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REVIEW

Role of MicroRNAs in NAFLD/NASH

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Abstract MicroRNAs (miRNAs) are highly conserved, small, 18–25 nucleotide, non-coding RNAs that regulate gene expression at the post-transcriptional level. Each miRNA can regulate hundreds of target genes, and vice versa each target gene can be regulated by numerous miRNAs, suggesting a very complex network and explaining how miRNAs play pivotal roles in fine-tuning essentially all biological processes in all cell types in the liver. Here, we summarize the current knowledge on the role of miRNAs in the pathogenesis and diagnosis of nonalcoholic fatty liver disease (NAFLD) and nonalcoholic steatohepatitis (NASH) with an outlook to the broader aspects of metabolic syndrome. Furthermore, we discuss the role of miRNAs as potential biomarkers and therapeutic targets in NAFLD/NASH.

Keywords MicroRNAs · NASH · NAFLD

Introduction

MicroRNAs (miRNAs) are highly conserved, small, 18–25 nucleotide, non-coding RNAs that regulate gene expression at the post-transcriptional level [1]. In most cases, miRNAs bind to the 3′ un-translated region (UTR) of the target mRNA repressing the translation by destabilizing mRNA and/or silencing translation [1]. However, in some instances they can interact with their targets in a non-3′ UTR-dependent manner [2] and cause the up-regulation of their targets [3, 4].

The biogenesis of miRNAs is a strictly controlled, multi-step process [5]. First, miRNAs are transcribed from the genome as pri-miRNAs in the nucleus by RNA polymerase II. Then, pri-miRNAs are cleaved into pre-miRNAs by the DROSHA-DGCR8 complex. The pre-miRNAs are exported to the cytoplasm, where they are cleaved by DICER-TRBP-PACT into mature miRNAs. Finally, the mature guide miRNA strand is loaded onto the RNA-induced silencing complex (RISC) along with AGO2 and GW182 and binds to the target mRNA, while the other strand gets downgraded [5]. However, a Dicer-independent, non-canonical pathway of activation exists too [5].

The first functional miRNA, lin-4, was discovered by Lee et al. [6]. Since then, in the last two decades, the number of miRNAs grew exponentially and today there are thousands of identified mammalian miRNAs [7]. Each miRNA can regulate hundreds of target gene transcripts, and each target gene can be regulated by numerous miRNAs [8]. miRNAs regulate about 50 % of all protein coding genes in mammals [9], fine-tuning essentially all biological processes in all cell types in the liver [8]. Altered hepatic miRNA profile has been described in NAFLD/NASH both in humans and in animal models [10–15].

Here, we summarize the current knowledge on the role of miRNAs in the pathogenesis and diagnosis of nonalcoholic fatty liver disease (NAFLD) and nonalcoholic steatohepatitis (NASH) with an outlook to the broader aspects of metabolic syndrome. Furthermore, we discuss the role of miRNAs as potential biomarkers and therapeutic targets in NAFLD/NASH.

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Circulating MicroRNAs as Biomarkers in NASH

The presence of cell-free nucleic acid in plasma and serum has been acknowledged for more than 70 years [16]. While nucleases are found in abundance in the extracellular environment, the association of RNA molecules with proteins, lipids, lipoproteins, etc. increases their stability. AGO2-bound miRNAs can be detected 2 months after cell lysis [17]. The stability of miRNAs in the circulation makes them attractive in biomarker discovery [17–20]. Indeed, several studies have evaluated the specificity of miRNAs and their correlation with liver diseases [16]. miRNAs are found in the circulation in complex with proteins, mainly AGO2, but also AGO1, AGO3, and AGO4 [17, 21] or packaged in extracellular vesicles, mostly exosomes [22, 23]. The release of circulating miRNAs can occur via passive process during cell death or via active release of microvesicles from alive cells [16]. A tissue- and environment-specific process has been suggested by previous studies [24]. We showed that the liver-specific miR-122 is found mostly in the exosome-rich fraction in alcoholic and CpG-induced liver injury, while it is predominant in the protein-rich fraction in acetaminophen-induced toxic liver injury. The inflammatory miR-155 showed similar pattern [25]. This suggests that the distribution of miRNAs in the different compartments, protein versus vesicles, could provide additional information regarding the pathogenesis and increase the likelihood to find a specific miRNA pattern as biomarker. A desirable outcome of biomarker discovery would be to identify miRNAs (or a cluster of them) that could distinguish simple steatosis in NAFLD from steatohepatitis in NASH and/or from NASH with fibrosis.

A recent study assessed circulating miRNAs in NASH patients and found that among 84 circulating miRNAs, miR-122, miR-192, mir-19a, miR-19b, miR-125b, and miR-375 were significantly up-regulated [26]. Comparing simple steatosis with steatohepatitis, the expression of miR-122, miR-192 (up-regulated by TGFβ), and miR-375 (key regulator of glucose homeostasis) correlated with disease severity. Interestingly, the majority of miR-122 was not bound to AGO2 despite that in the lipid loaded hepatocytes miR-122 and AGO2 were co-localized. AGO2 expression differed between patients with simple steatosis and NASH. The authors also found that serum miR-122 and miR-192 positively correlated with serum CK-18 levels and miR-122 performed better than CK18 in predicting liver fibrosis [26].

The increased serum mir-122 levels in NAFLD patients were confirmed by other studies [27, 28] and positive correlation was reported between serum miR-122 levels and hepatic steatosis [27]. In addition higher serum miR-21, miR-34a and miR-451 were found in NAFLD patients [27].

In search of a NAFLD-specific miRNA profile, Tan et al. identified a panel (miR-122 5p, miR-1290, miR-37 3p, miR-192 5p) that showed high diagnostic accuracy and was suggested to be better predictor than ALT or FIB-4 [29].

More importantly, circulating miRNAs are not only "passive" biomarkers. There is increasing evidence that these circulating miRNAs also play important role in the intercellular communication and disease development [30] making them attractive therapeutic targets. Our group recently reported the biodistribution and biological function of extracellular vesicle-associated miR-155 [31].

MiRNA Expression in the Liver in NASH

Although progress has been made in understanding the factors causing NAFLD, more needs to be done to dissect the underlying regulatory networks. Recent evidence indicates the role of miRNAs in energy metabolism [32–37] and liver functions [8]. In addition to the metabolic disturbance, cell death regulation and inflammation are also major determinants of the progression of NAFLD and NASH [38, 39]. Because miRNAs closely regulate cell proliferation and survival as well as the complex process of inflammation, understanding of miRNA circuits and their abnormalities in NASH may promote understanding and modulation of disease.

In human livers with NASH, 23 miRNAs were found to be under- or over-expressed compared to normal livers [10]. The predicted targets of those miRNAs were in cell proliferation, apoptosis, inflammation, oxidative stress, and metabolism. Liver expression of miRNA-122 was significantly decreased in NASH [10]. Notably, differential miRNA expression was found not only in the liver, but also in visceral adipose tissue (VAT) of NAFLD patients [40]. Furthermore, altered gene expression of miRNA processing enzymes (Dicer1, Drosha, DGCR8) was reported in VAT in NASH patients [41].

Numerous animal models are used in NAFLD/NASH research. In livers of ob/ob mice that develop steatosis without prominent inflammation, 8 miRNAs (miR-34a, miR-31, miR-103, miR-107, miR-194, miR-335-5p, miR-221, and miR-200a) were up-regulated and three miRNAs (miR-29c, miR-451, and miR-21) were down-regulated [42]. Together, those findings suggested a connection between miRNAs and metabolic disorders.

High-fat diet (HFD) model that mimics the Western diet, resulting in obesity, insulin resistance, hepatic steatosis and later inflammation also leads to altered miRNA expression. In HFD-fed rats, 44 up-regulated and 12 down-regulated miRNAs were identified [11]. Among those 6 (miR-200a, miR-200b, miR-200c, miR-146a, miR-146b, and miR-152) were up-regulated in vitro in

hepatocytes after free fatty acid (FFA) treatment [11]. Studies suggest that the composition of the HFD, including the cholesterol content, affects the miRNA profile [12].

Methionine–choline-deficient (MCD) model of NASH leads to early inflammation, steatohepatitis, and fibrosis, but without the characteristic obesity and peripheral insulin resistance. Our group previously reported a distinct miRNA profile in the livers of MCD diet-fed mice [13]. MCD diet up-regulated 3 % and down-regulated 1 % of the analyzed miRNAs. Five of those miRNAs with altered expression (miR-182, miR-183, miR-199a-3p, miR-705, and miR-1224) were common in alcoholic and nonalcoholic steatohepatitis. All were up-regulated in the MCD model [13]. Recently another group described 71 miRNAs up-regulated (including miRNA-376a, miRNA-127, miRNA-34a, miRNA-300, miRNA-342-3p) and 60 miRNAs down-regulated (including miRNA-122, miRNA-194, miRNA-101b, miRNA-705) in the MCD mouse model [43]. Metformin, which has been shown to improve hepatic steatosis [44], resulted in attenuation of the MCD diet-induced changes in miRNA profile [43]. A recent study from our group found that miR-122 expression was decreased in the liver in the MCD-induced steatohepatitis in mice and the reduction of miR-122 occurred in hepatocytes [28]. Interestingly, expression of pri-miR-122 was also decreased in NASH, suggesting a transcriptional inhibition of miR-122 in NASH. Concomitantly, there was an increase in serum miR-122 levels suggesting loss of liver miR-122 and its potential redistribution to the circulation [28].

Choline-deficient amino acid-define (CDAA) model-induced steatohepatitis and hepatocellular carcinoma (HCC) affects hepatic miRNA expression too. The study of Wang et al. [14] showed differential expression of 30 miRNAs at the different stages of NASH and NASH-related HCC development. The down-regulation of miR-122 and up-regulation of miR-155 (master regulator of inflammation), miR-221/222 [miRNAs involved in epithelial–mesenchymal transition (EMT) and carcinogenesis], and miR-21 (mediator of fibrogenesis) was confirmed by RT-PCR [14]. C/EBPβ and PTEN were identified as potential miR-155 and miR-21 targets, respectively [14].

Alterations of various other mRNAs have been described in human NAFLD/NASH and in animal models of steatohepatitis, and the number is increasing day-by-day.

Functional Effects of miRNA Dysregulation in NASH

While changes in liver miRNA expression in nonalcoholic liver disease and NASH have been reported in human disease as well as in animal models, knowledge is limited regarding the functional significance and causative factors in changes in miRNAs in NASH.

miRNAs in Lipid and Cholesterol Metabolism and Hepatic Steatosis

In the liver, miRNA-122 is expressed in high abundance in hepatocytes where it constitutes for about 80 % of total miRNAs. miR-122 plays multifunctional roles in regulation of lipid metabolism, cell cycle, and in HCV replication [45–48]. It is known to regulate genes involved in fatty acid biosynthesis, and administration of this miRNA antagonist in mice resulted in reduced levels of plasma cholesterol, increased hepatic fatty acid oxidation, and decreased synthesis of hepatic fatty acid and cholesterol [45, 46]. Similarly, miR-122-deficient mice had lower serum cholesterol, LDL, triglyceride, and HDL levels [49, 50]. Silencing of miR-122 in HFD-fed mice reduced hepatic steatosis [45]. However, interestingly, miR-122-deficient mice developed steatohepatitis and HCC despite the lower lipid levels [49, 50]. In humans, miR-122 antagonists are under development.

Hepatic lipid accumulation and inflammation usually occur together. miR-155 is a master regulator of inflammation. Interestingly, mice deficient in miR-155 showed attenuated steatosis but no change in liver damage indicated by serum ALT or inflammation after MCD diet-induced steatohepatitis [51]. Reduction in liver triglycerides and steatosis in miR-155-deficient mice was associated with reduced expression of genes involved in lipid metabolism including Adrp, Dgat2, Cpt1a, Fabp4, Ldtr, Hmgcr, and Ppara [51]. The complexity is well demonstrated by the fact that in another model of NASH, miR-155 deficiency resulted in enhanced hepatic steatosis [52].

miR-21 has been also extensively studied in NASH [53–55]. It has been shown that miR-21 deficiency reduces expression of genes regulating lipogenesis and cell cycle transition via p53 [53]. Furthermore, miRNA-21 inhibition can restore PPARα expression in NASH leading to beneficial outcomes on liver pathology [55].

miR-33a has been found to be involved in fatty acid oxidation, cholesterol homeostasis, and bile acid regulation [56, 57]. The role of miR-33a is complex in these processes as SREBP2 and miR-33a activation resulted in down-regulation of cholesterol hepatic efflux transporters and bile acid synthesis [58]. Furthermore, miR-33-deficient mice develop obesity and liver steatosis presumably due to SREBP1, which is enhanced in the absence of miR-33a [59].

miR-34a has been studied as a potential central factor in NASH [60]. The most prominent target of miR-34a is sirtuin 1 (SIRT1), a NAD-dependent deacetylase that modulates hepatic steatosis and apoptosis [61]. In human NASH, both liver and serum miR-34a were found to be increased and it correlated with the severity of NASH [62].

In HFD-induced steatosis, miR-24 levels were increased in the liver and highly expressed in vitro in hepatocytes; fatty acid treatment up-regulated miR-24 expression [63].

These observations provided basis for additional studies with miR-24-ASO treatment that reduced triglyceride and cholesterol levels in vivo in HFD treated mice [63]; summarized in a recent review of Vincent and Sanyal [64].

miRNAs in Hepatic Inflammation in NASH

There is increasing evidence suggesting the role of innate immunity in NASH [38]. Toll-like receptor (TLR) signaling induces the expression of a variety of miRNAs, and various miRNAs modulate TLR or other PRR signaling both positively and negatively [65].

MiR-155 has been described as a master regulator of inflammation due to its complex effects on key components of intracellular signaling molecules in inflammation [66]. Previous studies from our group have described that miR-155 is increased in Kupffer cells in alcoholic liver disease where it contributes to LPS sensitization and increased TNFα production [3]. Similar to alcoholic liver disease, we found that miR-155 expression was also significantly increased in the livers of mice after MCD diet-induced steatohepatitis [51]. Increased miR-155 was present in hepatocytes as well as in liver mononuclear cells and in Kupffer cells isolated from livers with steatohepatitis compared to control mice. Furthermore, LMNCs and Kupffer cells showed pre-sensitization to further miR-155 increase induced by ex vivo LPS treatment. The pro-inflammatory cytokine, TNFα, is regulated by miR-155 by increasing TNFα mRNA stability and increasing TNFα protein production [3, 66]. Consistent with this, we found that increased miR-155 in LMNC in NASH correlated with increased TNFα production that was further increased by LPS both in LMNC and KCs [51]. In vivo, miR-155 deficiency failed to protect mice from inflammation induced by MCD diet, protein levels of TNFα, MCP-1, and IL-1β, and NF-kB DNA binding was comparable between miR-155 KO and wild-type mice in steatohepatitis [51].

In contrast to miR-155, miR-146a is a negative regulator of TLR signaling and inflammation. While in HFD increased miR-146a expression was reported [11], in the MCD diet model it was significantly down-regulated [67]. Latter one might contribute to the inflammation in NASH via the lack of brake on the pro-inflammatory processes.

Down-regulation of miR-451 was shown to inhibit fatty acid-induced pro-inflammatory cytokine production in NASH via AMPK/Akt pathway [68].

miRNAs in the Progression of NASH and Development of Fibrosis

NASH leads to liver fibrosis and was found to be the etiology of cirrhosis in most patients with "idiopathic" cirrhosis [69]. A broad variety of miRNAs have been identified regulating fibrogenesis via hepatic stellate cell (HSC) activation, EMT etc. The MCD diet model of NASH is characterized by steatosis, inflammation, and development of fibrosis after 6–8 weeks [51] thus widely accepted for studying the progression of NASH.

miR-21 has been extensively studied in fibrosis in NASH [70–72]. It has been shown that miR-21 deficiency reduces expression of genes regulating cell cycle transition via p53 [53]. miR-21 inhibition reduced liver fibrosis by inducing apoptosis of CD24+ progenitor cells [71], and it also regulates ERK1 signaling in HSC activation and hepatocyte EMT [72]. A positive feedback regulation was reported between miR-21 and TGFβ [8].

Fibrosis is the consequence of chronic tissue damage and inflammation, thus we studied the role of miR-155 in NASH fibrosis. Interestingly, miR-155-deficient mice showed reduced fibrosis indicated by attenuated expression of collagen, αSMA and TIMP1, despite the comparable level of inflammation on MCD diet [51]. Reduced fibrosis was associated with reduction in caspase3 protein expression in miR-155 KO mice after MCD diet feeding compared to wild types. The attenuated fibrosis correlated with lower levels of TGFβ and PDGF that both contribute to fibrosis [51]. Further, we found attenuation in liver expression of vimentin and up-regulation of C/EBP DNA binding in miR-155 KO mice compared to wild-type controls with steatohepatitis. Overall, these observations suggested that miR-155 is dysregulated in NASH in multiple cell types in the liver and plays a complex role in regulation of fibrosis in NASH.

miR-34a has been studied as a potential central factor in NASH; this miRNA controls cell cycle arrest, apoptosis, and activation of senescence [61, 73]. The most prominent target of miR-34a is SIRT1, a NAD-dependent deacetylase that modulates apoptosis [61]. Increased miR-34a results in suppression of SIRT1 and subsequent increase in p53 acetylation that leads to increased apoptosis.

While miR-122's role in lipid metabolism is well established, less data are available on their role in fibrosis. As miR-122-deficient mice develop steatohepatitis, fibrosis, and HCC despite the attenuated dyslipidemia [49, 50], studies investigating the role of miR-122 in fibrosis are warranted. Recently, we identified that reduced miR-122 in the liver and hepatocytes has functional consequences. A miR-122 target, MAP3K3 was increased in livers with steatohepatitis at mRNA level, and this was due to the effect of hepatocyte miR-122 [28] (Fig. 1).

The clinical syndrome of NASH is often associated with obstructive sleep apnea and transient episodes of hypoxia [74, 75], latter one intensively studied in fibrogenesis. We found a significant increase in HIF-1α protein levels as well as in HIF-1α nuclear binding in livers with steatohepatitis and fibrosis after 8 weeks of the MCD diet

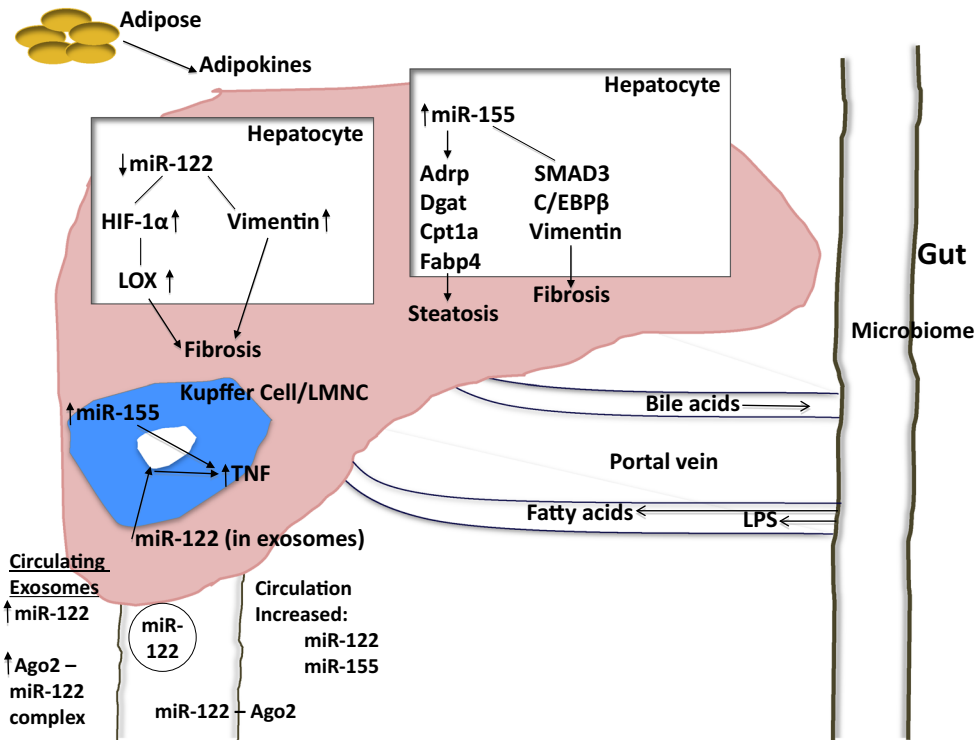

Fig. 1 Summarizing our data on the role of miR-122 and miR-155 in NASH

feeding compared to control mice [28]. Our studies further revealed a causal relationship between HIF-1 increase and miR-122 decreases in NASH. We found that HIF-1α is a miRNA-122 target in hepatocytes, and HIF-1α levels change in a reciprocal direction with miR-122 inhibition or over-expression in hepatocytes [28]. HIF-1α regulates multiple genes at the transcriptional levels. We discovered that parallel to HIF-1 up-regulation in steatohepatitis, the HIF-1α target gene, lysil oxidase, was also significantly increased in livers with steatohepatitis and fibrosis (8-week MCD diet). Liver fibrosis is associated with remodeling. Our study showed that the EMT marker, vimentin was also significantly increased in steatohepatitis. We further showed that vimentin mRNA expression was regulated by miR-122 in hepatocytes from mice with MCD diet-induced steatohepatitis [28]. Together, these results suggested that the decreased hepatic miR-122 levels in MCD diet-induced NASH with fibrosis are associated with increased HIF-1 and vimentin expression and that might contribute to the pathomechanisms of NASH. These new findings highlight the functional role of miR-122 in steatohepatitis that is beyond its originally defined role on regulation of lipid metabolism.

In steatohepatitis induced by alcohol, it was shown that miR-122 is packaged in exosomes and both the numbers of exosomes and miR-122 are increased in the circulation in alcoholic hepatitis in mouse models and in human patients [76]. Furthermore, a functional role for the exosome-packaged miR-122 was identified that indicated that hepatocyte-derived miR-122 is delivered to monocytes and macrophages via exosomes resulting in increases in monocyte/macrophages miR-122 levels. The delivered miR-122 has functional effects on monocytes/macrophages by increasing LPS-induced TNFα production in these inflammatory cells [76]. One can speculate that similar mechanisms may occur in NASH that would provide exosome-mediated intercellular communication between hepatocyte-derived exosomes and liver macrophages via delivery of miR-122 to sensitize the macrophages to inflammatory signals.

Taken together, these observations suggest cell-specific roles for miRNAs in NASH and identify new opportunities for miRNA-based intervention in disease modulation.

MicroRNAs in NASH-Associated HCC

Hepatocellular cancer occurs in livers with cirrhosis where prolonged injury and repair give rise to HCC development. However, in NASH, HCC was found without the presence of cirrhosis both in human disease as well as in animal models suggesting a unique association between NASH and HCC [69]. This association calls for even greater need for biomarkers of HCC. The role of miRNAs in HCC has

been the focus of intense investigations both as a mechanistic element in the pathogenesis of HCC and as potential biomarkers [77]. A cluster of up- an down-regulated miRNA have been identified in HCC associated with NASH. Increased levels of serum miR-122 and liver expression of miR-16, miR-33, miR-21, miR-31, miR-221/222, miR-181a/b, let-7a/a, and miR-10b were found [78]. Other miRNAs showed down-regulation in HCC including miR-122 in the liver tissue, miR-34a, miR-200a/b, miR-99am let-7c/g, and miR-199 a/b-3p (summarized by Gori et al.) [78]. Some of the potential targets via miRNAs promote or inhibit hepatocarcinogenesis are the following: the tumor suppressor gene C/EBPβ (miR-155) [14], PTEN (miR-21) [14] and the HBP1-p53-SREBP1c axis (miR-21) [70]. miR-122 targets numerous genes involved in hepatocarcinogenesis, including cyclin G, c-myc, Wnt1, etc. and exhibit an anti-tumor function [79].

Because of these associations, it is not unexpected that miRNA-based therapies are under investigations. In preclinical models, many of these dysregulated miRNAs have been targeted as summarized in by Callegari et al. [80]. Most of the preclinical approaches are based on attempts to restore miR-122 or miR-124. Others aim at inhibition of miR-221 or miR-494; outcomes of these preclinical studies will be informative for further considerations in human disease modification.

MicroRNAs in Obesity and Adipose Tissue Homeostasis

Obesity, the excessive expansion of white adipose tissue, results in chronic low-grade inflammation, and the adipocytes along with adipose tissue macrophages secrete multiple mediators that act in an autocrine/paracrine or endocrine manner. These adipokines are crucial in the cross talk between the adipose tissue and other organs, such as the liver and in the development of insulin resistance and metabolic syndrome [32, 33].

Obesity results in unique or dysregulated miRNA profile, and some of those differentially regulated miRNAs correlate with body weight and some metabolic parameters [32, 33, 81–84]. miRNAs can regulate adipocyte differentiation [85], adipose tissue inflammation [33], as well as hypothalamic regulation of energy homeostasis [35].

miR-132 activates IL-8 and MCP-1 expression via NF-κB in human pre-adipocytes [86]; miR-221 and miR-222 positively correlate with TNFα expression [87], while others have negative effect. miR-126 and miR-193b directly or indirectly inhibit CCL2 secretion, respectively [88]. miR-223 suppresses adipose tissue inflammation via inhibiting infiltration of M1 polarized macrophages [89] and miR-883b-5p via repressing LPS-binding protein (LBP) and TLR4 signaling [90]. Furthermore, miRNAs, beyond regulating adipokine secretion, can be secreted in microvesicles released from adipocytes [91].

miRNAs in Intestinal Homeostasis and Microbiome

There is increasing evidence that changes in gut microbiome are associated with disease development [92], including obesity [93, 94] and NAFLD/NASH [95–98]. The liver's unique circulation allows direct blood influx from the gastrointestinal tract, and as a first pass organ, it is exposed to the highest concentration of gut-derived microbial components [96]. Increased gut permeability is well-known feature of NAFLD [99, 100]. Inflammasome-associated changes in gut microbiome, including expansion of the *Porphyromonadaceae* family, results in enhanced steatosis and inflammation via increased influx of TLR4 and TLR9 ligands [95]. Furthermore, deficiency of toll-like receptor 5 (TLR5) affects the composition of gut microbiota and leads to the development of metabolic syndrome [101]. The fact that probiotics might be useful in the therapy of steatohepatitis [102, 103] further supports that gut microbiome has significant influence on the pathogenesis of NASH.

MicroRNAs modulate the expression of genes involved in microbial recognition, PRRs and their downstream signaling, and vice versa, miRNAs are induced by microbial products [104]. The crucial role of miRNAs in the regulation of intestinal homeostasis and gut permeability is well demonstrated by the fact that mice with intestinal epithelial cell (IEC)-specific Dicer deficiency have increased gut permeability [105]. In a pro-inflammatory environment, IECs expressed higher level of miR-122, which increased intestinal permeability via directly targeting occludin [106]. Among others, miR-375 and miR-146a were also implicated in IECs homeostasis [104]. On the role of miRNAs in intestinal immune cells, only few studies are available. miR-155 KO mice have defective intestinal humoral response and delayed bacterial clearance [107]. Our group previously reported that miR-155 deficiency prevented alcohol-induced endotoxin increase in circulation, as well as inflammation in proximal intestine [108]. Similar studies in NASH are warranted. Others have suggested that miR-155 might have anti-inflammatory effect, unique function in intestinal LP T cells [109]. miR-29 in the intestinal dendritic cells targets Th17 response [110], and Th17 axis has been implicated in the pathogenesis of obesity and NAFLD [111]. miR-10a, highly expressed in Treg cells, was suggested to play role in maintenance of immune tolerance [112].

Overall, these suggest that miRNAs might contribute to NASH pathogenesis via regulating the gut microbiome and intestinal homeostasis.

miRNAs in the Metabolic Syndrome

NAFLD/NASH is strongly associated with metabolic syndrome or syndrome X, a cluster of conditions leading to advanced atherosclerosis and cardiovascular diseases as first described by Reaven GM [113]. There are several existing definitions, but all include central obesity, insulin resistance (increased fasting plasma glucose or previously diagnosed type 2 diabetes), dyslipidemia (increased plasma triglycerides and/or low HDL cholesterol), and high blood pressure [114]. Beyond these basic criteria, a link has been implicated between metabolic syndrome and numerous other diseases, such as hyperuricemia and gout [115], asthma [116] and obstructive sleep apnea (OSA) [117], disorders of the reproductive system (polycystic ovary syndrome (PCOS) in females [118], erectile dysfunction in males [119]), psoriasis [120, 121], microalbuminuria and chronic kidney disease [122], certain type of cancers [123], etc.

The role of miRNAs in obesity and dyslipidemia has been discussed above. miRNAs involved in insulin resistance were recently reviewed by others [37]. The role of miRNAs in cardiovascular diseases, being involved in every stage of atherosclerosis, from endothelium dysfunction, cellular adhesion, plaque development, progression, rupture, and thrombus formation, has been recently summarized by Nishiguchi et al. [124]. The role of miRNAs in asthma [125, 126], PCOS [127], psoriasis [128], and chronic kidney diseases [129] has been recently reviewed elsewhere and is beyond the focus of our review.

Here, we briefly discuss the role of miRNAs in hyperuricemia as it has been implicated in the pathogenesis of steatohepatitis. There is increasing evidence that hyperuricemia plays role not only in gout but also in other systemic diseases, including atherosclerosis [124] and alcoholic steatohepatitis [130, 131]. Uric acid crystals can serve as endogenous danger signals and activate the inflammasome complex [132], a pro-inflammatory multiprotein platform that has been previously shown to play role in the pathogenesis of NAFLD/NASH [133]. Uric acid also induces inflammation in adipose tissue via the reduction of adiponectin [134]. Furthermore, uric acid results in insulin resistance and hepatic steatosis by generation of mitochondrial oxidative stress in the liver and direct effect on pancreatic islet cells [135]. Fructose, a major component of the Western diet, increases nucleotide turnover and thus uric acid generation [135]. Elevated serum uric acid levels were found to correlate with hepatic fat content [136, 137].

There are only few studies investigating the role of miRNAs in relation to hyperuricemia. MicroRNA-146a expression was induced by monosodium urate (MSU) crystals in one study, and over-expression of miR-146a resulted in blunted pro-inflammatory cytokine including IL-1β, TNFα, MCP-1, and IL-8 production [19]. Interestingly, while PBMCs from patients with acute gout were shown to have increased miR-146a expression [138], we found significantly lower serum miR-146a levels in NAFLD patients (unpublished observation). Notably, NAFLD is a chronic condition, and in the abovementioned study, miR-146a levels were increased in acute gout, but not in hyperuricemia [138]; furthermore, we do not have data on miR-146a expression in PBMCs in NAFLD. Hyperuricemia was also shown to inhibit angiogenesis [139] and endothelial cell migration [140] via miR-92a and miR-663, respectively. In fatty liver, early activation of angiogenesis positively correlates with fibrosis [141]. Overall these suggest that uric acid might activate pro-inflammatory processes but turn on brakes as well via regulation of certain miRNAs.

Summary

MicroRNAs represent newly discovered regulators and potential biomarkers in the NAFLD and NASH. Due to their pleiotropic functions in regulation of most biological processes, dysregulation of miRNAs contribute to pathogenesis of NAFLD/NASH at various levels of disease development and progression (Fig. 1). Some miRNAs regulate glucose and lipid metabolisms, while others govern cell death and survival pathways. There is a lot more to learn about the role of miRNAs in NASH-associated cell survival and inflammation as they relate to liver fibrosis and remodeling. Progress is on the way in defining potential biomarkers that could aid clinicians to differentiate between benign fatty liver in NAFLD and steatohepatitis with and without hepatocyte necrosis. Further understanding of the meaning and potential role of dysregualted miRNAs, particularly of those in the systemic circulation, will provide better understanding of the overall disease process and aim in development of potential miRNA-based new therapeutic interventions.

Key Points

- MicroRNAs are small, non-coding RNAs that regulate gene expression at post-transcriptional level.
- Each mature miRNAs can target many genes, fine-tuning essentially all biological processes in all cell types, including in the liver.

- Disease-specific tissue miRNA signatures have been identified in various liver diseases including NAFLD, NASH, and HCC.
- MicroRNAs contribute to pathogenesis of NAFLD/NASH at various levels of disease development and progression.
- MicroRNA-based therapies are under investigations in preclinical trials.
- The stability of miRNAs in the circulation makes them attractive in biomarker discovery.
- A desirable outcome would be to identify miRNAs (or a cluster of them) that could distinguish simple steatosis in NAFLD from steatohepatitis in NASH and/or from NASH with fibrosis.

Compliance with ethical standards

Conflict of interest None.

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