

## Review Article

# MicroRNAs in modulating non-alcoholic steatohepatitis

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**Abstract:** Nonalcoholic fatty liver disease (NAFLD) has become a major worldwide health problem recently. The initial nonalcoholic fatty liver (NAFL) is usually considered to be benign, but it has the progressive potential, which can progress into nonalcoholic steatohepatitis (NASH), fibrosis, cirrhosis, and hepatocellular carcinoma. NASH is characterized by steatosis and inflammation, and is the indicator of liver injury. Though the exact mechanisms of NASH are still inclusive, studies implicated the inflammatory process may relate to a multiple disorders, such as abnormal lipid metabolism and secretion of adipokines, dysfunction of Kupffer cells (KCs), alteration of intestinal environment and autophagy response, et al. MicroRNAs (miRNAs), the small non-coding RNA molecules that mainly function in regulating RNA silencing and post-transcriptional of gene expression, are reported to be involved in the development of NASH in recently studies. In this review, we highlight miRNAs studies targeting NASH and related pathological processes, and try to analyze and discuss their therapeutic and predictive potentials on NASH patients.

**Keywords:** MicroRNAs, non-alcoholic steatohepatitis, liver, inflammation, regulation

### Introduction

Nonalcoholic fatty liver disease (NAFLD) is characterized by excessive ectopic triglyceride (TG) accumulation in the liver. The initial nonalcoholic fatty liver (NAFL) is usually considered to be benign, but it has the progressive potential, which can progress into nonalcoholic steatohepatitis (NASH), fibrosis, cirrhosis, and hepatocellular carcinoma [1]. As hepatic component of metabolic syndrome (MS), NAFLD is also the risk factor of type 2 diabetes, coronary heart disease, and atherosclerosis [2]. With the epidemics of other MS components such as diabetes, obesity, et al, NAFLD has become a major worldwide health problem, which is estimated to affect 20%-30% of the adult population [2]. NASH is the indicator of liver injury, thus preventing NAFLD progressive is with great importance. Up to now, the exact mechanisms of NASH are still largely unknown, studies implicated the inflammatory process may relate to a multiple disorders, such as abnormal lipid metabolism and secretion of adipokines, dysfunction of Kupffer cells (KCs), alteration of intestinal environment and autophagy response, et al.

MicroRNAs (miRNAs) are reported to be involved in the process of NAFLD/NASH in rodents and humans. MiRNA is a small non-coding RNA molecule (18-24 nucleotides in length) that was first discovered by Ambros and colleagues in 1993 [3]. MiRNAs often function in regulating RNA silencing and post-transcriptional of gene expression via base-pairing with complementary sequences within mRNA molecules. The miRNAs controlled systems have been reviewed everywhere, in brief, genes that encode miRNAs are transcribed from DNA by polymerase II and polymerase III to a primary transcript (pri-miRNAs), pri-miRNA is processed into a short precursor (pre-miRNA) by the ribonuclease (RNase) III family proteins Drosha and Dicer, and then exported into the cytoplasm for further procession into a mature, single stranded miRNA [4]. In this review, we will highlight miRNAs studies that may involve in the development of NASH, and propose the possible relations, aiming to tease the mess among the mist.

### MiRNAs and liver development

MiRNAs are reported to be involved in almost every aspect of biology, including cell differen-

## microRNAs in NASH

**Table 1.** MiRNAs involve in NASH

MiRNAs	Source	Expression	Function	Target genes	Experimental model	Ref
miR-155	Liver CD11b <sup>+</sup> Macrophages	Up-regulated	Lipid metabolism Inflammation	LXR $\alpha$	HFD mice	[21]
miR-155	Liver KCs	Up-regulated	Lipid metabolism Fibrogenesis Cell apoptosis		MCD mice	[43]
miR-155	Liver Hepatocytes KCs	Up-regulated	Lipid metabolism Vitamin metabolism Drug metabolism	Ces3/TGH	HFD mice	[20]
miR-451	Liver	Down-regulated	Inflammation	Cab39	HFD mice	[19]
miR-21	Liver	Up-regulated	Inflammation Sinusoidal endothelial injury	Grhl3	DIO+BDCM mice DIO+CCl <sub>4</sub> mice MCD mice	[89]
miR-21	Liver	Up-regulated	Fibrogenesis Inflammation	SMAD7	Human DIO+BDCM mice	[90]
miR-122	Serum	Up-regulated	Lipid metabolism Inflammation		MCD mice	[26]
miR-122	Serum Liver Hepatocytes	Up-regulated Down-regulated Down-regulated	Fibrogenesis	HIF-1a Vimentin MAP3K3	MCD mice	[27]
miR-122	Liver	Down-regulated	Lipid metabolism	SREBP1c/2 FAS HMGCR	Human	[23]
miR199a-5p	Liver	Up-regulated	Fibrogenesis	NCOR1	MCD mice	[29]
miR-296-5p	Liver	Up-regulated	Cell apoptotic	PUMA	Human	[24]
miR-34a	Liver	Up-regulated	Lipid metabolism	HNF4 $\alpha$	Human	[33]
miR-34a	Liver	Up-regulated	Cell apoptotic		Human	[34]
miR-33a	Liver	Up-regulated	Fibrogenesis		HFD mice MCD mice	[36]
miR-146a-5p	Liver	Down-regulated	Fibrogenesis	Wnt1/5a	MCD mice	[28]
miR-146b	Liver	Up-regulated	Inflammation	TRAF6 IRAK1	HFD mice	[35]
miR-144	Liver	Down-regulated	Inflammation	TLR2	HFD rats	[37]

tiation, proliferation, metabolism and apoptosis. The liver consists of different types of cells, including parenchymal cells (hepatocytes) and nonparenchymal cells, such as cholangiocytes, liver sinusoidal endothelial cells (LSECs), hepatic stellate cells (HSCs) and KCs. During liver development, a cluster of miRNAs are known to be involved. Direct evidence is from Dicer conditional knock-out (KO) animals, which exhibited significantly down-regulated miRNAs, progressed hepatocyte damage, steatosis, overwhelming apoptosis, etc [5]. Transforming growth factor  $\beta$  (TGF- $\beta$ ) is one of the key factors that govern the process of liver development. Studies showed that miR-30, miR-302, miR-20 might be the regulators of TGF- $\beta$  [6]. During hepatocytes maturation, miR-122, the liver specific and most abundant miRNA, directly or indirectly stimulates the expression levels of 24

hepatocyte-specific genes [7]. MiR-122 overexpression in transgenic mice promoted hepatocyte nuclear factor 6 (HNF6) expression, while HNF6 could directly bind to the miR-122 promoter during liver development and stimulate its expression, thus promote hepatocytes maturation [7]. Down-regulation of miR-122 has been reported to restrict the hepatocytic differentiation potential of liver progenitor cells [8].

Using E-cadherin positive cells isolated from mouse fetal liver, Gailhouste *et al.* found that forced expression of miR-148a could promote cell maturation, further analysis identified that miR-148a directly inhibits the major DNA methylation enzyme DNA (cytosine-5-)-methyltransferase 1 (DNMT1) expression [9]. In addition, miR-21, miR-23b and miR-221 are known regulators that promote hepatocyte proliferation, and the miRNAs that inhibit hepatocyte prolif-

eration include miR-26a, miR-33, miR-34a, miR-127 [6].

Liver has the capability of repairing itself, in the process of liver regeneration, a series of miRNAs were reported to regulate hepatocytes proliferation, such as miR-26a [10], miR-127 [11], miR-150 [12], miR-378 [13], miR-199a-5p [14], miR-33 [15], miR-122 [16], miR-376b [17] miR-125b [18] among others.

#### MiRNAs in NASH development

NASH is characterized by inflammation in the liver with concurrent fat accumulation, the exact mechanisms under the progression from steatosis to steatohepatitis are largely unknown, but it is generally considered that the innate immune responses occur in the early stage, and inflammation processes follow [19], whereas multiple disorders such as abnormal lipid metabolism and secretion of adipokines, dysfunction of KCs, alteration of intestinal environment and autophagy response, etc, are correlatively involved.

Recent studies have shown that miRNAs play important roles in the development of NAFLD/NASH (**Table 1**). MiR-155 is identified to be involved in the initial lipid accumulation in the liver. Hepatic miR-155 plays a pivotal role in regulating the biological process of lipid metabolism, applying Cre/lox P system, conditional overexpression of miR-155 transgene mice were generated, studies showed that hepatic miR-155 overexpression alleviate high fat diet (HFD) induced NAFLD, specifically dyslipidemia improvement and hepatic lipid accumulation reduction, and carboxylesterase 3/triacylglycerol hydrolase (Ces3/TGH) was identified as a direct miR-155 target gene that might contribute to the attenuation of the disorders [20]. MiR-155 KO mice were more liable to develop hepatic steatosis compared to wild type (WT) controls, which was associated with the disorder of glucose and lipid metabolism, miRNA target prediction algorithms identified and validated that liver X receptor (LXR)/retinoid X receptor (RXR) pathway as the top canonical pathway and LXR $\alpha$  as a direct miR-155 target gene [21].

Even in NAFLD patients, miRNA expression patterns were different between NAFL and NASH stage [22]. In biopsy proved NASH patients, 46 among the 474 probed miRNAs were differen-

tially expressed in comparison to healthy subjects, the microarray analysis was further verified by quantitative RT-PCR, which identified that the levels of liver miR-34a and miR-146b were up-regulated whereas miR-122 was down-regulated. However, correlation analysis showed that the severity of NASH was not correlated with these miRNAs expression [23]. Other study showed that the reduction of miR-122 can target sterol-regulatory element binding proteins-1c (SREBP-1c), SREBP-2, fatty acid synthase (FAS) and hydroxymethylglutaryl-CoA reductase (HMGCR) to influence lipid metabolism [23]. In comparison with NAFL or healthy individuals, liver miR-296-5p was increased in NASH patients, in vitro experiments proved that miR-296-5p can regulate palmitate (PA)-mediated lipoapoptosis, thus it seems the enhancement of miR-296-5p expression might play a role in minimizing apoptotic damage in human fatty liver disease [24].

Methionine and choline deficient (MCD) diet is a common type method to induce NASH, it was reported that a series of miRNAs altered in MCD induced NASH mice [25]. Dynamic MCD diet feeding demonstrating that serum miR-122 has a liner relationship with hepatic lipid accumulation and inflammatory infiltrates [26], but different from the changes in serum, miR-122 expression was down-regulated in liver and hepatocytes of MCD dieting mice, the miR-122 reduction was positively related with the severity of the liver injury [27]. Using miR-122 overexpression and inhibition techniques, hypoxia-inducible factor-1 $\alpha$  (HIF-1 $\alpha$ ), vimentin and mitogen-activated protein kinase kinase 3 (MAP3K3) were identified as miR-122 targets in primary murine hepatocytes, which might contribute to the process of fibrosis development in the liver [27]. Hepatic miR-146a-5p was also reported to be down-regulated during fibrogenesis process, and overexpression miR-146a-5p could inhibit HSCs activation and reduce collagen deposition [28]. While hepatic miR-122 and miR-146a-5p expression decreased in MCD induced NASH mice, the level of miR-199a-5p was increased, miR-199a-5p was identified to be an active regulator in protein serine/threonine kinase activity, transcription, the Wnt and mitogen-activated protein kinase signaling and insulin signaling pathways. Bioinformatic analysis further

revealed that nuclear receptor co-repressor 1 (NCOR1) to be one of the potential targets of miR-199a-5p, which might participate in HSC activation during NASH progression [29]. In methyl deficient diet (MDD) induced NASH animals, hepatic miR-34a, miR-155, miR-200b and miR-221 were both up-regulated, while miR-122, miR-192 and miR-203 were down-regulated [30].

Mounting evidence confirmed miR-451 could regulate dendritic cell cytokines and suppress neutrophil chemotaxis [31, 32], with these closely integrated with inflammation characteristics, miR-451 is undoubtedly logical to play a role in NASH. Actually, miR-451 reduction existed in the liver of patients with NASH and mouse with diet induced (9 month HFD) steatohepatitis, and also in PA-exposed HepG2 cells [19]. In PA treated HepG2 cells, miR-451 overexpression could inhibit IL-8 and TNF $\alpha$  secretion and NF- $\kappa$ B activation by direct targeting Cab39 and adenosine 5'-monophosphate (AMP)-activated protein kinase (AMPK)/protein kinase B (Akt)/NF- $\kappa$ B pathway [19]. Studies showed that hepatic miR-34a was increased in NASH patients [33, 34]. Actually, the miR-34a increase could be also observed in HFD, *ob/ob*, *db/db* and streptozocin-treated mice. By treating WT mice or miR-34a KO mice with miR-34a mimic. Xu et al found that miR-34a regulates the expression of hepatocyte nuclear factor 4 $\alpha$  (HNF4 $\alpha$ ) in HepG2 cells, they further verified that miR-34a directly inhibition of HNF4 $\alpha$  gene in regulating lipid metabolism [33]. Rui et al discovered that miR-34a increase could modulate SIRT1/p53 proapoptotic pathway, whereas UDCA treatment could reverse the pathological change [34].

MiR-146 is primarily involved in regulating of inflammation and the innate immune function. In lipopolysaccharide (LPS) treated macrophages or oleic acid (OA) treated HepG2 cells, miR-146b could directly inhibit the expression of interleukin-1 receptor-associated kinase (IRAK1), and tumor necrosis factor receptor-associated factor 6 (TRAF6), thus suppress NF- $\kappa$ B activation, indicating the anti-inflammatory property of miR-146. Treating HFD induced NASH mice with miR-146b could suppress hepatic TRAF6, IRAK1, TNF- $\alpha$ , IL-6 expression and also attenuate lipid accumulation in the liver [35].

In addition, studies also found the increased expression of miR-33a and miR-144 in animal models of NASH, and these miRNAs were supposed to be toll-like receptors (TLRs) dependent, and also involved in regulating inflammatory pathways in the development of NASH [36, 37].

#### MiRNAs alteration in Kupffer cells

KCs are specialized macrophages located in the liver lining the walls of the sinusoids, and can take up and destroy foreign material such as bacteria. KCs are activated in response of liver injury. Receptors on the KCs internalize endotoxin (LPS), thus activates the transcription of pro-inflammatory cytokines (TNF $\alpha$ ) and production of superoxides (pro-oxidant), which are the typical biomarkers of NASH. KCs could be regulated by miRNAs, among these, miR-155 is one of the research hotspots. MiR-155 is a key regulator of anti-inflammatory mechanisms that control innate and adaptive hepatic immune responses [38]. In response to chronic infection or stress, hepatic miR-155 can be elevated. LPS or TLR3 agonists challenge could up-regulate miR155 level in isolated KCs while pre-treatment with IL-10 or TGF- $\beta$  suppress TLR3-induced miR-155 expression [38]. Inhibition miR-155 in KCs showed decrease MHC-II, CD40, and CD86 expression, thus suppressed antigen-presenting function and affected SOCS1/JAK/STAT inflammatory pathways [39]. The study using RAW264.7 macrophages got the consistent results, indicating the immunosuppression function of KCs/microphages could be positively regulated by miR-155 inhibition [40]. In liver ischemia-reperfusion (I/R) injury mice, miR-155 deficiency results in the development of M2 macrophages and produces a regulatory inflammatory response with higher level of IL-10, but lower levels of TNF- $\alpha$ , IL-6, and IL-12p40, KCs isolated from these miR-155 KO mice also produce lower TNF- $\alpha$  and more IL-10 upon LPS challenge in comparison to those from WT control mice [41]. While administrating exosomes loaded with synthetic miR-155 mimic into miR-155 KO mice, the restoration of miR-155 in KCs showed augment monocyte chemotactic protein 1 (MCP1) mRNA increase, illustrating the pro-inflammatory properties of miR-155 in KCs [42]. In addition, miR-155 targets LXR $\alpha$  in regulating lipid metabolism and liver injury in miR-155 loss of function mice model, the effect of



miR-155 regulation takes place in KCs, but not in other liver cell lineages, since increased LXR $\alpha$  expression specifically presents in CD11b<sup>+</sup> macrophages [21]. Other study also proved that MCD diet could cause increased miR-155 expression of KCs in mice, however, miR-155 KO failed to reduce inflammation though the levels of liver steatosis and fibrosis biomarkers were reduced [43].

In co-culture of primary hepatocytes and KCs, transfecting miR-146a mimic into KCs attenuates primary hepatocyte apoptosis by suppressing KCs activation and TNF- $\alpha$  production after hypoxia/reoxygenation (H/R) [44]. Another study showed that miR-146a and miR-146b could decrease the expression of IRAK1 and TRAF6, and inhibit pro-inflammatory cytokines secretion, such as TNF- $\alpha$  and IL-6 in KCs [45]. MiR142-3p was up-regulated in KCs after LPS stimulation, and miR-142-3p inhibitor could improve LPS-mediated adenylyl cyclase 9 (AC9) inhibition in KCs, which may result in inactivation of KCs [46]. Pifithrin (PFT), a p53 inhibitor, proved to be an anti-inflammatory reagents during hepatic I/R injury, in PFT treated KCs, miR-34a expression was reduced along with the decreased expression of TNF- $\alpha$ , IL-6, and iNOS in response to LPS challenge, suggesting that the repression of p53/miR-34a recruit can inhibit KCs activation and inflammation response [47]. MiR-223 was primarily located in KCs [48], miR-223 mimic could decrease IL-1 $\beta$  secretion and KCs activation after concanavalin A treatment, which might associated with the repressing of AIM2 (absent in melanoma 2) expression [49].

#### **MiRNAs in regulating intestinal environment**

The host provides habitats for the diverse microbial ecosystems. The gastrointestinal tract harbors a large quantities of microbial population ( $10^{14}$  bacteria), which compose the diverse microbial ecosystems. Recent studies demonstrated that the gut microbiota is involved in metabolic disorders and gut-derived endotoxin, which contributes to the formation of NASH [50, 51]. Studies showed that germ-free mice that colonized with the microbiota from pathogen-free mice implicated the change of host miRNAs in response to the microbiota colonization [52, 53], suggesting the microbiota can also participate in regulating miRNAs,

which could in turn modulate host gene expression [52, 53].

MiR-212 is highly expressed in intestine, and ethanol treatment could increase miR-212 expression in Caco2 cells, in vivo experiments further confirmed the over-expression of miR-212 can decrease the major component of tight junction zonula occludens 1 (ZO-1) protein levels, and up-regulate the expression of iNOS, which might contribute to result in intestinal barrier dysfunction [54, 55]. Chronic ethanol exposure increases intestinal miR-122a expression, which decreased occludin expression leading to increase intestinal permeability. Probiotic *Lactobacillus rhamnosus* GG (LGG) supplementation have been shown to promote intestinal epithelial integrity and attenuate ethanol-induced liver injury in mice, and these benefits of LGG were associated with miR-122a inhibition [56]. The miR-122a increase can be also observed in TNF- $\alpha$  treated Caco2 cells, miR-122a mimics could induce and increase tight junction permeability, whereas miR-122a inhibitor prevent TNF- $\alpha$  induced damage, further experiment identified occludin as the directly target in the process [57].

The dysfunction of intestinal barrier is also reported in miR-21 overexpression subjects, and the potential targets include RhoB and CDC42 (PTEN, Akt) genes [58, 59]. MiR-21 KO in C57BL/6J mice showed attenuated inflammation response, intestinal permeability and tissue injury [60]. In addition, miR-29 was also found to be up-regulated in colitis mice, and miR-29a/b KO could inhibit intestinal permeability by targeting NF- $\kappa$ B repressing factor (NKRFB) and Claudin 1 [61].

During the process of intestinal permeability alteration, some other miRNAs were down-regulated, such as miR-193a-3p [62], miR-107 [63], miR-10a [64] and miR-200c-3p [65], and the mimics of these miRNAs all showed benefits in improving the intestinal permeability.

#### **MiRNAs in regulating bile acid metabolism**

Bile acids are steroid acids found predominantly in the bile of mammals and other vertebrates. Bile acids, especially the lipophilic bile acids have emerged as potent modulators of lipid metabolism. By binding to farnesoid X receptor (FXR) and G protein-coupled bile acid receptor

1 (GPBAR1 or TGR5), lipophilic bile acids play multiple roles in mediating hepatic and peripheral lipid metabolism, and enhancing expression can promote insulin sensitivity and decrease hepatic gluconeogenesis and circulating scavenger receptors (SRB1) in the liver [66, 67]. A recent large multi-center, randomized, placebo-controlled trial identified obeticholic acid to be a potential reagent for treating NASH, and 72-week obeticholic acid intervention improved the histological features of NASH, which arise great interest of bile acids in the development of NASH [68]. Studies also provided evidence that miRNAs might play a role in regulating bile acid metabolism during NASH development.

The synthetic FXR agonist GW4064 decreases hepatic miR-34a levels in mice, and hepatic miR-34a levels are elevated in FXR-null mice, indicating the nuclear bile acid receptor FXR might regulate or be regulated by miR-34a in the liver. The expression of miR-34a is negatively related to the expression of SIRT1, a NAD-dependent deacetylase, and the orphan nuclear receptor, small heterodimer partner (SHP) might be one of the mediators in this process [66]. Compared to patients with mild disease, liver tissues of severe fibrosis patients showed greater amounts of hepatocyte death, lower levels of FXR, and increased miR-199a-3p. Activation of FXR with GW4064 in HepG2 cells, or inject mice with FXR ligand both inhibit the level of miR-199a-3p, which protects hepatocytes from injury by enhancing liver kinase B1 (LKB1) activation [69]. In human primary hepatocytes, three types of FXR agonists, chenodeoxycholic acid, GW4064, and fibroblast growth factor (FGF) 19 all induced increased miR-122a and miR-422a levels respectively. Since the putative recognition sequences for miR-122a and miR-422a are localized in the 3'-UTR of human cholesterol 7 alpha-hydroxylase (CYP7A1) mRNA, it is not unexpected that the miR-122a and miR-422a mimics inhibit, whereas their inhibitors stimulate CYP7A1 mRNA expression [70]. CYP7A1 is one of FXR target genes, and can be negatively regulated by FXR activation. The cytochrome P450 heme enzyme is the rate-limiting enzyme in the synthesis of bile acid from cholesterol via the classic pathway. CYP7A1-tg mice showed improved metabolic homeostasis and increased SREBP-2 expression, since miR-33a encoded by intron

16 of the SREBP-2 gene, hepatic miR-33a expression is co-induced by CYP7A1 overexpression, which is in association with the decreased bile acid pool and lowered serum cholesterol in mice [71].

GPBAR1/TGR5 is known to play an important role in alleviating obesity and improving glucose regulation, TGR5 activation by a natural compound induced miR-26 expression, and JNK pathway was identified to be the target of miR-26 [67]. Hydrophobic bile acids, particularly deoxycholic acid (DCA), activate apoptosis and are increased in NASH. In rat primary hepatocytes, DCA induced miR-34a expression in a dose- and time-dependent manner, and miR-34a inhibition significantly rescued apoptosis induced by DCA. In accordance with the vitro results, rats administrated with DCA also demonstrated increasing miR-34a expression [72]. MiR-199a-5p was increased in bile acid stimulated hepatocytes, as well as in the liver of bile duct-ligated mice. Overexpression of miR-199a-5p could ease hepatocytes from endoplasmic reticulum stress and cell death by repressing GRP78, ATF6 and IRE1 $\alpha$ , thus protects the liver from injury [73].

#### MiRNAs in autophagy process

Autophagy is a lysosomal degradation pathway that can degrade bulk cytoplasm and superfluous or damaged organelles to maintain cellular homeostasis. Recent evidence indicated that lipophagy, a specific autophagy that selectively degrades lipid droplets, involved in controlling hepatic lipid droplets and apoptosis. It has been shown that lipophagy inhibition increases TG contents in hepatocytes, whereas rapamycin, a reagent that induce lipophagy, could decrease the oleic acid-induced TG accumulation. Therefore, the specific autophagy has an important impact on the pathogenesis of NAFLD [74, 75].

MiRNAs and autophagy are mutually interplayed, and many miRNAs have reported to regulate autophagy. In SK-Hep-1, Huh 7, HepG2 cells, miR-26a is reported to be up-regulated during the autophagic process, and overexpression of miR-26a could enhance autophagic in SK-Hep-1 cells. In animal experiments, hepatic steatosis and liver injury were attenuated in miR-26a liver-specific overexpression mice after ethanol administration compared

with WT mice, this protective effect of miR-26a was declined when pretreated with autophagy inhibitor chloroquine. Further research found miR-26a could directly target several autophagy-related genes, such as dual specificity phosphatase 4 (DUSP4) and DUSP5 in Huh 7 cells [76]. Other studies have shown that miR-375 [77, 78], miR-101 [79, 80], miR-100 [81], miR-199-5p [82] and miR-423-5p [83] could regulate autophagy by targeting autophagy-related genes. What is more, autophagy could also influence miRNAs, during the autophagic process, miR-224 can be preferentially degraded and thus contribute to inhibit tumorigenesis [84, 85].

#### The interplay of miRNAs and adipokines

NAFLD is strongly associated with visceral adiposity, the secretion of adipokines from adipose tissue could promote necroinflammation, thus play important roles in the pathogenesis of NASH. Adipose tissue secrete a series of adipokines such as leptin, resistin, TNF- $\alpha$  and adiponectin. Several adipokines have been reported to be correlated with the change of miRNAs. In biopsy-proven NASH, the expression of miR-7-1, miR-132, miR-150, miR-433, miR-28-3p, miR-511, miR-517a, miR-671 in visceral adipose tissue are different from non-NASH patients [86, 87], indicating adipose tissue miRNAs may participate in the progression of NAFLD.

In the progression from steatosis to steatohepatitis, leptin was identified as one of the proinflammatory mediators. Studies showed that both liver miR-21 and leptin levels were increased in NASH animals, whereas the sinusoidal endothelial dysfunction was improved in miR-21 KO mice, further research using *ob/ob* (leptin KO) mice identified that miR-21 could also be regulated by leptin [88, 89]. In addition, miR-21 and leptin may involve in fibrogenic processes in experimental and human NASH. Mice that were deficient in leptin had decreased miR-21 levels, whereas miR-21 KO mice had decreased co-localization events of sma and mad-related protein (SMAD)2/3-SMAD4 in the nucleus, increased SMAD7 levels, and decreased fibrogenesis [90].

In RAW264.7 macrophages, adiponectin could increase miR-155 expression to regulate inflammatory response via mitogen-activated protein

kinase (MAPK)/NF- $\kappa$ B dependent pathway [91], using the same cell line, another study indicated that adiponectin could modulate miR-21 and programmed cell death protein 4 expression, which dependent on ERK and JNK/NF- $\kappa$ B pathways [92]. In 3T3-L1 adipocyte, miR-21 was up-regulated during adipocyte differentiation, overexpression of miR-21 could increase adipocyte differentiation and adiponectin expression by directly suppress AP-1 expression [93]. Treat human pre-adipocytes with leptin and TNF- $\alpha$  could down-regulate miR-221, which contribute to the process of insulin resistance [94]. MiR-221 inhibits nitric monoxide production in endothelial cells after adiponectin stimulation, however, NF- $\kappa$ B signaling can be activated upon the effect of miR-221 [95]. In *ob/ob* mice, miR-378 expression in white adipose tissue was increased, while the adiponectin expression was decreased. MiR-378 mimic could inhibit adiponectin expression in 3T3-L1 cells [96]. The increased miR-378 expression upon TNF- $\alpha$ , IL-6, and leptin stimulation in human adipocytes further confirmed the previous study [97]. In mature human adipocytes, TNF  $\alpha$  and IL 6 could increase, while resistin and leptin decrease the level of miR-1908, suggesting miR-1908 can be regulated by adipokines [98]. In addition, miR-143 [99, 100], miR-26b [101, 102], miR-492 [103], miR-145 [104], miR-146b-5p [105], miR-883b-5p [106] and miR-335 [107] all have reported to be regulated by one or more kinds of adipokines.

#### Prospects and conclusions

Despite numerous research efforts, the exact mechanisms of NAFLD are still elusive. Even patients under the same condition of NAFLD, the reason why some of them are progressive while others are not is still an unsolved problem. The upcoming "precision medicine" mode is largely based on pan-omics (genomics, proteomics, metabolomics, transcriptomics, etc) and systematic biology, and miRNAs application is one of the attracting highlights in this area. According to the aforementioned information, miRNAs almost involved in all the aspects of NASH development and progression, thus suggesting the importance in clarifying the potential mechanisms. Analysis of the miRNAs expression profiles can help explicating the pathological mechanisms of NASH, and also to benefit for diagnosing, preventing and treating NAFLD.

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### Disclosure of conflict of interest

The authors declare that they have no competing interests.

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