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REVIEW ARTICLE

The miR-23–27–24 cluster: an emerging target in NAFLD pathogenesis

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The incidence of non-alcoholic fatty liver disease (NAFLD) is increasing globally, being the most widespread form of chronic liver disease in the west. NAFLD includes a variety of disease states, the mildest being non-alcoholic fatty liver that gradually progresses to non-alcoholic steatohepatitis, fibrosis, cirrhosis, and eventually hepatocellular carcinoma. Small non-coding single-stranded microRNAs (miRNAs) regulate gene expression at the miRNA or translational level. Numerous miRNAs have been shown to promote NAFLD pathogenesis and progression through increasing lipid accumulation, oxidative stress, mitochondrial damage, and inflammation. The miR-23–27–24 clusters, composed of miR-23a–27a–24–2 and miR-23b–27b–24–1, have been implicated in various biological processes as well as many diseases. Herein, we review the current knowledge on miR-27, miR-24, and miR-23 in NAFLD pathogenesis and discuss their potential significance in NAFLD diagnosis and therapy.

Keywords: non-alcoholic fatty liver disease; miR-27; miR-24; miR-23

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INTRODUCTION

Non-alcoholic fatty liver disease (NAFLD) is diagnosed when steatosis is detected in >5% of hepatocytes under conditions without significant alcohol consumption (<30 g/day for men and <20 g/day for women) [1]. The severity of NAFLD varies from simple steatosis to non-alcoholic steatohepatitis (NASH), fibrosis, cirrhosis, and even hepatocellular carcinoma (HCC). NAFLD is the most common hepatic disorder in developed countries and is the second primary cause of liver transplantation in the USA after chronic hepatitis C [2]. It has a worldwide prevalence of 25.2%, with the highest incidence in the Middle East (32%) and the lowest in Africa (14%) [3, 4]. NAFLD progresses to NASH, which is characterized by steatosis, inflammation, and hepatocyte ballooning. It is one of the main risk factors associated with HCC [5]. NAFLD is an emerging critical public health problem with a significant clinical and economic burden worldwide.

The "multiple-hit" hypothesis has replaced the classic "two-hit" hypothesis to become the most widely accepted theory of NAFLD pathogenesis. It proposes that NAFLD arises from the occurrence of multiple metabolic syndromes, which include obesity, type-2 diabetes, and dyslipidemia in the liver [6]. The first hit involves excessive lipid accumulation in hepatocytes that is induced by insulin resistance, which is usually accompanied by metabolic syndromes. In the second hit, oxidative stress leads to mitochondrial dysfunction and then the release of inflammatory cytokines. Moreover, the "multiple-hit" hypothesis indicates that additional factors including genetic predisposition, intestinal microbiota, and diet also contribute to NAFLD's progression to fibrosis [7–9].

MicroRNAs (miRNAs) are 19–25 nucleotides long non-coding RNAs (lncRNAs) that regulate post-transcriptional gene expression. The first member of the miRNA family lin-4 was discovered in 1993. To date, the miRNA family has expanded to include hundreds of miRNAs [10]. MiRNAs are initially processed from miRNA genes by RNA polymerase II into primary miRNAs (pri-miRNAs) with lengths that span hundreds of nucleotides. The pri-miRNAs are subsequently processed by RNA polymerase III into 70–90 base-long precursor miRNAs (pre-miRNAs) (Fig. 1). The translocation of pre-miRNAs from the nucleus to the cytoplasm is facilitated by Exportin-5. In the cytoplasm, they undergo RNase III Dicer-mediated splicing into mature miRNAs, miRNA-5p, and miRNA-3p. The "passenger strand" is then degraded, whereas the "guide strand" binds to the RNA-induced silencing complex (RISC) before binding to the 3′ untranslated region (3′ UTR) of complementary sequences of target mRNAs, which leads to either mRNA degradation or translational repression [11]. MiRNAs, such as miR-122, miR-34a, miR-103 and miR-107, and miR-29 have been associated with NAFLD pathogenesis [12]. MiR-378 has also been identified as a critical player in hepatic inflammation and fibrosis during NASH progression in our previous study [13].

The miR-23–27–24 clusters in the human genome consist of the miR-23a cluster (miR-23a–27a–24–2) and miR-23b cluster (miR-23b–27b–24–1), on chromosomes 19 and 9, respectively. Both clusters have been implicated in various physiological and pathological processes [14–16]. Importantly, it has been reported that the three miRNAs have roles in NAFLD pathogenesis and promote its progression to fibrosis. The regulatory roles of miR-27 and miR-24 in NAFLD-associated lipid metabolism are summarized in Table 1. Here, we review the current knowledge on miR-27, miR-24, and miR-23, recent advances, and potential clinical significance in NAFLD pathogenesis.

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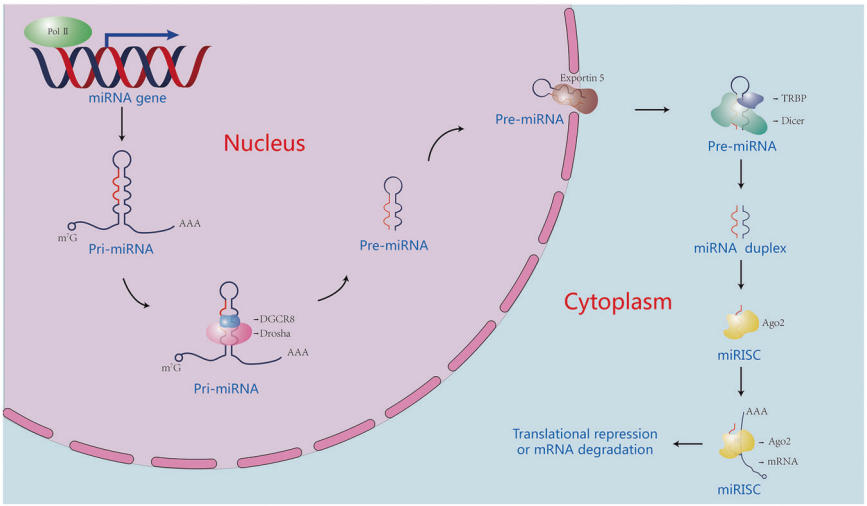

Fig. 1 Canonical miRNA biogenesis pathway. MiRNA genes are transcribed by RNA polymerase II into primary miRNAs (pri-miRNA), which are subsequently processed by Drosha and DiGeorge syndrome critical region gene 8 (DGCR8) to produce precursor miRNAs (pre-miRNAs). Pre-miRNAs are exported into the cytoplasm by Exportin-5 and form a miRNA duplex with the RNase III Dicer and TAR RNA- binding protein (TRBP). One strand of the duplex is degraded and the other strand is bound by the Argonaute proteins (AGO 1–4) to form the RNA-induced silencing complex (RISC). The loaded miRNA then binds with target mRNAs, which leads to translational repression or mRNA degradation.

MIR-27

MiR-27, which is a miRNA precursor that was discovered in animals and humans, contains two isoforms, miR-27a and miR-27b. MiR-27a is an intergenic miRNA at the 13836440–13836517 locus of chromosome 19 p13.12 with the mature sequence 5′-UUCACAGUGGCUAAGUUCCGC-3′, and miR-27b is an intronic miRNA at the 95085445–95085541 locus of chromosome 9 q22.32 with the mature sequence 5′-UUCACAGUGGCUAAGUUCUGC-3′. MiR-27a and miR-27b share high sequence homology, which differs by only one nucleotide [17]. Numerous studies have shown that miR-27 is associated with diverse diseases that include osteoarthritis, atrial fibrosis, multiple sclerosis, and malignant lesions [18–21]. Of note, miR-27 is correlated with NAFLD pathogenesis, which includes adipogenesis, lipid metabolism, oxidative stress, inflammation, mitochondrial dysfunction, and NAFLD progression. Thus, miR-27 is a potential target of NAFLD therapy. The high correlation between serum miR-27 levels in NAFLD patients and disease severity and prognosis suggests its potential as a NAFLD biomarker.

MiR-27 might be a novel therapeutic target for NAFLD

MiR-27 in adipogenesis. White adipose tissue (WAT) regulates systemic energy homeostasis through size remodeling. WAT increases in size and cell numbers through adipocyte hypertrophy and hyperplasia (known as adipogenesis) to store excess energy [22]. However, WAT in obese individuals might not be able to expand through adipocyte hyperplasia, and instead undergoes excessive enlargement of adipocytes and secretes proinflammatory adipokines [23]. Impaired WAT expansion might lead to ectopic fat accumulation and might be associated with insulin resistance, which is the central cause of metabolic syndromes [24, 25]. In combination, the previous studies suggest an association between the inhibition of adipogenesis and NAFLD pathogenesis.

Peroxisome proliferator-activated receptor (PPAR)γ is a key transcriptional regulator for adipogenesis. Recent studies demonstrated that miR-27 could inhibit adipogenesis by targeting PPARγ. High miR-27a/b levels in 3T3-L1 preadipocytes inhibited PPARγ and CCAAT/enhancer-binding protein α (C-EBP/α), thus suppressing adipocyte differentiation [26]. Both miR-27a and miR-27b showed antiadipogenic effects by binding to the 3′ UTR and inhibiting PPARγ gene expression [27, 28]. The lncRNA Gm15290, which was first discovered in hereditary catalepsy mouse's brain, interacted with miR-27b and upregulated PPARγ, therefore promoting adipogenesis [29, 30]. A feed-forward loop composed of miR-27a/b, PPARγ (pro-adipogenic transcription factor), and secretory carrier membrane protein 3 (SCAMP3; antiadipogenic) was proposed to regulate adipogenesis [31]. Within the feed-forward loop, miR-27a/b either directly downregulates SCAMP3 or indirectly through inhibiting PPARγ [31]. Moreover, miR-27a/b inhibition or depletion increased PPARγ expression and induced adipogenesis in sheep and zebrafish [32, 33].

Novel targets of miR-27 in adipogenesis have been identified. For example, the miR-27a/b-mediated downregulation of the cAMP-response element-binding protein (CREB) via direct binding of its 3′ UTR inhibited adipocyte differentiation in 3T3-L1 preadipocytes [34]. MiR-27a/b disrupted adipocyte commitment by targeting lysyl oxidase (Lox), which is a critical component in bone morphogenetic protein (BMP)-induced adipocyte commitment [35–38]. MiR-27a/b can suppress adipocyte differentiation by directly targeting prohibitin (PHB) in human adipose-derived stem cells (ASC), hence impairing mitochondrial function [39]. However, Murata et al. evidenced increased lipid deposition and higher triglyceride (TG) content in miR-27b-3p-transfected 3T3-L1 cells. From their results, miR-27b promoted adipocyte differentiation by upregulating acyl-CoA thioesterase 2 level in 3T3-L1 preadipocytes [40]. Increased miR-27b levels in adipose tissue could be due to the accumulation of miR-27b in extracellular vehicles released by donor cells. In combination, the dual roles of miR-27 in adipogenesis are summarized in Fig. 2. Further understanding of the role of miR-27 in adipogenesis is required.

MiR-27 in metabolism. The liver plays a vital role in lipid metabolism, which includes the uptake of circulating fatty acids, de novo lipogenesis, fatty acid oxidation, and the export of TGs [41]. The disruption of lipid homeostasis could lead to hepatic lipotoxicity and steatosis. The importance of miR-27 in hepatic lipid metabolism has recently been clarified. MiR-27a repressed hepatic de novo lipogenesis by inhibiting the expression of lipogenesis-associated enzymes fatty acid synthase (FAS) and stearoyl-CoA desaturase 1 (SCD1), hence attenuating NAFLD development [42]. The overexpression of miR-27a in HepG2 cells inhibited nuclear factor-erythroid 2-related factor 2 (Nrf2) and significantly increased TG levels, which promoted lipid accumulation [43]. Hepatitis C virus (HCV) activated miR-27a/b in Huh7.5

Table 1. Multifaceted roles of miR-27, miR-24, and miR-23 in NAFLD progression-associated processes.

miRNA	Function	Subject	Source	Expression pattern	Effect	Target genes	Reference
miR-27	Adipogenesis	Sheep	AT	Downregulated in differentiated ovine adipocytes	miR-27 overexpression suppressed ovine preadipocytes differentiation	PPARγ, RXRα	[32]
		Zebrafish	AT	–	miR-27 depletion promoted adipocyte hyperplasia	–	[33]
		Cell line		Downregulated during adipocyte differentiation	miR-27 overexpression suppressed adipocyte differentiation	PPARγ, C-EBP/α	[26, 27]
						CREB	[34]
						PHB	[39]
		Cell line		–	miR-27 overexpression promoted adipocyte differentiation	ACOT2	[40]
		Human/mouse	AT	Downregulated during adipogenesis	miR-27 overexpression suppressed adipocyte differentiation	PPARγ, C-EBP/α, SCAMP3	[28, 30, 31]
		Human/mouse	AT	Downregulated in obesity	miR-27 overexpression impaired lineage commitment	Lox	[38]
	Hepatic lipid metabolism	Mouse	Liver	Upregulated in high-fat diet-fed mice	miR-27 overexpression suppressed hepatic de novo lipogenesis	FAS, SCD1	[42]
		Mouse, Cell line	Liver	Upregulated in high-fat diet-fed mice/HCV-infected Huh7.5 cells	miR-27 overexpression increased lipid accumulation	Nrf2?	[43]
						PPARα, ANGPTL3	[44]
		Cell line		Upregulated in HCV-infected livers in a previous study	miR-27 overexpression repressed lipid accumulation	ABCA1	[45]
	Oxidative stress	Cell line		Downregulated in response to oxidative stress	miR-27 overexpression suppressed oxidative stress	NF-κB	[64]
		Cadaver	Eye	–	miR-27 inhibition promoted oxidative damage	–	[65]
		Mouse, Cell line	Kidney	Upregulated in renal ischemia/reperfusion injury	miR-27 overexpression promoted oxidative damage	Grb2	[66]
		Mouse, Cell line	Liver	Upregulated in high-fat diet-fed mice and HG-treated HepG2 cells	miR-27 overexpression increased reactive oxygen species production	Nrf2?	[43]
		Rat	Hippocampus	Upregulated in diabetic rats	miR-27 inhibition enhanced antioxidant genes expression	–	[67]
	Inflammation	Cell line		Downregulated in response to oxidative stress	miR-27 overexpression suppressed NF-κB activation	NF-κB	[64]
		Mouse	Liver	Downregulated in septic mice	miR-27 inhibition promoted inflammatory response	TAB3	[69]
		Mouse, Cell line	AT	Downregulated in high-fat diet-fed mice/upregulated in inflammatory conditions	miR-27 overexpression promoted inflammatory response	PPARγ	[70, 71]
		Mouse	BMDMs	Downregulated in LPS-treated BMDMs	miR-27 overexpression promoted inflammatory response	IL-10	[72]
		Cell line		Downregulated in LPS-treated RAW264.7 cells	miR-27 overexpression promoted inflammatory response	MCPIP1	[73]
	Mitochondrial dysfunction	Mouse, Cell line	Renal cortex	Upregulated in db/db mice and HG-treated HK-2 cells	miR-27 inhibition relieved mitochondrial dysfunction	PHB, TMBIM6	[76]
		Human	ASCs	Downregulated during adipocyte differentiation	miR-27 overexpression promoted mitochondrial dysfunction	PHB	[39]
		Mouse	Bone marrow	Downregulated in case of diabetes, high glucose, and oxidative stress	miR-27 overexpression suppressed mitochondrial apoptotic pathway	Bax, RUNX1	[77]
		Cell line		Upregulated in mitophagy	miR-27 overexpression mitophagy	PINK1	[78]
	Fibrogenesis	Rat	HSCs	Upregulated in activated HSCs	miR-27 inhibition decreased HSCs proliferation	RXRα	[82]
		Cell line		–	miR-27 overexpression upregulated HSCs proliferation-related proteins	–	[83]
		Mouse	HSCs	–	miR-27 overexpression promoted fibrogenesis	–	[84]
		Mouse, Cell line	Liver	Downregulated in *Schistosoma japonicum* infection	miR-27 inhibition alleviated schistosomiasis-induced hepatic fibrosis	KSRP	[87]
		Human, rat, Cell line	Serum, liver	Serum miR-27 level was upregulated in HBC patients and in rats with liver cirrhosis	miR-27 inhibition attenuated HSCs proliferation	PPARγ	[88]

Table 1. continued

miRNA	Function	Subject	Source	Expression pattern	Effect	Target genes	Reference
miR-24	Lipogenesis	Mouse, Cell line	Liver	Upregulated in high-fat diet-fed mice/fatty acid-treated HepG2 cells	miR-24 suppression decreased lipogenesis	Insig1	[105]
	Adipogenesis	Cell line		Downregulated during adipocyte differentiation	miR-24 overexpression inhibited adipocyte differentiation	–	[108]
		Cell line		Upregulated during preadipocytes differentiation	miR-24 overexpression inhibited adipocyte differentiation	MAPK7	[109]
	Hepatic lipid metabolism	Cell line		–	miR-24 upregulation inhibited de novo lipogenesis	SREBP1c	[110]
		Mouse, Cell line	Liver	Upregulated in high-fat diet-fed mice/fatty acid-treated HepG2 cells	miR-24 overexpression repressed lipid accumulation	SR-B1	[111]
	Fibrogenesis	Human, mouse, Cell line	Liver	Upregulated in early stage and downregulated in advanced stage of cholestatic liver disease	miR-24 inhibition promoted fibrosis genes expression	menin?	[112]
miR-23	Adipogenesis	Bovine	Progenitor cells	Downregulated during adipogenic differentiation	miR-23 overexpression inhibited adipogenic differentiation	ZNF423	[113]
		Cell line		Downregulated during adipocyte differentiation	miR-23 overexpression inhibited adipocyte differentiation	Stat1	[114]
	Hepatic lipid metabolism	Cell line		–	miR-23 overexpression promoted lipid accumulation	SIRT1	[115]
	Mitochondrial dysfunction	Cell line		–	miR-23 inhibition enhanced an mitochondrial protective gene expression	–	[120]
	Endoplasmic reticulum stress	Mouse, Cell line	Liver	–	miR-23 inhibition reduced endoplasmic reticulum stress	–	[121]
	Fibrogenesis	Rat	HSCs	Upregulated in rats with hepatic fibrosis	miR-23 inhibition suppressed hepatic fibrosis progression	PTEN	[116]
		Mouse	HSCs	–	miR-23 inhibition alleviated hepatic fibrosis	–	[117]
		Rat	HSCs	Upregulated in NDMA-induced hepatic fibrosis	miR-23 inhibition alleviated hepatic fibrosis	–	[118]
		Cell line		–	miR-23/27 overexpression alleviated hepatic fibrosis	gremlin1	[119]

ABCA1 ATP-binding cassette subfamily A member 1, *ACOT2* Acyl-coenzyme A thioesterase 2, *ANGPTL3* angiopoietin-like protein 3, *ASC* adipose-derived stem cell, *AT* adipose tissue, *Bax* BCL-2 associated X, *BMDM* bone marrow-derived macrophage, *C-EBP/α* CCAAT/enhancer-binding protein alpha, *CREB* cAMP-response element-binding protein, *FAS* fatty acid synthase, *Grb2* growth factor receptor-bound protein-2, *HCV* hepatitis C virus, *HG* high glucose, *HSC* hepatic stellate cell, *IL* interleukin, *Insig1* insulin-induced gene 1, *KSRP* KH-type splicing regulatory protein, *Lox* lysyl oxidase, *LPS* lipopolysaccharides, *MAPK7* mitogen-activated protein kinase 7, *MCPIP1* monocyte chemoattractant protein-induced protein-1, *NDMA N*-nitrosodimethylamine, *NF-κB* nuclear factor kappa-B, *Nrf2* nuclear factor-erythroid 2-related factor 2, *PHB* prohibitin, *PINK1* PTEN-induced kinase 1, *PPAR* peroxisome proliferator-activated receptor, *PTEN* phosphatase and tensin homolog deleted on chromosome 10, *RUNX1* runt-related transcription factor 1, *RXRα* retinoid X receptor α, *SCAMP3* secretory carrier membrane protein 3, *SCD1* stearoyl-CoA desaturase 1, *SIRT1* sirtuin1, *SR-B1* scavenger receptor type B1, *SREBP1c* sterol regulatory element-binding protein 1c, *Stat1* signal transducer and activator of transcription 1, *TAB3* TGF-beta-activated kinase 1 binding protein 3, *TMBIM6* transmembrane BAX inhibitor-1 motif-containing 6, *ZNF423* zinc finger protein 423.

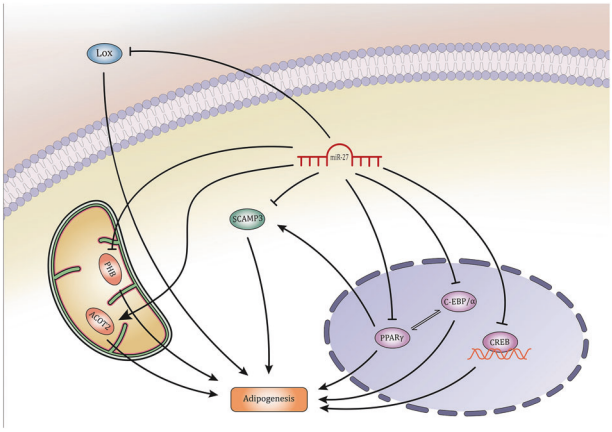

Fig. 2 The dual roles of miR-27 in adipogenesis. MiR-27 exerts an antiadipogenetic effect by targeting various genes including PPARγ, C-EBP/α, CREB, Lox, SCAMP3, and PHB. Besides, miR-27 induces ACOT2 expression and promotes adipogenesis. Normal arrows denote activation and blunt arrows denote inhibition. ACOT2 acyl-CoA thioesterase 2, C-EBP/α CCAAT/enhancer-binding protein alpha, CREB cAMP-response element-binding protein, Lox lysyl oxidase, PHB prohibitin, PPARγ peroxisome proliferator-activated receptor gamma, SCAMP3 secretory carrier membrane protein 3.

cells and repressed PPARα and angiopoietin-like protein 3 (ANGPTL3) expression, which induced TG accumulation [44]. It was reported that the upregulation of miR-27a upon HCV infection affected the levels of lipid metabolism genes that included PPARα [45]. Thus, this study proposed that miR-27a reduced TG levels in Huh7.5 cells. This could be due to oleic acid treatment of the cells, which induced a shift in the metabolic state [45].

In addition to NAFLD, miR-27 has been implicated in other metabolic abnormalities, such as childhood obesity, diabetes, insulin resistance, atherosclerosis, and impaired browning ability of visceral adipose tissue in obese patients, which involves the conversion of bad fat cells (white adipocytes) into good fat cells (brown-like adipocytes) [46–54]. Notably, miR-27a/b inhibited hepatic gluconeogenesis, which is the major contributor to hyperglycemia in diabetes [55]. In addition, miR-27a/b recently emerged as a primary regulator of cholesterol homeostasis and dysregulation could lead to hypercholesterolemia and atherosclerosis [56, 57]. Both miR-27a and miR-27b have been found to promote cholesterol homeostatic dysregulation. The former directly decreases the level of hepatic low-density lipoprotein receptor and affects circulating cholesterol clearance and the latter targets oxysterol binding protein-like 6 (OSBPL6) and inhibits cholesterol trafficking and efflux [58, 59]. However, miR-27a could lower serum cholesterol concentrations in high cholesterol diet-fed ApoE$^{-/-}$ mice by targeting the 3-hydroxy-3-methylglutaryl-coenzyme A reductase (HMGCR) gene, which encodes the rate-limiting enzyme in cholesterol synthesis [60]. The previously mentioned studies suggest that miR-27 might have different roles in the pathogenesis of hypercholesterolemia via different pathways.

MiR-27 has been implicated in multiple metabolic processes. Besides, it shows potential as a diagnostic marker and is a promising therapeutic target for metabolic abnormalities.

MiR-27 in oxidative stress, inflammation, and mitochondrial dysfunction. Lipotoxicity refers to the toxic effects that arise from lipid accumulation in cells. Hepatic lipotoxicity occurs due to the influx of free fatty acids that overwhelm the liver capacity, which leads to oxidative stress, inflammation, mitochondrial dysfunction, thus resulting in NASH pathogenesis and progression. The roles of miR-27 in oxidative stress, inflammation, and mitochondrial dysfunction will be reviewed in this section.

Oxidative stress, which arises due to an imbalance between the levels of reactive oxygen species (ROS) and antioxidants, is associated with NAFLD and NASH pathogenesis. Increased activity of mitochondrial cytochrome P450 2E1 (CYP2E1), which is a major source of ROS, was observed in NASH patients [61]. Angiotensin II (Ang II), which is a systemic vasoconstrictor, promotes NAFLD progression in transgenic Ren2 rat models by increasing hepatic ROS [62]. In addition, advanced glycation end products induce ROS production and enhance the proliferation and activation of hepatic stellate cells (HSCs), and contribute to NASH's progression to fibrosis [63].

The role of miR-27 in oxidative stress could be evidenced by its downregulation in response to hydrogen peroxide-induced oxidative stress in the RAW264.7 murine macrophage cell line [64]. MiR-27 either promotes or suppresses oxidative stress via diverse mechanisms in different diseases. For example, miR-27a-mediated activation of the PI3K/AKT and Wnt/β-catenin pathways protected human trabecular meshwork cells from oxidative damage [65]. However, miR-27a aggravated renal ischemia-reperfusion injury by inhibiting Grb2 and promoting oxidative stress [66]. Downregulating miR-27a increased Nrf2 levels and upregulated the expression of antioxidant genes. This improves memory dysfunction in diabetic rats [67]. In NAFLD, miR-27a inhibited the expression of Nrf2, which is a key modulator of the cellular antioxidant system [43]. Further research into the role of miR-27 in NAFLD-associated oxidative stress is thus required.

Inflammation protects an organism from a variety of harmful stimuli, such as infection, trauma, and autoimmune injury. However, when harmful stimuli cannot be eliminated and inflammation persists, chronic liver injury occurs, which drives the progression of NAFLD to NASH and more advanced disease stages [68]. MiR-27 influences inflammation via multiple pathways. Overexpressing miR-27b in the RAW264.7 murine macrophage cell line suppressed lipopolysaccharide (LPS)-induced activation of nuclear factor-κB (NF-κB), the master regulator of inflammation [64]. Paclitaxel alleviated the inflammatory response and attenuated sepsis-induced liver injury via the miR-27a/TAB3/NF-κB signaling pathway [69]. MiR-27a activated M1 macrophage polarization by blocking PPARγ, thus promoting inflammation [70]. LPS-induced miR-27b upregulation in human monocytes directly inhibited PPARγ and promoted the release of proinflammatory cytokines tumor necrosis factor (TNF)-α and interleukin (IL)-6 [71]. IL-10, monocyte chemoattractant protein-1-induced protein-1 (MCPIP1), and ubiquitin-specific peptidase 4 (USP4) have been implicated in the association between miR-27 and inflammation [72, 73]. However, how miR-27 regulates the inflammatory response in NAFLD needs to be elucidated. Mitochondria are semiautonomous double membranous cytoplasmic organelles that are present in eukaryotes. They play an essential role in energy metabolism, calcium ion (Ca^{2+}) homeostasis, and cell proliferation and apoptosis [74]. Mitochondrial dysfunction is characterized by altered activities in respiratory chain enzymes, decreased mitochondrial membrane potential, intracellular Ca^{2+} overload, and impaired energy production. Of note, mitochondrial dysfunction was observed in NAFLD pathogenesis and progression. Mechanisms that underlie mitochondrial changes involve the overproduction of mitochondrial ROS, increased lipid peroxidation, impaired mitochondrial mitophagy, and the activation of mitochondrial apoptotic pathways [75].

MiR-27 either promotes or inhibits mitochondrial dysfunction. Inhibition of miR-27a promoted the activities of adenosine triphosphate (ATP), complexes I and III of the mitochondrial respiratory chain in the kidney of db/db mice [76]. High miR-27a/b levels inhibited prohibitin in ASC, which affected mitochondrial membrane potential and reduced complex I activity [39]. However, miR-27b could enhance the survival of bone marrow progenitor cells by the inhibition of the mitochondrial apoptotic pathway in type-2 diabetic mice [77]. In addition, miR-27a/b inhibited the expression of PTEN-induced putative kinase 1 (PINK1) protein, which mediated mitophagy

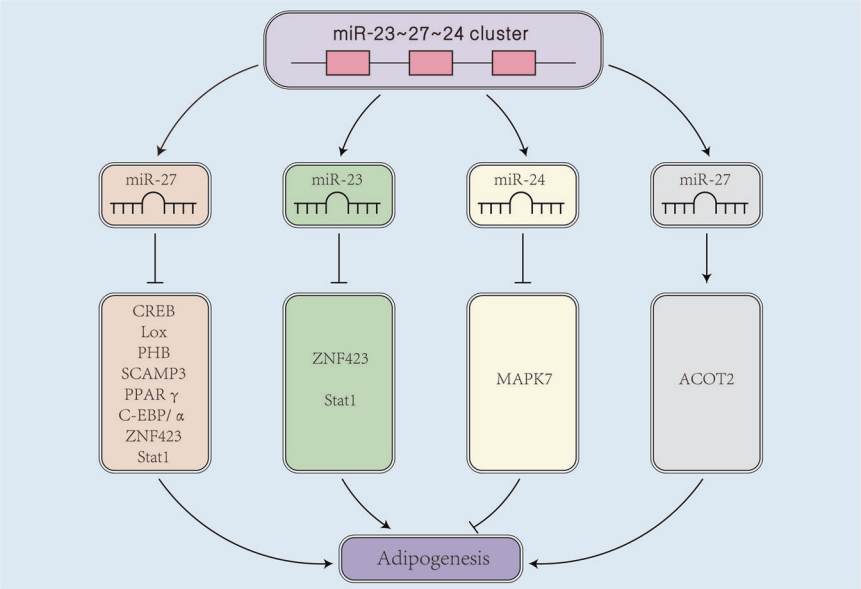

Fig. 3 The opposing roles of miR-23, miR-27, and miR-24 in adipogenesis. MiR-27 and miR-23 can both act as inhibitors in adipogenesis through various mechanisms. Besides, miR-27 and miR-24 can also promote adipogenesis by targeting ACOT2 and MAPK7, respectively. Normal arrows denote activation and blunt arrows denote inhibition. ACOT2 Acyl-coenzyme A thioesterase 2, C-EBP/α CCAAT/enhancer-binding protein alpha, CREB cAMP-response element-binding protein, Lox lysyl oxidase, MAPK7 mitogen-activated protein kinase 7, PHB prohibitin, PPARγ peroxisome proliferator-activated receptor gamma, SCAMP3 secretory carrier membrane protein 3, ZNF423 zinc finger protein 423, Stat1 signal transducer and activator of transcription 1.

[78]. Taken together, the association between miR-27 and mitochondrial dysfunction in NAFLD remains unclear and further studies in this area are required.

MiR-27 in fibrogenesis. During wound healing that follows chronic liver injury, excess extracellular matrix (ECM) proteins could be produced, which could lead to liver fibrosis [79]. Chronic hepatitis B and C infections, alcohol abuse, NASH, autoimmune disturbance, cholestasis, and hereditary and metabolic defects are potentially the main causes of liver fibrosis. HSC activation is central to the initiation and progression of liver fibrosis. Fibrogenic cytokines, such as transforming growth factor (TGF)-β, IL-1, and TNF-α, mediate HSC activation. Activated HSCs proliferate and migrate to the injury site and secrete a large amount of collagen and other ECM components, which replace functional hepatocytes and promote fibrous scar formation, ultimately culminating in liver failure [80]. Bone marrow-derived mesenchymal cells, portal fibroblasts, and fibrocytes differentiate into myofibroblasts that produce ECM components [81].

Researchers have attempted to determine the role of miR-27 in activating fibrogenesis. Ji et al. found that downregulating overexpressed miR-27a/b inhibited HSC proliferation and reversed the decrease in cytoplasmic lipid droplets, which is a characteristic of activated HSCs [82]. Furthermore, they demonstrated that miR-27a overexpression upregulated several proteins involved in HSCs proliferation, such as transcription elongation factor A protein-like 4 (TCEAL4) and eukaryotic translation initiation factor 3 subunit J (EIF3J) [83]. The adipocyte-derived hormone leptin-mediated upregulation of miR-27a/b-3p in HSCs has been shown to increase levels of α-smooth muscle actin (α-SMA) and α1(I) collagen and thus promote fibrogenesis [84]. TGF-β promotes fibrogenesis via multiple pathways [85]. Inhibition of the TGF-β/SMAD (drosophila mothers against decapentaplegic protein) pathway significantly downregulated miR-27a in HSCs [86]. MiR-27b inhibition destabilized TGF-β1 mRNA and alleviated schistosomiasis-induced hepatic fibrosis in HSCs [87]. Furthermore, miR-27a, combined with its target genes PPARγ, forkhead box-O (FOXO)1, adenomatous polyposis coli (APC), p53, and retinoid X receptor-alpha (RXRα), contributed to HSC activation [88]. These reports demonstrated miR-27's role in promoting fibrogenesis (Fig. 3). However, further research into the molecular mechanisms that underlie fibrosis and the role of miR-27 in pathogenesis is required.

MiR-27 as a potential biomarker for NAFLD

Further improvements in the existing methods to diagnose NAFLD are necessary. The gold standard diagnostic method, liver biopsy, is invasive and might cause severe complications [89]. Routine imaging techniques, such as abdominal ultrasonography computed tomography and magnetic resonance imaging, cannot discriminate NASH from simple steatosis [90]. In addition, laboratory testing of serum aminotransferases and other common blood biomarkers cannot diagnose NAFLD due to their low sensitivity and specificity [89, 91]. Thus, specific and sensitive diagnostic methods to identify novel non-invasive biomarkers for NAFLD are urgently required.

Recently, miRNAs have increased in prominence as promising biomarker candidates, which is mainly due to their non-invasiveness, tissue and disease specificity, and high stability in almost all body fluids. They are released into the circulation by the active secretion of exosomes from cells or through passive leakage during cell apoptosis and necrosis [92]. The released miRNAs are contained within extracellular vesicles or are attached to related proteins, such as Ago-2, nucleophosmin, or high-density lipoprotein (HDL), which protects miRs from degradation by ribonucleases [93]. In addition, miRNAs have been proposed as potential biomarkers for NAFLD [94–96]. For example, patients with severe NAFLD have significantly downregulated miR-27a levels [97]. In addition, a biomarker panel composed of miR-122, miR-1290, miR-27b, and miR-192 showed high clinical relevance in NAFLD diagnosis, which was independent of the NAFLD activity score status [98]. However, further large-scale studies should be performed to validate miR-27's diagnostic value in NAFLD.

MIR-24 AND MIR-23

MiR-24 was initially discovered in invertebrates and vertebrates in 2001 [99]. It occupies two gene loci in the human genome with the same sequence 5′-UGGCUCAGUUCAGCAGGAACG-3′, miR-24-1 at 9q22 and miR-24-2 at 19p13. MiR-23 has two different isoforms, miR-23a (5′-AUCACAUUGCCAGGGAUUUCC-3′) and miR-23b (5′-AUCACAUUGCCAGGGAUUACCAC-3′). MiR-24 and miR-23 are expressed in multiple tissues and are associated with a variety of malignancies [100–104]. The two miRNAs are implicated in NAFLD pathogenesis and development and thus may become promising therapeutic targets for NAFLD.

MiR-24 and miR-23 in NAFLD and its pathogenesis

Only a few studies have been carried out on miR-24's role in NAFLD. MiR-24 was significantly upregulated in the livers of mice on a high-fat diet and in oleic acid-induced human hepatocytes and HepG2 cells [105]. The insulin-induced gene (Insig) 1 that inhibits lipogenesis, was identified as a novel target of miR-24. MiR-24 knockdown in oleate-treated human hepatocytes and HepG2 cells led to significantly increased Insig1 level and inhibited hepatic lipid accumulation. Insig1 inhibits lipid synthesis by retaining the sterol regulatory element-binding protein (SREBP) in the endoplasmic reticulum and by mediating the degradation of HMGCR [106, 107]. Suppressing miR-24 induced significant Insig1 upregulation and downregulation of SREBP1c and SREBP2, therefore prevented lipid accumulation in primary human hepatocytes and HepG2 cells. This suggests that miR-24 is a potential target for NAFLD treatment [105].

Hitherto, there have been studies indicating the involvement of miR-24 in NAFLD pathogenesis. While miR-24 has been reported to inhibit 3T3-L1 adipocyte differentiation and maturity, it also serves a promoting role by targeting mitogen-activated protein kinase (MAPK) 7 [108, 109]. MiR-24 upregulation mediated by zinc fingers and homeoboxes 2 inhibited SREBP1c expression and thus suppressed de novo lipogenesis in HCC cells [110]. MiR-24 inhibited scavenger receptor class B type 1 and regulated the expression of lipogenic genes FASN, ACLY, and SCD1 in HepG2 cells [111]. Moreover, inhibiting miR-24 upregulated the expression of fibrosis genes in a primary sclerosing cholangitis mouse model [112]. Previous studies suggest that miR-24 might play multiple roles in NAFLD via diverse mechanisms.

Likewise, miR-23 plays multifaceted roles in NAFLD pathogenesis and progression. MiR-23 not only acts as an antiadipogenic regulator but can also cause lipid accumulation in HepG2 cells [113–115]. MiR-23a has been shown to promote hepatic fibrosis through various mechanisms, whereas miR-23b was only once reported to be involved in suppressing HSC activation [116–119]. Therefore, more evidence is required to explain whether the two isoforms exert opposite effects on hepatic fibrosis. Moreover, miR-23a downregulation caused by glucagon-like peptide-1 was reported to inhibit HepG2 cell apoptosis by enhancing a mitochondrial protective gene (peroxisome proliferator-activated receptor gamma coactivator 1a) expression [120]. Melatonin-induced miR-23a attenuation could reduce endoplasmic reticulum stress and thus alleviate hepatic steatosis [121]. The two studies indicate the protective effect of miR-23 against NAFLD development. Nonetheless, additional studies are required to confirm its precise role in the pathological process.

MiR-24 and miR-23 in other metabolic disorders

In addition to NAFLD, both miR-24 and miR-23 are involved in other metabolic diseases. Normal HDL (nHDL) in healthy subjects is proangiogenic and helps to improve total blood flow in the myocardium, and dysfunctional HDL (dHDL) from patients with coronary artery disease (CAD), which arises from oxidative stress, is proinflammatory [122–125]. nHDL-mediated miR-24 inhibition stimulated vinculin and endothelial nitric oxide synthase (eNOS) expression, which increased nitric oxide production and induced angiogenesis [126]. In contrast, dHDL suppressed angiogenesis and enhanced superoxide anion generation via miR-24-mediated inhibition of vinculin and eNOS [126]. This study suggested miR-24 was a potential therapeutic target for dHDL-impaired angiogenesis in patients with CAD. In addition, miR-24 either aggravates atherosclerosis by inhibiting selective lipid uptake from HDL cholesterol or alleviates the disease by inhibiting the proliferation and migration of vascular endothelial cells [127, 128]. MiR-24's role in diabetic vascular complications involves its suppression of vascular smooth muscle cell proliferation and migration in diabetes [129, 130]. Further studies should be conducted to determine the complex roles of miR-24 in metabolic disorders.

The existing data mainly suggest a correlation of miR-23 with atherosclerosis, insulin resistance, and diabetes. MiR-23 seems to be a culprit contributing to atherosclerosis by promoting inflammation or foam cell formation [131–133]. However, it plays an active part in preventing the development of diabetes. Lozano-Bartolomé et al. reported the role of miR-23a in protecting against insulin resistance in adipocytes [134]. Chang et al. found that miR-23a inhibited pyroptosis and thus relieved liver and kidney injuries in rats with type II diabetes [135]. Moreover, miR-23b could suppress oxidative stress in mice with diabetic nephropathy through targeting MAPK [136].

INTERACTIVE FUNCTIONS OF MIR-23, MIR-27, AND MIR-24

The individual roles of miR-23, miR-27, and miR-24 in NAFLD pathogenesis have been illustrated above. Besides, the high evolutionary conservation of miR-23–27–24 cluster among vertebrates indicates that the three miRNAs might function together in various disease states [137]. The synergistic effects of the miR-23a–27a–24–2 cluster in promoting tumorigenesis have previously been summarized in a review [138]. MiR-23a, miR-27a, and miR-24-2 have been found to target SMAD5 and thus prevent mouse embryonic stem cells apoptosis induced by BMP4 [139]. Recently, Su et al. observed upregulated expression of the three miR-23a cluster members in intestinal tissues of colitis mice and patients [140]. The authors concluded that Nrf2-promoted miR-23a–27a–24–2 facilitated intestinal damage repair in inflammatory bowel diseases by targeting Bach1 signaling. In addition, both miR-23a/b and miR-27a/b played a proangiogenic role in choroidal neovascularization [141]. These studies support the collaborative relationship among miR-23–27–24 cluster members.

However, antagonistic effects in regulating a specific biological process have also been observed among different miRNAs in the same cluster. One likely explanation is that various miRNA members play opposing roles to fine-tune the regulatory function of the whole cluster. The interactive roles of miR-23a, miR-27a, and miR-24-2 in bovine adipocyte adipogenesis have been reported [142]. MiR-23a and miR-24-2 could enhance adipogenesis by targeting antiadipogenic genes, while miR-23a, miR-27a, and miR-24-2 could target pro-adipogenic genes to suppress adipogenesis. The miR-23a–27a–24–2 cluster-mediated balanced regulation ultimately resulted in adipogenesis inhibition [142]. In this review, the interactive functions of miR-23, miR-27, and miR-24 in NAFLD development and progression are summarized and shown in Figs. 3–5.

CHALLENGES OF MIRNA-BASED THERAPY IN NAFLD

MiRNA-based therapeutic strategies can be divided into two types: miRNA inhibition (the use of anti-miRs or miRNA inhibitors to suppress "detrimental" miRNAs expression) and miRNA replacement (the use of miRNA mimics to promote "beneficial" miRNAs expression) [143]. Given the widespread regulatory roles of miRNAs in NAFLD, miRNA-based therapy has aroused great scientific and clinical attention. RG-125/AZD4076, an *N*-acetylgalactosamine (GalNAc)-conjugated anti-miR-103/107, has been

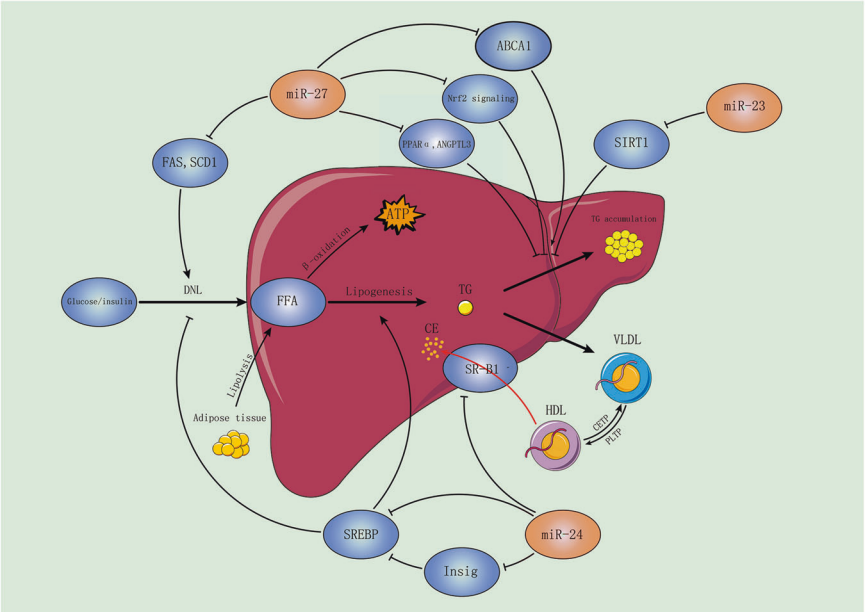

Fig. 4 Roles of miR-23, miR-27, and miR-24 in hepatic lipid metabolism. MiR-23 plays a promoting role in hepatic lipid accumulation by targeting SIRT1. MiR-24 promotes hepatic lipid accumulation by enhancing DNL and lipogenesis, which can be both mediated by SREBP signaling. Besides, miR-24 can suppress HDL uptake by targeting SR-B1. MiR-27 acts as a negative regulator in hepatic DNL by inhibiting FAS and SCD1. In addition, miR-27 can either facilitate hepatic lipid accumulation by targeting Nrf2 signaling or repressing PPARα and ANGPLT3 or inhibit the process by inhibiting ABCA1 expression. Normal arrows denote activation and blunt arrows denote inhibition. ABCA1 ATP-binding cassette subfamily A member 1, ANGPTL3 angiopoietin-like protein 3, ATP adenosine triphosphate, CE cholesteryl ester, CETP cholesteryl ester transfer protein, DNL de novo lipogenesis, FAS fatty acid synthase, FFA free fatty acid, HDL high-density lipoprotein, Insig1 insulin-induced gene 1, Nrf2 nuclear factor-erythroid 2-related factor 2, PLTP phospholipid transfer protein, PPARα peroxisome proliferator-activated receptor-alpha, SCD1 stearoyl-CoA desaturase 1, SIRT1 sirtuin1, SR-B1 scavenger receptor type B1, SREBP sterol regulatory element-binding protein, TG triglyceride, VLDL very low-density lipoprotein.

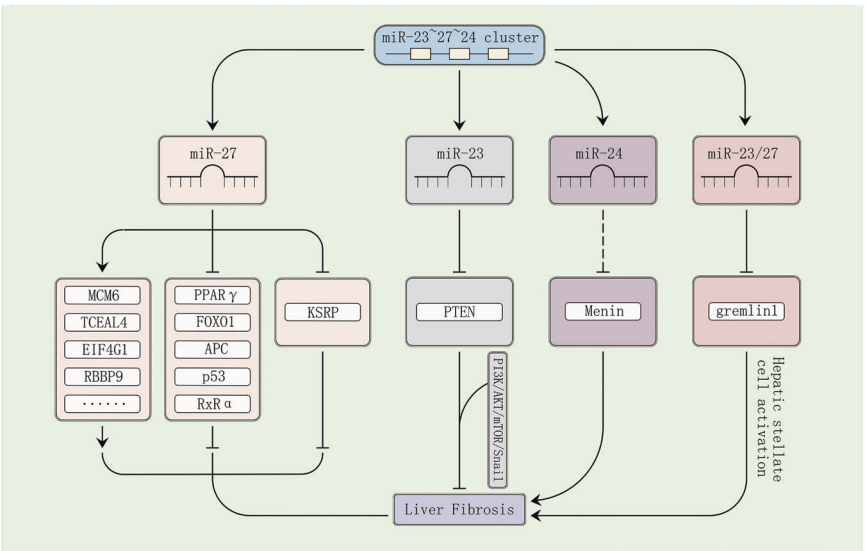

Fig. 5 The opposing roles of miR-23, miR-27, and miR-24 in liver fibrosis. MiR-27 and miR-23 separately promote liver fibrotic progression, while they act in concert as a suppressor in this process. Furthermore, miR-24 plays an inhibitory role in liver fibrosis. Normal arrows denote activation and blunt arrows denote inhibition. Solid lines represent identified mechanisms and dotted lines represent uncertain mechanisms. APC adenomatous polyposis coli, EIF4G1 eukaryotic translation initiation factor 4 gamma 1, FOXO1 forkhead box protein O1, KSRP KH-type splicing regulatory protein, MCM6 minichromosome maintenance complex component 6, p53 protein 53, PI3K/Akt/mTOR/Snail phosphatidylinositol 3-kinase/protein kinase B/mammalian target of rapamycin/Snail, PPARγ peroxisome proliferator-activated receptor gamma, PTEN phosphatase and tensin homolog deleted on chromosome 10, RBBP9 retinoblastoma-binding protein 9, RXRα retinoid X receptor α, TCEAL4 transcription elongation factor A (SII)-like 4.

tested in clinical trials for type-2 diabetes and NAFLD treatment (NCT02826525, NCT02612662). Numerous preclinical studies demonstrated that some compounds and molecules could block metabolic diseases progression by targeting miR-23, miR-27, or miR-24, which indicates great therapeutic potential of miRNA-based treatment (Table 2). However, the application of this therapy has been limited due to miRNA instability and potential off-target effects.

Table 2. Compounds and molecules targeting miR-23, miR-27, and miR-24 in metabolic diseases.

Agent	Metabolic disease studied	Model	MiRNA-targeted and -related roles	Effect	Reference
Trimetazidine	Diabetes	Alloxan-treated rats	miR-24: not mentioned	Alleviated dyslipidemia, inflammation, and hypotension	[148, 149]
GLP-1	Diabetes	HG-treated HepG2 cells	miR-23a: not mentioned	Protected against HG-induced cytotoxicity	[120]
Resveratrol	GDM	HFD-fed pregnant mice	miR-23a-3p: miR-23a-3p upregulation improved glucose uptake and lipid metabolism by targeting NOV	Improved glucose uptake and lipid metabolism	
Circ-0080425	DN	STZ-treated mice; HG-treated mouse MCs	miR-24-3p: miR-24-3p regulated cell proliferation and fibrosis by targeting FGF11	Inhibited cell proliferation and fibrosis	[150]
Lnc 4930556M19Rik	DN	HG-treated mouse podocytes	miR-27a-3p: miR-27a-3p inhibition alleviated podocyte injury by targeting TIMP3	Alleviated podocyte apoptosis, fibrosis, and inflammation	[151]
LINC01619	DN	HG-treated murine podocyte; STZ-treated rats	miR-27a: miR-27a triggered ER stress and podocyte injury by targeting FOXO1	Inhibited ER stress and podocyte injury	[152]
Omentin	DN	db/db mice	miR-27a: miR-27a inhibition suppressed oxidative stress and inflammation by targeting Nrf2	Relieved inflammatory response and oxidative stress; protected renal function	[153]
Arbutin	DN	HG-treated HK-2 cells	miR-27a: miR-27a inhibition induced cell apoptosis by blocking JNK and mTOR signaling pathway	Alleviated apoptosis and autophagy	[154]
Ginsenoside	DFU	HFD- and STZ-induced diabetic rats; HG-treated HUVECs	miR-23a: miR-23a overexpression inhibited cell migration and angiogenesis by targeting IRF-1	Promoted wound healing of DFUs	[155]
Quercetin	Diabetic-related cognitive dysfunction	STZ-treated rats	miR-27a: miR-27a overexpression increased Nrf2 and related antioxidant genes expression	Prevented memory dysfunction in diabetic rats	[67]
Gypenosides	AS	HFD-fed mice	miR-27a: miR-27a-related LPL downregulation suppressed proinflammatory cytokines secretion and lipid accumulation	Inhibited the development of AS	[53]
LINC01140	AS	oxLDL-treated human macrophages	miR-23b: miR-23b inhibition alleviated inflammatory response	Alleviated inflammatory response in macrophages	[156]
Theaflavin	AS	HFD-fed mice; HUVECs treated with cholesterol	miR-24: miR-24 inhibition promoted oxidative injuries	Alleviated oxidative injury and AS progress	[157]
Melatonin	Hepatic steatosis	Tunicamycin-treated mice	miR-23a: miR-23a inhibition alleviated ER stress	Alleviated ER stress-mediated hepatic steatosis	[121]

AS atherosclerosis, *DFU* diabetic foot ulcer, *DN* diabetic nephropathy, *ER* endoplasmic reticulum, *FGF11* fibroblast growth factor 11, *FOXO1* forkhead box protein O1, *GDM* gestational diabetes mellitus, *GLP-1* glucagon-like peptide-1, *HFD* high-fat diet, *HG* high glucose, *HUVEC* human umbilical vein endothelial cell, *IRF-1* interferon regulatory factor element 1, *JNK* c-Jun N-terminal kinase, *LPL* lipoprotein lipase, *MC* mesangial cell, *mTOR* mammalian target of rapamycin, *NOV* nephroblastoma overexpressed, *Nrf2* nuclear factor-erythroid 2-related factor 2, *oxLDL* oxidized low-density lipoprotein, *STZ* streptozotocin, *TIMP3* tissue inhibitor of metalloproteinase 3.

Off-target effects are generally classified into specific and non-specific off-target effects [144]. The former is caused by miRNAs binding to partially complementary sequences of non-targeted mRNAs. The latter refers to immunological activation, cellular toxicity, and other effects induced by exogenous miRNAs and their delivery systems [144]. It is well known that miRNAs can regulate various genes in different cell types, thus affecting multiple cellular processes. In this case, regulating a specific miRNA might result in beneficial effects in one cell type, but adverse effects in another. Moreover, the two strands generated from the same miRNA duplex (miRNA-5p and miRNA-3p) might exert opposite effects [145]. Hence, there is an urgent need to develop effective strategies for improving miRNA target specificity.

Since a single miRNA usually has a rather weak inhibitory effect on its target and high-dose exogenous miRNAs may cause unwanted consequences, Lai et al. proposed that off-target effects could be reduced by applying a low-dose combination of miRNAs that cooperatively regulate the same target gene [146]. As mentioned earlier, one mature miRNA recruited by RISC regulates gene expression, while the other is degraded. Kadekar et al. modified miR-34a by adding extra nucleotides to the 3′ ends of miR-34a-5p and miR-34a-3p, which play opposite roles in tumor invasion [147]. They demonstrated that the structural modification of miR-34a-5p and miR-34a-3p led to thermodynamic asymmetry, resulting in their RISC-binding preference alteration and thus regulating the anticancer effect of miR-34a [147]. Their findings provide evidence that proper miRNA modification can enhance therapeutic efficacy and reduce off-target effects. Furthermore, the drug delivery system is also crucial for the success of miRNA-based therapy. An ideal delivery system is in urgent need to prevent miRNA degradation, target specific organs, and minimize immune activation.

CONCLUDING REMARKS

The number of literature reports on the role of miRNAs in metabolic disorders has increased over the last few years. Given the critical roles of the miR-23 cluster in NAFLD pathogenesis, all three components are potential emerging therapeutic targets in NAFLD treatment. Moreover, altered miR-27 levels in serum or liver tissue of patients and animal models in NAFLD suggest its potential as a novel diagnostic biomarker. Further research into the sensitivity and specificity of NAFLD diagnosis and the improvement in liver function could accelerate the clinical application of the miR-23 cluster in NAFLD therapy.

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ADDITIONAL INFORMATION

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