World Journal of Hepatology

Submit a Manuscript: http://www.wjgnet.com/esps/
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
DOI: 10.4254/wjh.v7.i2.226

World J Hepatol 2015 February 27; 7(2): 226-234
ISSN 1948-5182 (online)

Micro RNAs in the development of non-alcoholic fatty liver disease

Glenn S Gerhard, Johanna K DiStefano

Glenn S Gerhard, Department of Medical Genetics and Molecular Biochemistry, Temple University School of Medicine, Philadelphia, PA 19140, United States
Johanna K DiStefano, Diabetes, Cardiovascular and Metabolic Diseases Division, Translational Genomics Research Institute, Phoenix, AZ 85004, United States
Author contributions: Gerhard GS and Distefano JK contributed to this paper.
Supported by The National Institutes of Health DK091601 (JKD and GSG), P30 DK072488 (GSG and CDS); and the Translational Genomics Research Institute.

Correspondence to: Johanna K DiStefano, PhD, Diabetes, Cardiovascular and Metabolic Diseases Division, Translational Genomics Research Institute, 445 North Fifth Street, Phoenix, AZ 85004, United States. jdistefano@tgen.org
Telephone: +1-602-3438812
Fax: +1-602-3438844
Received: August 16, 2014
Peer-review started: August 17, 2014
First decision: September 28, 2014
Revised: October 28, 2014
Accepted: November 17, 2014
Article in press: November 19, 2014
Published online: February 27, 2015

Abstract

Nonalcoholic fatty liver disease or nonalcoholic fatty liver disease (NAFLD) refers to a group of disorders that arise from the accrual of fat in hepatocytes. Although various factors have been associated with the development of NAFLD, including genetic predisposition and environmental exposures, little is known about the underlying pathogenesis of the disease. Research efforts are ongoing to identify biological targets and signaling pathways that mediate NAFLD. Emerging evidence has implicated a role for micro RNAs (miRNAs), short single-stranded molecules that regulate gene expression either transcriptionally, through targeting of promoter regions, or post-transcriptionally, by blocking translation or promoting cleavage of specific target mRNAs. Several miRNAs have been associated with NAFLD, although our understanding of the biology underlying their role is still emerging. The goal of this review is to present an overview of the current state of knowledge of miRNAs involved in the development of NAFLD across a range of *in vitro* and *in vivo* models, including miRNAs that contribute to pathological mechanisms related to fatty liver in humans. Much less is known about the specific targets of miRNAs in cells, nor the molecular mechanisms involved in the development and progression NAFLD and related outcomes. More recently, the identification and validation of miRNA signatures in serum may facilitate the development of improved methods for diagnosis and clinical monitoring of disease progression.

Key words: MiRNA; Nonalcoholic fatty liver disease; Cell culture; Mouse; Human

Core tip: Available data on miRNAs in nonalcoholic fatty liver disease (NAFLD) are largely derived from various cell culture and animal models. Reflecting an emerging field, little cross-model concordance is present and few human data are available for comparison with cell culture and animal model results. Although the generation of human data may be limited by the availability of tissue samples, recent reports of circulating miRNAs from NAFLD patients hold promise for significant progress for diagnosis and clinical

monitoring of disease progression.

Gerhard GS, DiStefano JK. Micro RNAs in the development of non-alcoholic fatty liver disease. *World J Hepatol* 2015; 7(2): 226-234 Available from: URL: http://www.wjgnet.com/1948-5182/full/v7/i2/226.htm DOI: http://dx.doi.org/10.4254/wjh.v7.i2.226

INTRODUCTION

Nonalcoholic fatty liver disease (NAFLD) represents a spectrum of conditions resulting from excessive accumulation of fat in hepatocytes, a condition known as steatosis. NAFLD can be categorized into nonalcoholic fatty liver representing simple steatosis and non-alcoholic steatohepatitis (NASH) with coincident hepatocyte injury, liver inflammation, and fibrosis. NAFLD is the major cause of chronic liver disease (CLD), which is associated with substantial morbidity and mortality in developed countries[1]. In tandem with the rising rates of obesity and type 2 diabetes mellitus (T2D), the prevalence of NAFLD is also increasing, and is expected to double by 2030 in the United States. NAFLD/NASH has thus become a leading cause of cryptogenic cirrhosis[2] and is currently the 3rd leading clinical indication for liver transplantation in the United States and expected to soon become the primary indication[3]. At present liver biopsy is the only method to accurately assess the severity of liver fibrosis[1] or predict progression of NAFLD to clinically severe forms[4-6], which limits early diagnosis of NAFLD patients who are at high risk for development of liver-related morbidity and mortality. Given the substantial public health burden of NAFLD, novel therapeutic targets are also urgently needed to facilitate the development of improved pharmacological therapies for the treatment and prevention of the disease.

Although genetic predisposition, environmental exposures, and lifestyle factors contribute to the development of NAFLD, little is known about the underlying pathogenesis of the disease. Ongoing research efforts to identify biological targets and signaling pathways that mediate NAFLD are expected to provide the insight necessary to begin to distinguish among different clinical forms of the disease.

Emerging evidence has implicated a role for epigenetic factors, including micro RNAs (miRNAs) in the development of NAFLD. MiRNAs are endogenous, single-stranded RNAs (21-25 nucleotides in length) that regulate gene expression either post-transcriptionally, by blocking translation or promoting cleavage of specific target mRNAs, or transcriptionally, through targeting of promoter regions[7]. Thus, they do not code for proteins, but instead serve to regulate the expression of certain genes. More than 2500 miRNAs may be encoded in the human genome, residing in intergenic regions, introns, and within exons[8]. MiRNAs go through a complex processing pathway following transcription by RNA polymerase Ⅱ (RNA Pol Ⅱ), which has been described in detail elsewhere[9]. MiRNAs can be found both in cells and circulating in the blood, and have the potential to be taken up at sites distant from the cell of origin; as such they may serve as biomarkers of disease processes. They may also act in an endocrine or paracrine fashion to regulate expression at multiple sites. It is not yet known whether miRNAs have specific cell surface receptors or whether they target cells that express their target mRNAs.

MiRNAs have been found to regulate processes relevant to the development and progression of NAFLD; however, our understanding of the biology underlying these processes is presently in its infancy. The goal of this review is to present an overview of the current state of knowledge of miRNAs involved in the development of NAFLD across a range of appropriate *in vitro* and *in vivo* models, with a special focus on miRNAs that contribute to pathological mechanisms related to fatty liver in humans.

In vitro studies

A number of studies have been conducted on miRNAs using cell culture models of NAFLD, primarily those derived from hepatocyte cell lines (Table 1). In one study, immortalized human liver-derived L02 cells, cultured with high levels of free fatty acids (HFFA-treated) to serve as a model for hepatic steatosis, were analyzed using miRNA micro-array[10]. A total of 17 and 15 miRNAs were up- or downregulated, respectively, in these HFFA-treated L02 cells. Of these, miR-10b was the most up-regulated miRNA, and HFFA-cultured L02 cells transfected with anti-miR-10b showed significantly decreased lipid content and the triglyceride level (*i.e.,* steatosis). Peroxisome proliferator-activated receptor-a (*PPARA*) was identified as a potential target for miR-10b, and expression of both transcript and protein levels of *PPARA* were reduced in steatotic L02 cells. Overexpression of miR-10b in HFFA-cultured L02 cells, led to decreased PPARA protein levels, while miR-10b knockdown increased PPARA, indicating that this miRNA may regulate the development of hepatic steatosis through mechanisms involving the *PPARA* pathway. Further investigation involving both animal and human studies will be necessary to confirm this relationship.

In an independent study to identify miRNAs involved in the formation of lipid droplets, the human hepatocellular carcinoma-derived cell line Huh7 was transiently transfected with a library of 327 miRNAs[11]. The Huh7 cell line spontaneously accumulates lipid droplets in culture and is lipogenic, making it an

Table 1 Summary of *in vitro* studies of miRNAs relevant to fatty liver

Model	miRNAs	Biological effect	Validated targets	Ref.
L02	miRNA-10b	Inc expr, decrease steatosis	PPARA	[10]
Huh7	miR-181d	Inc expr, decrease steatosis	Not determined	[11]
Huh7 and Hep3B	miR-122	Dec exp, decrease steatosis	SOCS3	[14]
HepG2	miR-613	Incr exp, increase steatosis	LXRα	[15]
HepG2 and primary human hepatocytes	miR-107	Incr expression, increase steatosis	FASN	[16]

appropriate *in vitro* model for steatosis[12]. Following primary and secondary screening, eleven miRNAs were identified that either increased or decreased intracellular lipid content. Of these, miR-181d showed the strongest influence on steatosis, decreasing lipid droplet formation by approximately 60%. Huh7 cells were also used, along with Hep3B human hepatocellular carcinoma cells, to link expression of miR-122 to decreased fatty acid and cholesterol levels[13]. Expression of the suppressor of cytokine signaling 3 (*SOCS3*) gene is also regulated by miR-122[14]. SOCS3 protein increases expression of sterol regulatory element-binding protein 1 (SREBP1), a transcription factor that regulates cholesterol and lipid metabolism. Silencing of miR-122 in Huh7 cells corresponded with reduced SOCS3 expression, which in turn decreased SREBP1 levels, while restoration of SREBP1 expression when miR-122 levels were depleted through RNA silencing could be achieved by over-expression of SOCS3.

In addition to L02 and Huh7 cells, HepG2 cells have also been used as a model in which to study the role of miRNAs in hepatic lipid metabolism. In these cells, over-expression of miR-613 reduced expression of the nuclear receptor liver X receptor α (LXRA) and several of its target genes including acetyl-CoA carboxylase, sterol-regulatory element binding protein 1c, and fatty acid synthase, and led to the formation of lipid droplets[15]. miR-613 was shown to bind to the 3′-untranslated region of the *LXRA* mRNA. Similarly, miR-107 was shown to bind to the 3′ UTR of the fatty acid synthase gene (*FASN*), reducing its expression and causing malonyl CoA and lipid accumulation in both HepG2 cells and primary hepatocytes[16].

As evident from the studies described, the inherent advantage of cultured cell systems for the study of miRNA physiology in NAFLD is in their ease and economy of manipulating levels of specific molecules. However, NAFLD occurs in the context of multiple cell types that constitute the liver and with molecular interactions with distant cell types and organs; therefore studies involving *in vivo* models have been important in enhancing our understanding of the role miRNAs play in the development of the disease.

In vivo studies

A number of studies of miRNAs have been conducted using animal models of fatty liver, primarily in mice and rats[17], in whom NAFLD is typically induced with a high fat diet. A summary of the main findings from these studies is shown in Table 2. In one study, microarray analysis of 350 miRNAs in liver samples of sprague-dawley rats with diet-induced NASH showed downregulation of miR-122, miR-451, and miR-27a and upregulation of miR-429, miR-200a, and miR-200b compared to animals fed a standard diet[18]. In a similar microarray-based study of diet-induced NASH in sprague-dawley rats, the authors observed that upregulation of miR-146a, miR-210, miR-29c, miR-103, miR-20b-5p, miR-106b, miR-212, miR-31, miR-10a, miR-203, miR-27b, miR-199a, miR-107, let-7b, and downregulation of miR-33, miR-145, miR-196b, miR-93, let-7d, miR-19 could differentiate between steatohepatitis and steatosis[7]. No common mRNA targets were found for the 14 upregulated miRNAs, but 12 common targets were found for the six downregulated miRNAs including stearoyl-coenzyme A desaturase 1 (Scd1). Hepatic expression of miR-15b was also shown to be upregulated in liver RNA of Sprague Dawley rats fed a high fat diet for 16 wk[19]. Surprisingly, no miRNAs were replicated across these rat studies, possibly due to differences in dietary composition and regimen, as well as phenotypic endpoint.

MiRNA microarray analysis of liver RNA from both C57BL/6J and DBA/2J inbred strains of mice fed a lipogenic methyl-deficient diet to induce a form of fatty liver injury similar to human NASH identified significant upregulation in the expression of miR-34a, miR-155, and miR-200b, and downregulation of miR-29c[17]. A strain-specific effect was seen, with more significant changes occurring in DBA/2J mice.

In livers of C57BL/6J mice fed a high fat diet for eight weeks, miR-467b expression was significantly decreased, corresponding to an increase in hepatic lipoprotein lipase (LPL) expression[20]. The authors utilized bioinformatics sequence analysis to identify LPL as a direct target of miR-467b and confirmed the miRNA-mRNA interaction *in vitro*. Interestingly, the interaction between miR-467b and its target gene was associated with insulin resistance, which strongly increases the risk of NAFLD. In a separate study, apoE(-/-) mice treated with intra-peritoneal injection of an miR-467b mimic or agomirna (synthetic chemically modified RNA duplexes) led to reduced lipid accumulation and inflammatory cytokine secretion by macrophages *via* downregulation of LPL expression,

Table 2 MiRNAs associated with nonalcoholic fatty liver disease

miRNA	Species	Model	Ref.
Downregulation of miR-122, miR-451, and miR-27a and upregulation of miR-429, miR- 200a, and miR-200b	Sprague-dawley rats	High fat diet induced NASH *vs* standard diet	[18]
Upregulation of miR-146a, miR-210, miR-29c, miR-103, miR-20b.5p, miR-106b, miR-212, miR-31, miR-10a, miR-203, miR-27b, miR-199a, miR-107, let-7b, and downregulation of miR-33, miR-145, miR-196b, miR-93, let-7d, miR-19	Sprague-dawley rats	High fat diet induced NASH *vs* steatosis	[7]
miR-15b	Sprague-dawley rats	High fat diet induced NASH *vs* standard diet	[19]
Decreased expression miR-29c, and increased expression miR-34a, miR-155, and miR-200b	C57BL/6J and DBA/2J mice	methyl-deficient diet induced NASH	[17]
Decreased miR-467b	C57BL/6J mice	High fat diet *vs* standard diet	[20]
Increased miR-103 and miR-107	C57BL/6J mice	High fat diet *vs* standard diet	[22]
Decreased miR-21	C57BL/6J mice	High fat diet *vs* standard diet	[23]
miR-122 decreased in all strains, while expression of miR-34a, miR-200b, and miR-181a	A/J, C57BL/6J, C3H/HeJ, 129S/SvImJ, CAST/EiJ, PWK/PhJ, and WSB/EiJ strains	choline- and folate-deficient diet	[24]
miR-122 (-/-)	129SvJ	Steatosis on a Normal Diet	[25]
miR-155 (-/-)	C57BL/6	High fat diet *vs* standard diet	[26]
Up regulation of miR-122, miR-24, miR-195a, miR-106b, miR-15b, miR-802, miR-185, miR-214, miR-378, and let-7c; downregulation of miR-224, miR-126, miR-7a, miR-128, miR-455, miR-452, miR-135b, miR-145, miR-18a, and miR-196a	*ob/ob*	Standard diet	[27]
Increased miR-16, miR-122, miR-126 decreased miR-27b	Gankyrin transgenic zebrafish	Standard diet	[29]

NASH: Non-alcoholic steatohepatitis.

leading to protection from atherosclerosis in these animals[21]. Together, these findings suggest that the miR-467b-LPL interaction may play an important role in lipid accumulation, which may exert diverse effects on the development of both hepatic steatosis and atherosclerotic vascular disease.

In LDL receptor knockout ($LDLR^{-/-}$) mice derived from a C57BL/6J background and fed a high-fat diet for 10 wk, increased expression of miR-103 and miR-107 was abolished by daily dosing of a mixture of concentrated plant-derived polyphenol compounds[22], although weight gain and liver steatosis were ameliorated. The expression of miR-122 was not altered by the high fat diet, but was decreased by dietary polyphenols. Further studies on polyphenol administration for 8 wk to C57BL/6J mice fed a high-fat diet demonstrated that lycopene also ameliorated hepatic steatosis and prevented down-regulation of miR-21[23]. Expression of fatty acid-binding protein 7 (FABP7) was downregulated *via* interaction of miR-21 with the FABP7 3′ UTR.

Administration of a choline- and folate-deficient diet for 12 wk to induce NAFLD-like liver injury in inbred male mice of the A/J, C57BL/6J, C3H/HeJ, 129S/SvImJ, CAST/EiJ, PWK/PhJ, and WSB/EiJ strains induced strain-related differences in levels of hepatic and plasma miRNAs[24]. Hepatic expression of miR-122 decreased in all strains, while expression of miR-34a, miR-200b, and miR-181a increased and was correlated with histological severity of liver injury. Serum levels of miR-34a, miR-122, miR-181a, miR-192, miR-200b, and miR-221 were correlated with histological severity across all strains, providing evidence that release of miRNAs may serve as biomarkers of liver injury.

Knockout studies have also been conducted targeting specific miRNAs, although few have been reported thus far. A recent study by Hsu *et al*[25], in which mice with either germline or liver-specific knockdown of miR-122 were generated, showed that deficient animals developed steatosis, steatohepatitis, fibrosis, and spontaneous tumors that were histologically similar to hepatocellular carcinoma. These findings support a critical role for miR-122 in mediating the progression of steatosis to more clinically severe phenotypes and the subsequent development of cancer.

An independent study also using knockout mice investigated the role of a second miRNA, miR-155, in the development of hepatic steatosis[26]. In that study, $miR\text{-}155^{-/-}$ mice fed a high fat diet for six months developed significantly more hepatic steatosis, which was associated with increased liver weight and lipid levels, than C57BL/6 wild-type controls. Hepatic expression of genes involved in glucose regulation, fatty acid uptake, and lipid metabolism were also elevated in the $miR\text{-}155^{-/-}$ mice. Among the differentially expressed genes, the authors identified and validated only one, Nr1h3 (LXRα) as a direct target of miR-155. Together these data indicate that miR-155 plays a protective role in liver lipid metabolism and that downregulation of miR-155 expression may contribute to the development of hepatic steatosis.

In addition to microarray and knockdown studies of specific miRNAs, approaches using next generation sequence analysis have also been used to quantify miRNA expression in NAFLD. Sequencing of liver

non-coding RNA in *ob/ob* and control mice identified 37 differentially expressed hepatic miRNAs[27]. Although miR-122 showed the greatest alteration in expression between the two groups, miR-24, miR-195a, miR-106b, miR-15b, miR-802, miR-185, miR-214, miR-378, and let-7c were also significantly upregulated. In contrast, levels of miR-224, miR-126, miR-7a, miR-128, miR-455, miR-452, miR-135b, miR-145, miR-18a, and miR-196a were significantly downregulated. To determine whether overexpression of miR-126 or inhibition of miR-24 played a mechanistic role, AML-12 liver cells were treated with free fatty acids. Up-regulation of hepatic miR-126 using a miR-126 mimic or down-regulation of hepatic miR-24 using antagomiR-24 was correlated with decreased fat accumulation, suggesting that both may potentially mediate liver steatosis. Additional studies will be necessary to address this possibility.

In addition to mice and rats, zebrafish are presently becoming recognized as suitable models for lipid-related diseases, including hepatic steatosis[28]. In this model, transgenic over-expression of gankyrin, a small ankyrin-repeat protein that plays a role in cellular proliferation, led to increased lipid content in > 90% of viable adult fish. Overexpression of gankyrin led to the development of hepatic steatosis and was associated with increased levels of miR-16, miR-122, and miR-126, and decreased miR-27b[29]. This study provides evidence supporting a link between gankyrin and miRNAs in modulating the development of hepatic steatosis in zebrafish; however, the role of this network in humans is not yet known.

HUMAN STUDIES

A large number of studies on the role of miRNAs in viral hepatitis and hepatocellular carcinoma in humans have been reported[30], which is in contrast to the relatively limited investigations of these molecules in modulating the pathogenesis of NAFLD and liver-related outcomes. Of the studies that have been published, most have been performed using very small sample sizes, thereby limiting the impact of any conclusions drawn from the results. For example, one study recently profiled liver miRNA in 15 individuals with NASH and 15 individuals with normal liver histology[31]. Out of the 474 miRNAs represented on the array, six showed differential expression between the two groups. The authors confirmed overexpression of miR-34a and miR-146 b and underexpression of miR-122 using RT-PCR, although miRNA levels were not associated with NASH severity. Examination of miR-122 target genes, including SREBP-1c, FAS, and HMG-CoA reductase, showed significant increases in mRNA and protein levels in individuals with NASH, which is consistent with *in vitro* findings in HepG2 cells following miR-122 silencing. Similarly, an inverse correlation between miR-122 and levels of SOCS3 and SREBP1 was observed in human liver samples[13]. In obese individuals, levels of miR-34a were approximately 2-fold higher in mild NASH, increasing to more than 3-fold higher in severe NASH relative to steatosis[32]. In comparisons of steatotic and severe NASH liver samples, levels of miR-122, miR-143, and miR-451 were decreased.

In addition to studies using liver tissue, miRNA levels in adipose tissue have also been investigated. A total of 664 miRNAs were profiled in visceral adipose tissue obtained from 12 extremely obese bariatric surgery patients with biopsy-proven NASH and 12 with without NASH[33]. Expression of miR-132, miR-150, miR-433, miR-28-3p, miR-511, miR-517a, and miR-671-3p were all significantly decreased in individuals with NASH. In addition, expression of miR-197 and miR-99 were also decreased in NASH peri-sinusoidal fibrosis compared to non-fibrosis. *IL6* was identified as a target gene for all seven miRNAs, and the authors found that serum IL6 levels were inversely correlated with levels of these candidates. A study with a similar sample size was also conducted using visceral adipose tissue obtained from patients undergoing bariatric surgery[34]. In that study, Drosha, DGCR8, and Dicer1, all of which represent key components of miRNA processing, and seven pri-miRNAs including pri-miR-125b-2, pri-miR-16-2, pri-miR-26a-1, pri-miR-26a-2, pri-miR-7-1, pri-miR-7-2, and pri-miR-7-3 were assayed. Of these, levels of Dicer1, Drosha, DGCR8, and pri-miR-7-1 were significantly increased in NASH patients compared to normal controls. These results indicate that even in the context of severe obesity, specific miRNAs may serve to differentiate liver function, although the small sample sizes of these studies limit the generalizability of the results.

In addition to expression in tissue, miRNAs can also circulate freely in blood or be packaged within microvesicles that provide a high level of protection from degradation. Some studies report a correlation of miRNA levels in tissue and biofluids. A summary of findings from studies of circulating miRNAs in NAFLD is shown in Table 3. For example, levels of miR-122 in serum and liver were significantly correlated (R = 0.461; P = 0.005) in patients with NAFLD[35], suggesting that miR-122 released from hepatic cells enters the bloodstream. Serum levels of miR-122 were lower in individuals with mild steatosis, compared to those with severe steatosis, but higher in patients with mild fibrosis compared to those with severe fibrosis. This result is in agreement with those of previous studies, reporting decreased levels of hepatic miR-122 at advanced stages of fibrosis in patients with liver disease[36]. The reason for the

Table 3 Changes in circulating miRNAs associated with nonalcoholic fatty liver disease in humans

Population	Study design	Source of miRNA	miRNAs	Effect	Ref.
NAFLD	Mild *vs* Severe steaosis; Severe *vs* Mild fibrosis	Serum	miR-122	Decrease	[35]
NAFLD	NAFLD *vs* normal	Serum	miR-122, miR-34a, miR-16, and miR-21	Increased	[36]
NAFLD	NAFLD *vs* normal	Serum	miR-15b	Increased	[19]
NAFLD	NAFLD *vs* normal	Serum	miR-122, miR-192, miR-19a, miR-19b, miR-125b, and miR-375	Increased	[37]

NAFLD: Nonalcoholic fatty liver disease.

discrepancy in miR-122 levels in NAFLD stage may represent the loss of hepatocytes in worsening liver injury. Because hepatocytes are the primary source of miR-122 and since worsening of liver fibrosis results in the replacement of hepatocytes with extracellular matrix, hepatic miR-122 levels may be expected to decrease with severe fibrosis. These results indicate that levels of miR-122 may have significant prognostic value for patients with NAFLD.

Similarly, Cermelli *et al*[36] investigated serum levels of four miRNAs commonly dysregulated in liver fibrosis: miR-122, miR-34a, miR-16, and miR-21. In a study sample comprised of 34 individuals with NAFLD and 19 healthy controls, serum levels of miR-122, miR-34a, and miR-16 were significantly higher in NAFLD patients. Levels of miR-21 showed no difference between the two groups. Interestingly, levels of miR-122 and miR-34a were positively correlated with disease severity from simple steatosis to steatohepatitis, supporting the potential value of these two miRNAs to serve as noninvasive biomarkers for progressive NAFLD.

Zhang *et al*[19] also recently examined miR-15b as a potential biomarker for NAFLD in 69 individuals with fatty liver and 42 healthy controls. Levels of miR-15b were higher in the NAFLD patients compared to the control group. However, because there were significant differences in BMI, blood glucose, triglyceride levels, total cholesterol, and ALT between the two groups, these findings must be interpreted with caution. Additional studies will be necessary to demonstrate the appropriateness of miR-15b as a biomarker for fatty liver disease.

A case hyphen control, multi-phased study that analyzed 84 miRNAs in serum of patients with biopsy-proven NAFLD and healthy controls identified a greater than 2-fold up-regulation of miR-122, miR-192, miR-19a, miR-19b, miR-125b, and miR-375 with steatosis or more advanced NAFLD[37]. Only miR-122 was associated with severe *vs* no or mild fibrosis. Interestingly, miR-122 was 10-fold and miR-192 2-fold down-regulated in the liver. In situ hybridization of miR-122 in liver showed that the miRNA staining was concentrated at the hepatocyte membrane, not more broadly distributed throughout the cytoplasm, consistent with preparation for export to the circulation.

CROSS STUDY COMPARISONS

About a dozen miRNAs have been analyzed in two or more model systems (Table 4). miR-122, the most studied, was also the most discrepant across systems. miR-122, the most abundantly expressed miRNA in hepatocytes has been associated with a variety of liver diseases[38], suggesting complex regulation. Indeed, each miRNA may play a role in regulating the expression of tens to hundreds of genes, which may be regulated by multiple miRNAs. This complex regulatory landscape may thus have species specificity, accounting for differences within or across model systems. Mechanistic differences in the protocols for inducing NAFLD may also be reflected in differential miRNA expression. For example, high fat diet induced NAFLD has substantial differences with a choline- and folate-deficient diet that are very different than dyslipidemic mouse knockout models. Indeed, evidence suggests strain background also has substantial effect on miRNA levels, further implicating complex regulation.

The discrepancy between a decrease observed for human liver and an increase in circulating miR-122 is more difficult to reconcile, although little is known about the metabolism of miRNAs in circulation. In addition, serum levels will reflect the total body levels of the miRNA, thus contributions from other tissues may compensate for decreased levels in the liver. The relative severity of NAFLD may also be related to the levels in the serum, particularly if there is significant ballooning degeneration and hepatocyte cell death with subsequent release of miRNAs.

Despite the discrepant results, the change in expression with NAFLD of 10 of the 12 miRNAs were found to be in the same direction, although seven were only studied in rats and mice. However, three zebrafish miRNAs were concordant with those found in either mice or rats. The general agreement in results between two rodent species, as well as between rodents and zebrafish, suggests that the biology may be conserved. Cross-model concordance between evolutionarily distant species

Table 4 Cross-study comparison of miRNA changes with nonalcoholic fatty liver disease

	Cell lines	Mice	Rats	Zebrafish	Human liver	Human serum
MiR-122		INC/NC/DEC	DEC	INC	DEC	INC
miR-34a	INC/DEC	INC	INC		INC	
miRNA-107		INC	INC			
miR-29c		INC	INC			
miR-200b		INC	INC			
miR-103		INC	INC			
mir-106b		INC	INC			
miR-15b		INC	INC			
miR-126		DEC		DEC		
MiR-145		DEC	DEC			
miR-451			DEC		DEC	
miR-27b			INC	INC		

also suggests functional relevancy. Unfortunately, few human data are available for comparison with animal model results. The need for additional human data is largely limited by the availability of tissue samples. Future studies based on large cohorts of well annotated and high quality biological samples and clinical data are needed.

CONCLUSION

miRNAs may act either independently or interactively with environmental exposures and lifestyle factors to affect susceptibility to hepatic fat accumulation. Thus, the link between miRNA and progression of fatty liver disease represents a key area of focus for research endeavors in the development of novel therapies targeting control and prevention of NASH. Given the substantial public health burden of NAFLD, which is increasing at alarming rates due to the rising prevalence of obesity, novel therapeutic targets are urgently needed to facilitate the development of improved pharmacological therapies for the treatment and prevention of the disease.

In NAFLD, a small number of miRNAs, most notably miR-122, have emerged as potential participants in the regulation of biological processes relevant to the disease. However, much less is known of the specific targets of candidate miRNAs, and how, in fact, they affect disease development and progression and variability in liver-related outcomes in affected individuals. Studies aimed at delineating specific miRNA/mRNA networks will enhance our understanding of the complex pathogenesis of NAFLD and enable exploitation of relevant miRNAs as novel targets for therapeutic interventions. Notably, identification and validation of circulating miRNA signatures may facilitate the development of improved methods for diagnosis and clinical monitoring of disease progression. At present, current findings, combined with the rapidly expanding field of miRNA research, are expected to yield new insights into the complex pathogenesis of NAFLD and may eventually lead to the identification of novel, noninvasive biomarkers for the disease.

REFERENCES

1 **Chalasani N**, Younossi Z, Lavine JE, Diehl AM, Brunt EM, Cusi K, Charlton M, Sanyal AJ. The diagnosis and management of non-alcoholic fatty liver disease: practice Guideline by the American Association for the Study of Liver Diseases, American College of Gastroenterology, and the American Gastroenterological Association. *Hepatology* 2012; **55**: 2005-2023 [PMID: 22488764 DOI: 10.1002/hep.25762]

2 **Starley BQ**, Calcagno CJ, Harrison SA. Nonalcoholic fatty liver disease and hepatocellular carcinoma: a weighty connection. *Hepatology* 2010; **51**: 1820-1832 [PMID: 20432259 DOI: 10.1002/hep.23594]

3 **Wong RJ**, Cheung R, Ahmed A. Nonalcoholic steatohepatitis is the most rapidly growing indication for liver transplantation in patients with hepatocellular carcinoma in the U.S. *Hepatology* 2014; **59**: 2188-2195 [PMID: 24375711 DOI: 10.1002/hep.26986]

4 **Kleiner DE**, Brunt EM, Van Natta M, Behling C, Contos MJ, Cummings OW, Ferrell LD, Liu YC, Torbenson MS, Unalp-Arida A, Yeh M, McCullough AJ, Sanyal AJ. Design and validation of a histological scoring system for nonalcoholic fatty liver disease. *Hepatology* 2005; **41**: 1313-1321 [PMID: 15915461 DOI: 10.1002/hep.20701]

5 **Pagadala MR**, McCullough AJ. The relevance of liver histology to predicting clinically meaningful outcomes in nonalcoholic steatohepatitis. *Clin Liver Dis* 2012; **16**: 487-504 [PMID: 22824477 DOI: 10.1016/j.cld.2012.05.006]

6 **Younossi ZM**, Stepanova M, Rafiq N, Makhlouf H, Younoszai Z, Agrawal R, Goodman Z. Pathologic criteria for nonalcoholic steatohepatitis: interprotocol agreement and ability to predict liver-related mortality. *Hepatology* 2011; **53**: 1874-1882 [PMID: 21360720 DOI: 10.1002/hep.24268]

7 **Jin X**, Chen YP, Kong M, Zheng L, Yang YD, Li YM. Transition from hepatic steatosis to steatohepatitis: unique microRNA patterns and potential downstream functions and pathways. *J Gastroenterol Hepatol* 2012; **27**: 331-340 [PMID: 21793903 DOI: 10.1111/j.1440-1746.2011.06864.x]

8 **Gori M**, Arciello M, Balsano C. MicroRNAs in nonalcoholic fatty liver disease: novel biomarkers and prognostic tools during the transition from steatosis to hepatocarcinoma. *Biomed Res Int* 2014; **2014**: 741465 [PMID: 24745023 DOI: 10.1155/2014/741465]

9 **Alvarez ML**, DiStefano JK. Towards microRNA-based therapeutics for diabetic nephropathy. *Diabetologia* 2013; **56**: 444-456 [PMID: 23135222 DOI: 10.1007/s00125-012-2768-x]

10 **Zheng L**, Lv GC, Sheng J, Yang YD. Effect of miRNA-10b in regulating cellular steatosis level by targeting PPAR-alpha

expression, a novel mechanism for the pathogenesis of NAFLD. *J Gastroenterol Hepatol* 2010; **25**: 156-163 [PMID: 19780876 DOI: 10.1111/j.1440-1746.2009.05949.x]

11 **Whittaker R**, Loy PA, Sisman E, Suyama E, Aza-Blanc P, Ingermanson RS, Price JH, McDonough PM. Identification of MicroRNAs that control lipid droplet formation and growth in hepatocytes via high-content screening. *J Biomol Screen* 2010; **15**: 798-805 [PMID: 20639500 DOI: 10.1177/1087057110374991]

12 **Fujimoto Y**, Itabe H, Sakai J, Makita M, Noda J, Mori M, Higashi Y, Kojima S, Takano T. Identification of major proteins in the lipid droplet-enriched fraction isolated from the human hepatocyte cell line HuH7. *Biochim Biophys Acta* 2004; **1644**: 47-59 [PMID: 14741744]

13 **Shibata C**, Kishikawa T, Otsuka M, Ohno M, Yoshikawa T, Takata A, Yoshida H, Koike K. Inhibition of microRNA122 decreases SREBP1 expression by modulating suppressor of cytokine signaling 3 expression. *Biochem Biophys Res Commun* 2013; **438**: 230-235 [PMID: 23891753 DOI: 10.1016/j.bbrc.2013.07.064]

14 **Yoshikawa T**, Takata A, Otsuka M, Kishikawa T, Kojima K, Yoshida H, Koike K. Silencing of microRNA-122 enhances interferon-α signaling in the liver through regulating SOCS3 promoter methylation. *Sci Rep* 2012; **2**: 637 [PMID: 22957141 DOI: 10.1038/srep00637]

15 **Zhong D**, Zhang Y, Zeng YJ, Gao M, Wu GZ, Hu CJ, Huang G, He FT. MicroRNA-613 represses lipogenesis in HepG2 cells by downregulating LXRα. *Lipids Health Dis* 2013; **12**: 32 [PMID: 23496987 DOI: 10.1186/1476-511X-12-32]

16 **Bhatia H**, Verma G, Datta M. miR-107 orchestrates ER stress induction and lipid accumulation by post-transcriptional regulation of fatty acid synthase in hepatocytes. *Biochim Biophys Acta* 2014; **1839**: 334-343 [PMID: 24560669 DOI: 10.1016/j.bbagrm.2014.02.009]

17 **Pogribny IP**, Starlard-Davenport A, Tryndyak VP, Han T, Ross SA, Rusyn I, Beland FA. Difference in expression of hepatic microRNAs miR-29c, miR-34a, miR-155, and miR-200b is associated with strain-specific susceptibility to dietary nonalcoholic steatohepatitis in mice. *Lab Invest* 2010; **90**: 1437-1446 [PMID: 20548288 DOI: 10.1038/labinvest.2010.113]

18 **Alisi A**, Da Sacco L, Bruscalupi G, Piemonte F, Panera N, De Vito R, Leoni S, Bottazzo GF, Masotti A, Nobili V. Mirnome analysis reveals novel molecular determinants in the pathogenesis of diet-induced nonalcoholic fatty liver disease. *Lab Invest* 2011; **91**: 283-293 [PMID: 20956972 DOI: 10.1038/labinvest.2010.166]

19 **Zhang Y**, Cheng X, Lu Z, Wang J, Chen H, Fan W, Gao X, Lu D. Upregulation of miR-15b in NAFLD models and in the serum of patients with fatty liver disease. *Diabetes Res Clin Pract* 2013; **99**: 327-334 [PMID: 23287814 DOI: 10.1016/j.diabres.2012.11.025]

20 **Ahn J**, Lee H, Chung CH, Ha T. High fat diet induced downregulation of microRNA-467b increased lipoprotein lipase in hepatic steatosis. *Biochem Biophys Res Commun* 2011; **414**: 664-669 [PMID: 21986524 DOI: 10.1016/j.bbrc.2011.09.120]

21 **Tian GP**, Tang YY, He PP, Lv YC, Ouyang XP, Zhao GJ, Tang SL, Wu JF, Wang JL, Peng J, Zhang M, Li Y, Cayabyab FS, Zheng XL, Zhang DW, Yin WD, Tang CK. The effects of miR-467b on lipoprotein lipase (LPL) expression, pro-inflammatory cytokine, lipid levels and atherosclerotic lesions in apolipoprotein E knockout mice. *Biochem Biophys Res Commun* 2014; **443**: 428-434 [PMID: 24309104 DOI: 10.1016/j.bbrc.2013.11.109]

22 **Joven J**, Espinel E, Rull A, Aragonès G, Rodríguez-Gallego E, Camps J, Micol V, Herranz-López M, Menéndez JA, Borrás I, Segura-Carretero A, Alonso-Villaverde C, Beltrán-Debón R. Plant-derived polyphenols regulate expression of miRNA paralogs miR-103/107 and miR-122 and prevent diet-induced fatty liver disease in hyperlipidemic mice. *Biochim Biophys Acta* 2012; **1820**: 894-899 [PMID: 22503922 DOI: 10.1016/j.bbagen.2012.03.020]

23 **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**: 1665-1674 [PMID: 22968990 DOI: 10.1002/mnfr.201200182]

24 **Tryndyak VP**, Latendresse JR, Montgomery B, Ross SA, Beland FA, Rusyn I, Pogribny IP. Plasma microRNAs are sensitive indicators of inter-strain differences in the severity of liver injury induced in mice by a choline- and folate-deficient diet. *Toxicol Appl Pharmacol* 2012; **262**: 52-59 [PMID: 22561871 DOI: 10.1016/j.taap.2012.04.018]

25 **Hsu SH**, Wang B, Kota J, Yu J, Costinean S, Kutay H, Yu L, Bai S, La Perle K, Chivukula RR, Mao H, Wei M, Clark KR, Mendell JR, Caligiuri MA, Jacob ST, Mendell JT, Ghoshal K. Essential metabolic, anti-inflammatory, and anti-tumorigenic functions of miR-122 in liver. *J Clin Invest* 2012; **122**: 2871-2883 [PMID: 22820288 DOI: 10.1172/JCI63539]

26 **Miller AM**, Gilchrist DS, Nijjar J, Araldi E, Ramirez CM, Lavery CA, Fernández-Hernando C, McInnes IB, Kurowska-Stolarska M. MiR-155 has a protective role in the development of non-alcoholic hepatosteatosis in mice. *PLoS One* 2013; **8**: e72324 [PMID: 23991091 DOI: 10.1371/journal.pone.0072324]

27 **Liang T**, Liu C, Ye Z. Deep sequencing of small RNA repertoires in mice reveals metabolic disorders-associated hepatic miRNAs. *PLoS One* 2013; **8**: e80774 [PMID: 24260478 DOI: 10.1371/journal.pone.0080774]

28 **Hölttä-Vuori M**, Salo VT, Nyberg L, Brackmann C, Enejder A, Panula P, Ikonen E. Zebrafish: gaining popularity in lipid research. *Biochem J* 2010; **429**: 235-242 [PMID: 20578994 DOI: 10.1042/BJ20100293]

29 **Her GM**, Hsu CC, Hong JR, Lai CY, Hsu MC, Pang HW, Chan SK, Pai WY. Overexpression of gankyrin induces liver steatosis in zebrafish (Danio rerio). *Biochim Biophys Acta* 2011; **1811**: 536-548 [PMID: 21722753 DOI: 10.1016/j.bbalip.2011.06.011]

30 **Xin X**, Zhang Y, Liu X, Xin H, Cao Y, Geng M. MicroRNA in hepatic fibrosis and cirrhosis. *Front Biosci* (Landmark Ed) 2014; **19**: 1418-1424 [PMID: 24896361]

31 **Cheung O**, Puri P, Eicken C, Contos MJ, Mirshahi F, Maher JW, Kellum JM, Min H, Luketic VA, Sanyal AJ. Nonalcoholic steatohepatitis is associated with altered hepatic MicroRNA expression. *Hepatology* 2008; **48**: 1810-1820 [PMID: 19030170 DOI: 10.1002/hep.22569]

32 **Castro RE**, Ferreira DM, Afonso MB, Borralho PM, Machado MV, Cortez-Pinto H, Rodrigues CM. miR-34a/SIRT1/p53 is suppressed by ursodeoxycholic acid in the rat liver and activated by disease severity in human non-alcoholic fatty liver disease. *J Hepatol* 2013; **58**: 119-125 [PMID: 22902550 DOI: 10.1016/j.jhep.2012.08.008]

33 **Estep M**, Armistead D, Hossain N, Elarainy H, Goodman Z, Baranova A, Chandhoke V, Younossi ZM. Differential expression of miRNAs in the visceral adipose tissue of patients with non-alcoholic fatty liver disease. *Aliment Pharmacol Ther* 2010; **32**: 487-497 [PMID: 20497147 DOI: 10.1111/j.1365-2036.2010.04366.x]

34 **Sharma H**, Estep M, Birerdinc A, Afendy A, Moazzez A, Elariny H, Goodman Z, Chandhoke V, Baranova A, Younossi ZM. Expression of genes for microRNA-processing enzymes is altered in advanced non-alcoholic fatty liver disease. *J Gastroenterol Hepatol* 2013; **28**: 1410-1415 [PMID: 23663110 DOI: 10.1111/jgh.12268]

35 **Miyaaki H**, Ichikawa T, Kamo Y, Taura N, Honda T, Shibata H, Milazzo M, Fornari F, Gramantieri L, Bolondi L, Nakao K. Significance of serum and hepatic microRNA-122 levels in patients with non-alcoholic fatty liver disease. *Liver Int* 2014; **34**: e302-e307 [PMID: 24313922 DOI: 10.1111/liv.12429]

36 **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**: e23937 [PMID: 21886843 DOI: 10.1371/journal.pone.0023937]

37 **Pirola CJ,** Fernandez Gianotti T, Castano GO, Mallardi P, San Martino J, Mora Gonzalez Lopez Ledesma M, Flichman D, Mirshahi F, Sanyal AJ, Sookoian S. Circulating microRNA signature in non-alcoholic fatty liver disease: from serum non-coding RNAs to liver histology and disease pathogenesis. *Gut* 2014

[PMID: 24973316 DOI: 10.1136/gutjnl-2014-306996]
38 **Szabo G**, Bala S. MicroRNAs in liver disease. *Nat Rev Gastroenterol Hepatol* 2013; **10**: 542-552 [PMID: 23689081 DOI: 10.1038/nrgastro.2013.87]

P- Reviewer: Dip N, Mueller WC **S- Editor**: Ji FF **L- Editor**: A **E- Editor**: Lu YJ

Published by **Baishideng Publishing Group Inc**
8226 Regency Drive, Pleasanton, CA 94588, USA
Telephone: +1-925-223-8242
Fax: +1-925-223-8243
E-mail: bpgoffice@wjgnet.com
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
http://www.wjgnet.com