MicroRNAs (miRNAs) are a group of small non-coding RNAs that range in length from 20 to 25 nucleotides. MicroRNAs are specific for multiple cellular functions, including cell generation, differentiation, multiplication, carcinogenesis, and apoptosis. Many researchers have recently reported that the aberrant expression of miRNAs in hepatic tissue was related to the pathogenesis of liver disease, including viral hepatitis, hepatocellular carcinoma, and fatty liver disease. Multiple studies have proposed that an analysis of circulating miRNAs may be useful for diagnosing etiologies or staging the progression of liver disease, as well as for therapeutic purposes, for example, nucleic acid therapy. This review summarizes and discusses recent advances in the knowledge of miRNAs for chronic liver diseases, with special interest in viral hepatitis, liver fibrosis, and biomarkers.

**Key words:** biomarker, HBV, HCC, HCV, liver fibrosis, miRNA

## INTRODUCTION

MicroRNAs are non-coding RNAs that control gene expression by annealing to complementary target mRNAs. MicroRNAs form RNA-induced silencing complexes with their target mRNAs in the cytoplasm, which inhibits the transfer and translation of mRNA. This process is referred to as RNAi. The 5’ 6–8 nucleotides of miRNA, which form the “seed sequence”, anneal to a complementary 3’-untranslated region of the target mRNA during RNAi, which prevents subsequent processes. The mRNAs that are controlled by miRNAs are referred to as target genes. Hundreds of possible miRNA target genes exist in the human genome, and miRNAs likely control up to 60% of human mRNAs.\(^1\) Therefore, miRNAs and their target genes cooperatively control life processes.\(^2,3\) Collectively, miRNAs participate in complex regulatory networks and control gene expression in all biological processes, including cell development, immune responses, aging, and cell death. A total of 1881 precursors and 2588 mature human miRNAs are registered in miRbase version 21.0 (http://www.mirbase.org).

## BASIS OF MIRNAS IN LIVER

MicroRNA expression patterns differ dramatically in internal organs. MicroRNA-122 comprises ~70% of miRNAs in hepatic tissue.\(^4\) The function of miR-122 in liver varies, but it plays a crucial role in the regulation of cholesterol and fatty acid metabolism. Liver-enriched transcription factors, including HNF6 and 4a, regulate miR-122 expression\(^5\) and control cell proliferation and differentiation in hepatocyte and cholangiocyte lineages.\(^6\) The molecular mechanism of steatosis was first revealed during gene expression profiling in miR-122 knockout mice, in which genes involved in lipid synthesis in the liver, including Agpat1 and Cidec, were overexpressed.\(^7,8\)

Catfild et al.\(^9\) reported that miR-122 influenced circadian rhythm by controlling the expression of metabolic regulators of the PPAR family. MicroRNA-122 expression also affects liver inflammation.\(^10\) Several studies revealed that the expression of miR-122 was reduced in experimental models and clinical samples of HCC and that the loss of miR-122 was associated with tumor invasiveness and cancer progression.\(^7,8,11,12\) MicroRNA-33,\(^13\) miR-27,\(^14\)
miR-378, miR-34, and miR-21 are also involved in liver metabolism.

Collectively, miRNAs are important mediators of metabolic stress during hypoxia, hyperglycemia, hypertriglyceridemia, hypercholesterolemia, and caloric restriction, and miR-122 regulates metabolic stress through cationic amino acid transporter 1.

MICRONA IN VIRAL HEPATITIS AND THERAPY

Hepatitis C virus

MicroRNA-122 controls HCV replication, and most studies of miR-122 in liver tissue were carried out in the context of HCV replication. The suppression of miR-122 controls HCV replication for the following reasons: (i) the binding site of miR-122 is downstream of the IRES, which controls the early stages of HCV replication; (ii) miR-122 controls the isoprenoid biosynthesis pathway, which may regulate HCV replication; and (iii) miR-122 recognizes the 5′- and 3′-untranslated regions in the HCV genome as target genes. MicroRNA-122 forms an oligomeric complex in which one miR-122 molecule binds to the 5′-terminus of the HCV RNA with 3′-overhanging nucleotides, which masks the 5′-terminal sequences of the HCV genome. This effect of miR-122 on HCV is unusual. MicroRNA-199a targets domain II of the HCV IRES, which inhibits HCV replication. MicroRNA-196 and miR-448 recognize NS5A and the core, respectively, to control replication, and let-7b recognizes domain IV of the IRES, NS5B (Fig. 1).

The expression of miR-130a is significantly higher in HCV-infected hepatocytes and liver biopsy specimens than in uninfected controls. MicroRNA-130a reduces the expression of interferon-induced transmembrane 1 to inhibit HCV replication.

Hepatic miRNA expression patterns in patients with CHC prior to therapy with pegylated interferon and ribavirin are consistent with therapeutic outcomes. The expression of eight miRNAs (miR-27b, miR-378, miR-422b, miR-34b, miR-145, miR-143, miR-652, and miR-18a) differed significantly between samples from sustained virological response and non-responder groups, and the accuracy of this distinction was 70.5%.

The expression of miRNAs is unique in HBV and HCV infections, and it is associated with liver disease progression. Seventeen miRNAs (miR-130a, miR-200a, miR-200b, miR-223, miR-326, miR-187, miR-199-5p, miR-199a*, miR-125b, miR-17-3p, miR-92, miR-139, miR-122a, miR-30e, miR-30a-3p, and miR-99a) were downregulated in HCC cells, which led to the upregulation of cancer-associated pathways involved in the cell cycle, adhesion, proteolysis, transcription, and translation. Six miRNAs (miR-21, miR-98, miR-183, miR-301, miR-221, and miR-222) were upregulated in HCC, which downregulated the antitumor immune response (Table 1).

Nucleic acid therapies recently emerged as potential strategies for the treatment of CHC, and these therapies showed high efficacy and few adverse effects. Treatment of locked nucleic acid-modified miRNAs, complementary to miR-122 (miravirsen), caused prolonged dose-dependent

Figure 1  Direct interaction of microRNAs (miR) with the hepatitis C virus genome. Schematic detailing the binding of miR-122 to the hepatitis C virus untranslated region (red arrows) and the roles of miR-122 and other miRNAs that inhibit replication (blue bars). IRES, internal ribosome entry site.
reductions in HCV levels without viral resistance in CHC genotype 1 infections.

Hepatitis B virus

MicroRNAs are known regulators of HBV replication, and HBV-related proteins regulate miRNA expression. Multiple miRNAs affect HBV replication through epigenetic mechanisms and direct regulation of transcription factors. Other miRNAs recognize specific target genes in the HBV genome.

Endothelin 1 is significantly upregulated in HBx-induced HCC in mice. Endothelin 1 plays an important role in HCC progression through activation of the PI3K/AKT pathway, and it is regulated by miR-1. MicroRNA-15b, miR-18a, miR-122, miR-141, and miR-372 regulate HBV transcription factors to control replication. MicroRNA-15b directly downregulated HNF1α mRNA, which resulted in the transactivation of HBV enhancer I. MicroRNA-18a suppressed the synthesis of estrogen receptor α and stimulated cell proliferation through expression of the estrogen receptor 1 gene. MicroRNA-122 is a broad regulator of HBV replication, and it directly regulates N-myc downstream-regulated gene 3, which suppresses replication. The HBV mRNA-mediated inhibition of miR-122 causes the upregulation of PTG1-binding protein, which promotes HCC tumor growth and cell invasion. MicroRNA-141 represses HBV replication by targeting PPARA, and miR-372/373 promotes HBV by targeting nuclear factor I/B.

The miR-15a/miR-16-1 cluster recognizes a region of overlap between HBV polymerase and HBx, and miR-122 recognizes the polymerase region across multiple HBV subtypes. The HBV preS1 region is a target of miR-210 (Fig. 2), and miR-1231 suppresses HBV replication by targeting the core mRNA (Table 2).

A current clinical trial is evaluating an RNAi-based drug that recognizes the 3′-end of gene X. The siRNA ARC-520 is a dynamic polyconjugate in which the RNAi trigger is conjugated to cholesterol. The conjugate is co-injected with a hepatocyte-targeted, membrane-active peptide. Phase IIa of the clinical trial revealed that the drug was well tolerated and caused a significant dose-dependent reduction in hepatitis B surface antigen (HBsAg) in patients with chronic hepatitis B. This drug candidate is not based on miRNA, but it relies on a similar technology.

Table 1 List of microRNAs (miR) associated with hepatitis C virus (HCV)-related liver disease

| Direct recognition | Function | Mechanism |
|--------------------|----------|-----------|
| let-7b              | Downregulation of HCV replication | Direct recognition of IRES and NS5B region |
| miR-122             | Upregulation of HCV replication | IRES region binding control of isoprenoid biosynthesis |
| miR-130a            | Downregulation of HCV | control of interferon-induced transmembrane 1 |
| miR-196             | Downregulation of HCV | Direct recognition of NS5A region |
| miR-199a            | Downregulation of HCV | Direct recognition of IRES region |
| miR-448             | Downregulation of HCV | Direct recognition of core region |
| miRNA               | Biomarker | Response |
| miR-18a, 34a, 143, 145, 652 | IFN response prediction | Upregulation in liver tissue of IFN non-responder |
| miR-27b, 378, 422b  | IFN response prediction | Downregulation in liver tissue of IFN non-responder |
| miR-17-3p, 30a-3p, 30e, 92, 99a, 122a, 125b, 130a, 139, 187, 199a, 199a*, 199-s, 200a, 200b, 223, 326 | Biomarker | Expression pattern |
| miR-21, 98, 183, 221, 222, 301 | HCC marker | Downregulation in HCC cells |
| miR-21              | Upregulation in HCC cells | |

HCC, hepatocellular carcinoma; IFN, interferon; IRES, internal ribosome entry site; NS, non-structural protein.
MICRORNA IN LIVER FIBROSIS

Pathophysiology of liver fibrosis

Liver fibrosis is a pathological manifestation of chronic liver disease (CLD) caused by any etiology, including HCV or HBV infection, alcoholic or non-alcoholic steatohepatitis, autoimmune hepatitis, and biliary cholangitis. Extracellular matrix materials replace the hepatic parenchyma in liver fibrosis. Fibrotic septum is composed of HSCs, myofibroblasts, vasculature, and inflammatory cells, but HSCs are the most important cells for the production of extracellular matrix materials and pro-fibrogenic mediators. Hepatic stellate cells become activated following chronic liver trauma and transdifferentiate to myofibroblast-like cells, which are characterized by a lower amount of vitamin A, abundant expression of α-SMA and growth factor receptors, and incremental production of collagens. The following factors initiate HSC activation: (i) damage-associated molecular patterns, that is, the chromatin-associated protein high-mobility group box 1, DNA and RNA, S100 molecules, and reactive oxygen species derived from damaged hepatocytes; (ii) paracrine production of stimuli, such as reactive oxygen species, cytokines, platelet-derived growth factor, TGF-β, and monocyte chemotactic protein-1 by sinusoidal endothelial cells, Kupffer cells, and cholangiocytes; and (iii) signal molecules from platelets and the immune system. Activated HSCs secrete an increased amount of MMPs and their inhibitors, TIMPs.49–51

Activation of HSCs is strictly controlled by transcription factors, such as activated protein-1, Jun D, Sp1, Kruppel-like factor 6, and NF-κB, under the control of Smad, Ras, Raf-1, and mitogen-activated protein kinase. Hypermethylation of PTEN occurs in activated HSCs. Activation of extracellular signal-regulated kinase and AKT is restricted by PTEN, and repression of PTEN in activated

Table 2

| miRNA   | Function                        | Mechanism                                      
|---------|---------------------------------|------------------------------------------------|
| miR-1   | Promotion of HBV x protein expression | Control of endothelin 1                        |
| miR-15b | Upregulation of HBV replication | Downregulated HNF1α                             |
| miR-18a |                                  | Control of estrogen receptor α                  |
| miR-372 |                                  | Control of nuclear factor 1/B                   |
| let-7   | Suppression of HBV x protein expression | Control signal transducer and activator of transcription 3 |
| miR-152 |                                  | Control of DNA methyltransferase 1              |
| miR-548p| Downregulation of HBV replication | Control of hepatitis B x-interacting protein (HBXIP) |
| miR-122 |                                  | Control of n-myc downstream-regulated gene 3    |
| miR-141 |                                  | Control of PPARα                                |
| miR-15a/16–1 | Downregulation of HBV replication | Direct recognition of polymerase and HBx region |
| miR-122 |                                  | Direct recognition of polymerase region         |
| miR-125a-5p |                                | Direct recognition of core region              |
| miR-199a-3p |                                |                                                |
| miR-210 |                                |                                                |
| miR-1231|                                |                                                |

HBV x protein, hepatitis B virus x protein; HNF1α, hepatic nuclear factor 1α; PTTG1, pituitary tumor-transforming 1; PPARα, peroxisome proliferator-activated receptor α.

© 2016 The Authors. Hepatology Research published by John Wiley & Sons Australia, Ltd on behalf of Japan Society of Hepatology.
HSCs promotes cell cycle, proliferation, and migration. Hypermethylation of RAS protein activator like 1 (RASL1), IkB, PPAR-γ and patched 1 (PTCH1) by methyl-CpG-binding protein 2 also contributes to HSC activation.\textsuperscript{52,53}

\textbf{+B:Role of miRNAs in stellate cell function}

MicroRNAs are the post-transcriptional regulators of gene expression and participate in HSC activation. The miRNAs implicated in HSC activation include miR-199a, miR-199b, miR-221, miR-27, miR-21, miR-125, miR-195, miR-214, and miR-221/222 as profibrotic miRNAs and miR-29, miR-15b, miR-200, miR-16, miR-133b, and miR-122 as antifibrotic miRNAs.\textsuperscript{54}

\textbf{MicroRNA-21}

MicroRNA-21 is an “onco-miR”, and it is frequently overexpressed in solid tumors. The human \textit{MIR21} gene is localized on the plus strand of chromosome 17q23.2, and it is transcriptionally upregulated by NF-κB. MicroRNA-21 is upregulated in liver fibrosis in human CLD and experimentally induced rodent models. Zhang \textit{et al.} reported that upregulated miR-21 expression in activated HSCs is maintained through a feedback loop that consists of miR-21, programmed cell death protein 4, and activation protein-1, and it promotes the TGF-β signaling pathway.\textsuperscript{55} He \textit{et al.} showed that recombinant adenovirus serotype 8-mediated downregulation of miR-21 protected mice with a lethal infection of \textit{Schistosoma japonica} through attenuation of the progression of liver fibrosis and hepatocyte damage. They also confirmed the involvement of IL-13 in addition to TGF-β in miR-21 induction for activated HSCs.\textsuperscript{56} MicroRNA-21 is also involved in ethanol-induced liver injury and HSC activation mediated by IL-6/Stat3 and targets Fas ligand (TNF superfamily, member 6) and death receptor 5.\textsuperscript{57}

\textbf{MicroRNA-29}

The miR-29 group is composed of miR-29a, 29b, and 29c, in which the former two miRNAs are processed from chromosome 7 and the latter two are transcribed from chromosome 1. MicroRNA-29 generally targets myeloid leukemia cell differentiation protein, DNA methyltransferases DNMT3A and DNMT3B, and zinc finger protein 36 homolog. MicroRNA-29 may also play an antifibrotic role in the liver and other organs. Roderburg \textit{et al.} and our group previously reported that miR-29 was downregulated in rodent models of liver fibrosis induced by CCl\textsubscript{4} or bile-duct ligation and human cirrhotic livers and that miR-29 expression decreased dependently to HSC activation in primary culture.\textsuperscript{58,59} Forced overexpression of miR-29 showed marked suppression of collagen 1 A1 mRNA and protein expression and attenuated HSC activation, which indicates an anti-fibrotic action of miR-29 in the liver. The downregulation of the expression of heat shock protein 47 and lysyl oxidase is involved in the inhibition of collagen maturation by miR-29b.\textsuperscript{60} A single systemic delivery of miR-29a expressing adenovirus prevented and reversed fibrosis development in CCl\textsubscript{4}-treated mice.\textsuperscript{61} MicroRNA-29b caused cell cycle arrest in the G\textsubscript{1} phase through reduction of cyclin D1 levels and induced HSC apoptosis by inhibition of the PI3K/AKT pathway. The antifibrotic action of hepatocyte growth factor and class II histone deacetylase inhibitor may be mediated by the induction of miR-29.\textsuperscript{62}

\textbf{MicroRNA-200a}

MicroRNA-200 consists of a family containing miR-200a, miR-200b, miR-200c, miR-141, and miR-429. Human miR-200a, miR-200b, and miR-429 are transcribed from chromosome 1, and miR-200c and miR-141 are transcribed from chromosome 12. The miR-200 family is involved in tumor biology through the inhibition of epithelial–mesenchymal transition or promotion of bladder cancer, breast cancer, melanoma, and ovarian cancer metastasis. Murakami \textit{et al.} first identified an increase in miR-200 and miR-199 in the liver of mice given CCl\textsubscript{4} for 4–8 weeks and human CLD that depended on the progression of fibrosis. The overexpression of miR-200 in LX-2 cells resulted in increased expression of TIMP-1 and MMP-13.\textsuperscript{63} In contrast, Sun \textit{et al.} reported that miR-200a expression was downregulated in CCl\textsubscript{4}-induced hepatic fibrosis in a rat model and that overexpression of miR-200a attenuated TGF-β-dependent HSC activation.\textsuperscript{64} Therefore, the role of miR-200 in liver fibrosis remains controversial.

\textbf{MicroRNA-222}

MicroRNA-222 and its parologue miR-221 are onco-miRs and regulators of angiogenesis. MicroRNA-221 is also involved in liver cancer. Ogawa \textit{et al.} first showed that miR-221/222 expression was upregulated in HCV-infected patients and patients with NASH dependent on the progression of liver fibrosis and that it closely correlated with the mRNA expression of collagen 1 A1 and α-SMA. Upregulation of miR-221/222 expression was also confirmed in a mouse liver fibrosis model induced by thioacetamide. MicroRNA-221/222 expression increased during HSC activation, and it regulated cyclin-dependent kinase 1B expression.\textsuperscript{65} Shen \textit{et al.} reported the modulation of liver fibrosis by miR-222 in a mouse model of biliary atresia.\textsuperscript{66} Dong \textit{et al.} identified that protein phosphatase 2 A subunit B was a target of miR-222.\textsuperscript{67}
Others
Du et al. recently reported that miR-146-5p played a role in HSC activation through the targeting of Wnt1 and Wnt5a in a mouse model of non-alcoholic fibrosing steatohepatitis.68 Povero et al. showed that the presence of miR-128-3p in extracellular vesicles derived from fat-laden hepatocytes targeted PPAR-γ and promoted pro-fibrogenic gene expression, such as collagen 1, α-SMA, and TIMP-2.69 Therefore, miRNAs are also implicated in fibrosis development in NASH.

MICRORNAS AS BIOMARKERS FOR LIVER PATHOPHYSIOLOGY
Liver biopsy is the gold standard for the diagnosis of the etiology of liver diseases, liver inflammation, liver fibrosis, and liver cancer, but non-invasive methodologies are being developed using serum markers and imaging technologies. The successful recent progression in the field of liver fibrosis diagnosis is the result of the development of useful fibrosis scores, such as AST to Platelet Ratio Index (APRI), Fibrosis-4 (FIB-4), FibroTest, and FibroMeter, and ultrasound and magnetic resonance imaging-based measurements of liver stiffness.70 However, the diagnostic markers of HCC have remained unchanged, including the use of α-fetoprotein and des-γ-carboxy prothrombin in the serum and the detection of the space occupying lesions by using ultrasound, computed tomography, and magnetic resonance-based imaging. Nevertheless, efforts have been made to establish a specific, repetitive, and easy method for diagnosing liver disease using circulating miRNAs.

Hepatocellular carcinoma
Li et al. first indicated the utility of the combination of miR-25, miR-375, and let-7f as a biomarker to separate HBV-derived HCC from controls (area under the curve, 99.67 ± 0.15%; sensitivity, 97.9%; specificity, 99.1%).71 Circulating miR-141 and miR-200a were significantly downregulated in HCC patients compared with patients with liver cirrhosis (P < 0.007) and healthy controls (P < 0.002) in a recent report.72 Lin et al. showed the utility of an miRNA classifier containing miR-29a, miR-29c, miR-133a, miR-143, miR-145, miR-192, and miR-505 in identifying small-sized, early-stage, and α-fetoprotein-negative HCC derived from HBV-infected patients.73 Wen et al. indicated four miRNAs, including miR-20a-5p, miR-320a, miR-324-3p, and miR-375, in addition to serum α-fetoprotein levels, as potential preclinical biomarkers of HBV-derived HCC.74 These and other reports indicate that miRNAs may be biomarkers for the detection of HCC, but candidate miRNAs are different across the cohorts studied. Further research using larger patient cohorts and several etiologies are definitely required in future works.

Non-alcoholic steatohepatitis
Four independent groups reported the use of serum miR-122 level as a biomarker for NAFLD and NASH. Miyaki et al. reported a significant correlation of serum miR-122 levels with hepatic miR-122 expression in NAFLD patients and an inverse correlation of serum miR-122 levels with hepatic fibrosis.75 Pirola et al. identified an increase in miR-122 (7.2-fold change in NASH compared with control) and miR-192 (4.4-fold change in NASH compared with control) among 84 circulating miRNAs tested and the overexpression of miR-122 in lipid-laden hepatocytes using in situ hybridization.76 Similar observations were reported by Becker on the utility of serum miR-122, miR-192, and miR-2177 and Tan using a serum miRNA panel (miR-122-5p, miR-1290, miR-27b-3p, and miR-192-5p) for the diagnosis of NAFLD.78

Autoimmune hepatitis and PBC
Circulating miRNA profiles were examined in type-1 autoimmune hepatitis patients, and an increase in miR-21 and miR-122 was reported in these patients compared with healthy controls. Reductions in miR-21 and miR-122 were observed in patients treated with corticosteroids and patients with advanced liver fibrosis.79 Primary biliary cholangitis patients are diagnosed using a serum miRNA panel consisting of miR-122-5p, miR-141-3p, and miR-26b-5p with high accuracy (sensitivity, 80.5%; specificity, 88.3%). The sensitivity and specificity of this panel was less than the serum levels of an antimitochondrial antibody, but these values were higher than the serum levels of an antinuclear antibody and alkaline phosphatase.78 Ninomiya et al. reported a unique miRNA expression pattern in the sera of PBC patients, and PBC patients were distinguished from a viral hepatitis group by the downregulation of miR-505-3p, miR-139-5p, and miR-197-3p expression.80

FUTURE THERAPY FOR LIVER DISEASE USING MI RNAS
MicroRNAs can be possible candidates for future therapies targeting liver disease. Several trials have recently been reported regarding the possibility of liver fibrosis treatment with miRNA. MicroRNA-214 participated in the development of hepatic fibrosis by modulating the epidermal growth factor receptor and TGF-β signaling pathways. By using platelet-derived growth factor-c transgenic mouse, LNA-anti-miR-214 showed the preventive
effect for hepatic fibrosis.\textsuperscript{81} Inhibition of miR-21 also reduced liver fibrosis through concomitant reduction of CD24\textsuperscript{+} liver progenitor cell and S100 A4\textsuperscript{+} cancer-associated stromal cells.\textsuperscript{82} Novel nucleic acid therapies for cancer have also emerged as potential clinical applications. MicroRNA-34a\textsuperscript{83} let-7,\textsuperscript{84} and miR-16\textsuperscript{85} have been well documented as tumor suppressor miRNA. MicroRNA-34 is a representative tumor suppressor miRNA acting through the p53 pathway.\textsuperscript{86} Mirna Therapeutics (Austin, TX, USA) has been promoting the development of MRX34, which is a custom nanoparticle liposome containing a miR-34a mimic compound. Application of MRX34 for several mouse cancer models, including prostate cancer\textsuperscript{87} and lung cancer,\textsuperscript{88} was carried out preclinically. Moreover, according to the promising result using a mouse HCC model, Mirna Therapeutics is progressing the clinical trial for non-resectable HCC and metastatic liver tumor. In addition, cholesterol conjugated let-7a mimics showed downregulation of the Ras gene, resulting in antitumor function on a hepatoma cell line and orthotopic xenograft model.\textsuperscript{89}

CONCLUSION

MICRONAS CONTRIBUTE TO the pathogenesis of liver disease of any etiology and regulate the expression of multiple genes that control inflammation, fibrosis, and cancer development. MicroRNA-122 is most abundant in hepatocytes and primarily regulates their metabolism, but multiple miRNAs also modify the functions of other hepatic constituent cells. Circulating miRNAs in the serum also reflect pathological conditions of the liver and may serve as plausible and non-invasive biomarkers for the diagnosis of the etiology and progression rate of liver disease.

ACKNOWLEDGMENTS

K. WAS SUPPORTED by a Grant-in-Aid for Scientific Research from the Japan Society for the Promotion of Science (Grant No. 25293177, 2013–present) and a grant for research on hepatitis and BSE from the Ministry of Health, Labor and Welfare (2013–present). Y.M. was supported by a grant from KOSEI-KAKENHI (H25-B-sou-Kan-en-general-018).

REFERENCES

1. Friedman RC, Farh KK, Burge CB, Bartel DP. Most mammalian miRNAs are conserved targets of microRNAs. Genome Res 2009; 19: 92–105.
2. Ambros V. The functions of animal microRNAs. Nature 2004; 431: 350–5.
3. Bartel DP. MicroRNAs: genomics, biogenesis, mechanism, and function. Cell 2004; 116: 281–97.
4. Landgraf P, Rusu M, Sheridan R et al. A mammalian microRNA expression atlas based on small RNA library sequencing. Cell 2007; 129: 1401–14.
5. Deng XG, Qiu RL, Wu YH et al. Overexpression of miR-122 promotes the hepatic differentiation and maturation of mouse ESCs through a miR-122/FoxA1/HNF4a-positive feedback loop. Liver int 2014; 34: 281–95.
6. Laudadio I, Manfrid I, Achouri Y et al. A feedback loop between the liver-enriched transcription factor network and miR-122 controls hepatocyte differentiation. Gastroenterology 2012; 142: 119–29.
7. Hsu SH, Wang B, Kota J et al. Essential metabolic, anti-inflammatory, and anti-tumorigenic functions of miR-122 in liver. J Clin Invest 2012; 122: 2871–83.
8. Tsai WC, Hsu SD, Hsu CS et al. MicroRNA-122 plays a critical role in liver homeostasis and hepatocarcinogenesis. J Clin Invest 2012; 122: 2884–97.
9. Gatfield D, Le Martelot G, Vejnar CE et al. Integration of microRNA miR-122 in hepatic circadian gene expression. Genes Dev. 2009; 23: 1313–26.
10. Lanford RE, Hildebrandt-Eriksen ES, Petri A et al. Therapeutic silencing of microRNA-122 in primates with chronic hepatitis C virus infection. Science 2010; 327: 198–201.
11. Cheung O, Puri P, Eicken C et al. Nonalcoholic steatohepatitis is associated with altered hepatic MicroRNA expression. Hepatology 2008; 48: 1810–20.
12. Wang S, Qiu L, Yan X et al. Loss of microRNA 122 expression in patients with hepatitis B enhances hepatitis B virus replication through cyclin G(1)-modulated P53 activity. Hepatology 2012; 55: 730–41.
13. Sun X, Feinberg MW. MicroRNA-management of lipoprotein homeostasis. Circ Res 2014; 115: 2–6.
14. Ji J, Zhang J, Huang G, Qian J, Wang X, Mei S. Overexpressed microRNA-27a and 27b influence fat accumulation and cell proliferation during rat hepatic stellate cell activation. FEBS Lett 2009; 583: 759–66.
15. Carrer M, Liu N, Grueter CE et al. Control of mitochondrial metabolism and systemic energy homeostasis by microRNAs 378 and 378*. Proc Natl Acad Sci USA 2012; 109: 15330–5.
16. Lee H, Park S, Lee Y et al. A pathway involving farnesoid X receptor and small heterodimer partner positively regulates hepatic sirtuin 1 levels via microRNA-34a inhibition. J Biol Chem 2010; 285: 12604–11.
17. Ahn J, Lee H, Jung CH, Ha T. Lycopene inhibits hepatic steatosis via microRNA-21-induced downregulation of fatty acid-binding protein 7 in mice fed a high-fat diet. Mol Nutr Food Res 2012; 56: 1665–74.
18. Bhattchararya SN, Habermacher R, Martine U, Closs EI, Filipowicz W. Relief of microRNA-mediated translational repression in human cells subjected to stress. Cell 2006; 125: 1111–24.
19. Patella F, Rainaldi G. MicroRNAs mediate metabolic stresses and angiogenesis. Cell Mol Life Sci. 2012; 69: 1049–65.
20 Jopling CL, Yi M, Lancaster AM, Lemon SM, Sarnow P. Modulation of hepatitis C virus RNA abundance by a liver-specific microRNA. *Science*. 2005; 309: 1577–81.

21 Henke JJ, Goergen D, Zheng J et al. MicroRNA-122 stimulates translation of hepatitis C virus RNA. *EMBO J.* 2008; 27: 3300–10.

22 Norman KL, Sarnow P. Modulation of hepatitis C virus RNA abundance and the isoprenoid biosynthesis pathway by microRNA miR-122 involves distinct mechanisms. *J Virol.* 2010; 84: 666–70.

23 Nasher N, Singaravelu R, Goodmurphy M, Lyn RK, Pezacki JP. Competing roles of microRNA-122 recognition elements in hepatitis C virus RNA. *Virology.* 2011; 410: 336–44.

24 Machlin ES, Sarnow P, Sagan SM. Masking the 5′ terminal nucleotides of the hepatitis C virus genome by an unconventional microRNA-target RNA complex. *Proc Natl Acad Sci USA.* 2011; 108: 3193–8.

25 Murakami Y, Aly HH, Tajima A, Inoue I, Shimotohno K. Regulation of the hepatitis C virus genome replication by miR-199a. *J Hepatol.* 2009; 50: 453–60.

26 Pedersen IM, Cheng G, Wieland S et al. Interferon modulation of cellular microRNAs as an antiviral mechanism. *Nature.* 2007; 449: 919–22.

27 Cheng JC, Yeh YJ, Tseng CP et al. Let-7b is a novel regulator of hepatitis C virus replication. *Cell Mol Life Sci.* 2012; 69: 2621–33.

28 Bhanja Chowdhury J, Shrivastava S, Steele R, Di Bisceglie AM, Ray R, Ray RB. Hepatitis C virus infection modulates expression of interferon stimulatory gene IFITM1 by upregulating miR-130 A. *J Virol.* 2012; 86: 10221–5.

29 Murakami Y, Tanaka M, Toyoda H et al. Hepatic microRNA expression is associated with the response to interferon treatment of chronic hepatitis C. *BMC Med Genomics.* 2010; 3: 48.

30 Ura S, Honda M, Yamashita T et al. Differential microRNA expression between hepatitis B and hepatitis C leading disease progression to hepatocellular carcinoma. *Hepatology.* 2009; 49: 1098–112.

31 Janssen HL, Reesink HW, Lawitz EJ et al. Treatment of HCV infection by targeting microRNA. *N Engl J Med.* 2013; 368: 1685–94.

32 Lu JW, Liao CY, Yang WY et al. Overexpression of endothelin 1 triggers hepatocarcinogenesis in zebrafish and promotes cell proliferation and migration through the AKT pathway. *PloS One.* 2014; 9: e85318.

33 Wu G, Huang P, Ju X, Li Z, Wang Y. Lin28B overexpression mediates the repression of let-7 by hepatitis B virus X protein in hepatoma cells. *Int J Clin Exp Med* 2015; 8: 15108–16.

34 Wang Y, Lu Y, Toh ST et al. Lethal-7 is down-regulated by the hepatitis B virus x protein and targets signal transducer and activator of transcription 3. *J Hepatol.* 2010; 53: 57–66.

35 Hu XM, Yan XH, Hu YW et al. MicroRNA-548p suppresses hepatitis B virus X protein associated hepatocellular carcinoma by downregulating oncoprotein HBXIP. *Hepatol Res* 2015. doi: 10.1111/hepr.12618.

36 Huang J, Wang Y, Guo Y, Sun S. Down-regulated microRNA-152 induces aberrant DNA methylation in hepatitis B virus-related hepatocellular carcinoma by targeting DNA methyltransferase 1. *Hepatology.* 2010; 52: 60–70.

37 Dai X, Zhang W, Zhang H et al. Modulation of HBV replication by microRNA-15b through targeting hepatocyte nuclear factor 1α. *Nucleic Acids Res.* 2014; 42: 6578–90.

38 Liu WH, Yeh SH, Lu CC et al. MicroRNA-18a prevents estrogen receptor-α expression, promoting proliferation of hepatocellular carcinoma cells. *Gastroenterology.* 2009; 136: 683–93.

39 Fan CC, Wang CM, Tian C et al. miR-122 inhibits viral replication and cell proliferation in hepatitis B virus-related hepatocellular carcinoma and targets NDRG3. *Oncol Rep.* 2011; 26: 1281–6.

40 Li C, Wang Y, Wang S et al. Hepatitis B virus RNA-mediated miR-122 inhibition upregulates PTG1-binding protein, which promotes hepatocellular carcinoma tumor growth and cell invasion. *J Virol.* 2013; 87: 2193–205.

41 Hu W, Wang X, Ding X et al. MicroRNA-141 represses HBV replication by targeting PPARA. *PloS One.* 2012; 7: e34165.

42 Guo H, Liu H, Mitchelson K et al. MicroRNAs-372/373 promote the expression of hepatitis B virus through the targeting of nuclear factor I/B. *Hepatology.* 2011; 54: 808–19.

43 Wang Y, Jiang L, Ji X, Yang B, Zhang Y, Fu XD. Hepatitis B viral RNA directly mediates down-regulation of the tumor suppressor microRNA miR-15a/miR-16-1 in hepatocytes. *J Biol Chem.* 2013; 288: 18484–93.

44 Chen Y, Shen A, Rider PJ et al. A liver-specific microRNA binds to a highly conserved RNA sequence of hepatitis B virus and negatively regulates viral gene expression and replication. *FASEB J* 2011; 25: 4511–21.

45 Potenza N, Papa U, Mosca N, Zerbini F, Nobile V, Russo A. Human microRNA hsa-miR-125a/miR-16-1 interferes with expression of hepatitis B virus surface antigen. *Nucleic Acids Res.* 2011; 39: 5157–63.

46 Zhang GL, Li YX, Zheng SQ, Liu M, Li X, Tang H. Suppression of hepatitis B virus replication by microRNA-199a-3p and microRNA-210. *Antiviral Res.* 2010; 88: 169–75.

47 Kohno T, Tsuge M, Murakami E et al. Human microRNA hsa-miR-1231 suppresses hepatitis B virus replication by targeting core mRNA. *J Viral Hepat.* 2014; 21: e89–97.

48 Gish RG, Yuen MF, Chan HL et al. Synthetic RNAi triggers and their use in chronic hepatitis B therapies with curative intent. *Antiviral Res.* 2015; 121: 97–108.

49 Friedman SL. Hepatic stellate cells: protein, multifunctional, and enigmatic cells of the liver. *Physiol Rev.* 2008; 88: 125–72.

50 Friedman SL. Evolving challenges in hepatic fibrosis. *Nat Rev Gastroenterol Hepatol.* 2010; 7: 425–36.

51 Yin C, Evasion KJ, Ashina K, Stainier DY. Hepatic stellate cells in liver development, regeneration, and cancer. *J Clin Invest.* 2013; 123: 1902–10.
Takashima M, Parsons Cj, Ikejima K, Watanabe S, White ES, Rippe RA. The tumor suppressor protein PTEN inhibits rat hepatic stellate cell activation. J Gastroenterol. 2009; 44: 847–55.

Mann J, Chu DC, Maxwell A et al. MeCP2 controls an epigenetic pathway that promotes myofibroblast transdifferentiation and fibrosis. Gastroenterology 2010; 138: 705–14., 14 e1-4.

Lee YA, Wallace MC, Friedman SL. Pathobiology of liver fibrosis: a translational success story. Gut. 2015; 64: 830–41.

Zhang Z, Zha Y, Hu W 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: 37082–93.

He X, Xie J, Zhang D et al. Recombinant adeno-associated virus-mediated inhibition of microRNA-21 protects mice against the lethal schistosome infection by repressing both IL-13 and transforming growth factor-β 1 pathways. Hepatology. 2015; 61: 2008–17.

Francis H, McDaniel K, Han Y et al. Regulation of the extrinsic apoptotic pathway by microRNA-21 in alcoholic liver injury. J Biol Chem. 2014; 289: 27526–39.

Roderburg C, Urban Gw, Bettermann K et al. Micro-RNA profiling reveals a role for miR-29 in human and murine liver fibrosis. Hepatology. 2011; 53: 209–18.

Sekiya Y, Ogawa T, Yoshizato K, Ikeda K, Kawada N. Suppression of hepatic stellate cell activation by microRNA-29b. Biochem Biophys Res Commun. 2011; 412: 74–9.

Zhang Y, Ghazwani M, Li J et al. MiR-29b inhibits collagen maturation in hepatic stellate cells through down-regulating the expression of HSP47 and iysyl oxidase. Biochem Biophys Res Commun. 2014; 446: 940–4.

Knabel MK, Ramachandran K, Karhadkar S et al. Systemic delivery of scAAV8-encoded miR-29a ameliorates hepatic fibrosis in carbon tetrachloride-treated mice. PloS One. 2015; 10: e0124411.

Kwiecinski M, Noetel A, Elfmova N et al. Hepatocyte growth factor (HGF) inhibits collagen I and IV synthesis in hepatic stellate cells by miRNA-29 induction. PloS One. 2011; 6: e24568.

Murakami Y, Toyoda H, Tanaka M et al. The progression of liver fibrosis is related with overexpression of the miR-199 and 200 families. PloS One. 2011; 6: e16081.

Sun X, He Y, Ma TT, Huang C, Zhang L, Li J. Participation of miR-200a in TGF-β1-mediated hepatic stellate cell activation. Mol Cell Biochem. 2014; 388: 11–23.

Ogawa T, Enomoto M, Fujii H et al. MicroRNA-221/222 up-regulation indicates the activation of stellate cells and the progression of liver fibrosis. Gut. 2012; 61: 1600–9.

Shen WJ, Dong R, Chen G, Zheng S. MicroRNA-222 modulates liver fibrosis in a murine model of biliary atresia. Biochem Biophys Res Commun. 2014; 446: 155–9.

Dong R, Zheng Y, Chen G, Zhao R, Zhou Z, Zheng S. miR-222 overexpression may contribute to liver fibrosis in biliary atresia by targeting PPP2R2A. J Pediatr Gastroenterol Nutr. 2015; 60: 84–90.

Du J, Niu X, Wang Y et al. MiR-146a-5p suppresses activation and proliferation of hepatic stellate cells in nonalcoholic fibrosing steatohepatitis through directly targeting Wnt1 and Wnt5a. Sci Rep. 2015; 5: 16163.

Povero D, Panera N, Eguchi A et al. Lipid-induced hepatocyte-derived extracellular vesicles regulate hepatic stellate cell via microRNAs targeting PPAR-γ. Cell Mol Gastroenterol Hepatol 2015; 1: 646–63 e4.

Castora I, Pinzani M. Biopsy and non-invasive methods for the diagnosis of liver fibrosis: does it take two to tango? Gut. 2010; 59: 861–6.

Li LM, Hu ZB, Zhou ZX et al. Serum microRNA profiles serve as novel biomarkers for HBV infection and diagnosis of HBV-positive hepatocarcinoma. Cancer Res. 2010; 70: 9798–807.

Dhayat SA, Husing A, Senninger N et al. Circulating microRNA-200 family as diagnostic marker in hepatocellular carcinoma. PloS One. 2015; 10: e0140066.

Lin XJ, Chong Y, Guo ZW et al. A serum microRNA classifier for early detection of hepatocellular carcinoma: a multicentre, retrospective, longitudinal biomarker identification study with a nested case–control study. Lancet Oncol. 2015; 16: 804–15.

Wen Y, Han J, Chen J et al. Plasma miRNAs as early biomarkers for detecting hepatocellular carcinoma. Int J Cancer. 2015; 137: 1679–90.

Miyazaki H, Ichikawa T, Kamo Y et al. Significance of serum and hepatic microRNA-122 levels in patients with non-alcoholic fatty liver disease. Liver Int. 2014; 34: e302–7.

Pirola CJ, Fernandez Gianotti T, Castano GO et al. Circulating microRNA signature in non-alcoholic fatty liver disease: from serum non-coding RNAs to liver histology and disease pathogenesis. Gut. 2015; 64: 800–12.

Becker PP, Rau M, Schmitt J et al. Performance of serum microRNAs-122, -192 and -21 as biomarkers in patients with non-alcoholic steatohepatitis. PloS One. 2015; 10: e0142661.

Tan Y, Pan T, Ye Y et al. Serum microRNAs as potential biomarkers of primary biliary cirrhosis. PloS One. 2014; 9: e111424.

Migita K, Komori A, Kozuru H et al. Circulating microRNA profiles in patients with type-1 autoimmune hepatitis. PloS One. 2015; 10: e0136908.

Ninomiya M, Kondo Y, Funayama R et al. Distinct microRNAs expression profile in primary biliary cirrhosis and evaluation of miR 505-3p and miR197-3p as novel biomarkers. PloS One. 2013; 8: e66086.

Okada H, Honda M, Campbell JS et al. Inhibition of microRNA-214 ameliorates hepatic fibrosis and tumor incidence in platelet-derived growth factor C transgenic mice. Cancer Sci. 2015; 106: 1143–52.

Zhang J, Jiao J, Cermelli S et al. miR-21 inhibition reduces liver fibrosis and prevents tumor development by inducing apoptosis of CD24+ progenitor cells. Cancer Res. 2015; 75: 1859–67.

Welch C, Chen Y, Stallings RL. MicroRNA-34a functions as a potential tumor suppressor by inducing apoptosis in neuroblastoma cells. Oncogene. 2007; 26: 5017–22.
84 Akao Y, Nakaoka Y, Naoe T. let-7 microRNA functions as a potential growth suppressor in human colon cancer cells. *Biol Pharm Bull.* 2006; 29: 903–6.

85 Calin GA, Ferracin M, Cimmino A et al. A microRNA signature associated with prognosis and progression in chronic lymphocytic leukemia. *N Engl J Med.* 2005; 353: 1793–801.

86 He L, He X, Lim LP et al. A microRNA component of the p53 tumour suppressor network. *Nature.* 2007; 447: 1130–4.

87 Liu C, Kelnar K, Liu B et al. The microRNA miR-34a inhibits prostate cancer stem cells and metastasis by directly repressing CD44. *Nat Med.* 2011; 17: 211–5.

88 Trang P, Wiggins JF, Daige CL et al. Systemic delivery of tumor suppressor microRNA mimics using a neutral lipid emulsion inhibits lung tumors in mice. *Mol Ther.* 2011; 19: 1116–22.

89 Liu YM, Xia Y, Dai W et al. Cholesterol-conjugated let-7a mimics: antitumor efficacy on hepatocellular carcinoma *in vitro* and in a preclinical orthotopic xenograft model of systemic therapy. *BMC Cancer.* 2014; 14: 889.