moustafa T, Stradner M, Strohmaier HM, et al. Characterization of HULC, a novel gene with striking up-regulation in hepatocellular carcinoma, as noncoding RNA. Gastroenterology 2007; 132(1):330–342.
65. Janssen HL, Reesink HW, Lawitz EJ, Zeuzem S, Rodriguez-Torres M, Patel K, et al. Treatment of HCV infection by targeting microRNA. N Engl J Med 2013; 368(18):1685–1694.
66. Hsu SH, Yu B, Wang X, Lu Y, Schmidt CR, Lee RJ, et al. Cationic lipid nanoparticles for therapeutic delivery of siRNA and miRNA to murine liver tumor. Nanomedicine 2013; 9(8):1169–1180.
67. Zhang M, Zhou X, Wang B, Yung BC, Lee LJ, Ghoshal K, et al. Lactosylated gramicidin-based lipid nanoparticles (Lac-GLN) for targeted delivery of anti-miR-155 to hepatocellular carcinoma. J Control Release 2013; 168(3):251–261.