MicroRNAs and lipid metabolism

Binod Aryal, Abhishek K. Singh*, Noemi Rotllan, Nathan Price, and Carlos Fernández-Hernando*

Purpose of review
Work over the past decade has identified the important role of microRNAs (miRNAS) in regulating lipoprotein metabolism and associated disorders including metabolic syndrome, obesity, and atherosclerosis. This review summarizes the most recent findings in the field, highlighting the contribution of miRNAs in controlling LDL-cholesterol (LDL-C) and HDL-cholesterol (HDL-C) metabolism.

Recent findings
A number of miRNAs have emerged as important regulators of lipid metabolism, including miR-122 and miR-33. Work over the past 2 years has identified additional functions of miR-33 including the regulation of macrophage activation and mitochondrial metabolism. Moreover, it has recently been shown that miR-33 regulates vascular homeostasis and cardiac adaptation in response to pressure overload. In addition to miR-33 and miR-122, recent GWAS have identified single-nucleotide polymorphisms in the proximity of miRNA genes associated with abnormal levels of circulating lipids in humans. Several of these miRNAs, such as miR-148a and miR-128-1, target important proteins that regulate cellular cholesterol metabolism, including the LDL receptor and the ATP-binding cassette A1.

Summary
MicroRNAs have emerged as critical regulators of cholesterol metabolism and promising therapeutic targets for treating cardiometabolic disorders including atherosclerosis. Here, we discuss the recent findings in the field, highlighting the novel mechanisms by which miR-33 controls lipid metabolism and atherogenesis, and the identification of novel miRNAs that regulate LDL metabolism. Finally, we summarize the recent findings that identified miR-33 as an important noncoding RNA that controls cardiovascular homeostasis independent of its role in regulating lipid metabolism.

Keywords
atherosclerosis, cholesterol metabolism, miR-148a, miR-33, miRNAs

INTRODUCTION

The leading cause of death worldwide is cardiovascular disease (CVD). Atherosclerosis is the cause of the most common forms of CVD such as heart attack and stroke [1,2]. Among others, the most important risk factors and mediators of atherosclerosis are the circulating levels of LDL-cholesterol (LDL-C) and HDL-cholesterol (HDL-C) [1,2].

MicroRNAs (miRNAs) are approximately 22-nt long noncoding sequences involved in the post-transcriptional regulation of gene expression [3–5]. Since their initial discovery in *Caenorhabditis elegans*, these short noncoding RNAs have been involved in the regulation of a wide spectrum of biological processes, from development and metabolic regulation, to aging and disease progression [3–5]. miRNAs control the expression of numerous mRNA targets, and a single mRNA can be regulated by several miRNAs. Indeed, it has been proposed that the expression of more than half of the human genes is regulated by numerous miRNAs. miRNAs assemble with Argonaute proteins into miRNA-induce silencing complexes (miRISCs) to direct post-transcriptional silencing of complementary mRNA targets [3–5].

The role of miRNAs in the biology and pathophysiology of CVDs has been extensively studied

Vascular Biology and Therapeutics Program, Integrative Cell Signaling and Neurobiology of Metabolism Program, Section of Comparative Medicine, and Department of Pathology, Yale University School of Medicine, New Haven, Connecticut, USA

Correspondence to Carlos Fernández-Hernando, New York University, New York, NY, USA. Tel: +1 203 737 4615;
e-mail: carlos.fernandez@yale.edu

*Binod Aryal and Abhishek K. Singh contributed equally to the writing of this article.

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KEY POINTS

- miRNAs regulate lipid metabolism, and genetic variations in miRNA loci have been associated with alterations in circulating cholesterol and triglycerides (TAG) in humans.
- miR-33 controls HDL-C biogenesis and cholesterol efflux by regulating ABCA1 expression. Moreover, miR-33 regulates cellular functions associated with cardiovascular disease, including macrophage activation, mitochondrial biogenesis, and autophagy.
- Further studies are necessary to determine whether silencing of miR-148a and miR-128-1 will attenuate atherosclerosis, obesity, and insulin resistance.

over the past decades [6,7]. Of note, miRNAs have recently been uncovered as regulators of circulating lipids, a major factor that influences the risk of CVD. Decreased levels of HDL-C are associated with increased risk for developing CVD. A number of miRNAs regulate different steps of HDL-C metabolism, from synthesis to clearance, of which miR-33a/b are the most well studied [8–13]. Whereas the role of miRNAs in regulating HDL-C metabolism has been deeply studied, the importance of miRNAs in controlling LDL metabolism has fallen behind. Nonetheless, a number of recent studies have given light on the importance of miRNAs in controlling plasma LDL-C levels, such as miR-148a, miR-128-1, or miR-30c, which regulate genes involved in VLDL secretion, cholesterol biosynthesis, and hepatic LDL receptor (LDLR) expression [14▪▪,15▪,16,17▪▪]. Thus, miRNAs have arisen as critical regulators of cholesterol metabolism and promising therapeutic targets for the treatment of CVD. Their conservation between species suggests that the biological pathways in which miRNAs play a role may have been conserved. For that reason, miRNAs have a therapeutic potential, and different approaches have been undertaken to examine this possibility. In this review, we summarize the most important and novel roles of miRNAs in regulating lipoprotein metabolism.

MICRORNA REGULATION OF VLDL-C AND LDL-C METABOLISM

Increased levels of LDL-C leads to the infiltration and retention of these lipoproteins in the arterial wall, which is a critical initiating event in the development of atherosclerosis [1,2]. Early studies uncovered the role of miR-122 in regulating LDL-C levels in mice and nonhuman primates [18,19]. Antagonism of miR-122 in both animal models significantly reduces plasma LDL-C and VLDL-C levels (30–45% reduction) [18,19]. These results were further confirmed in two independent miR-122-deficient mouse models [20,21]. Whereas the reduction in circulating ApoB-containing lipoproteins in miR-122-deficient mice and mice treated with miR-122 inhibitors is very well established, the molecular mechanisms and the mRNA targets that mediate this effect remain unknown. Moreover, while the effect of miR-122 inhibition in reducing circulating LDL-C was very promising, the deleterious effects observed in mice deficient in miR-122 including hepatic steatosis and hepatocellular carcinoma [20,21] reduced the enthusiasm for developing miR-122 inhibitors for treating dyslipidemias. In addition to miR-122, recent studies have identified additional miRNAs that control plasma LDL-C levels. Using two independent approaches, human genome-wide association study (GWAS) and a high-throughput genome-wide screening assay *in vitro*, the Näär and Fernández-Hernando laboratories identified miR-148a and miR-128-1 as important regulators of LDL-C and HDL-C metabolism [14▪▪,17▪▪]. Additional recent studies found miR-30c to be an important regulator of VLDL secretion through targeting of the microsomal transfer protein (MTP), which controls ApoB lipidation and hepatic VLDL production [15▪,16]. Here, we summarize how these miRNAs control lipoprotein metabolism.

miR-148a

miR-148a is highly expressed in mouse and human hepatic tissue, and conserved among vertebrate species. miR-expression quantitative trait loci analysis of human livers strongly support the fact that single-nucleotide polymorphism (SNP) status in the promoter region of miR-148a strongly correlated with altered levels of total cholesterol (TC), LDL-C, and triglycerides (TAGs) in circulation[14▪▪,17▪▪, 22–24]. miR-148a directly targets the 3′UTR of *LDLR*, along with other genes involved in lipid metabolism, such as ATP-binding cassette A1 (*ABCA1*), peroxisome proliferator-activated receptor gamma coactivator 1-alpha (*PGC1α*), AMP-activated protein kinase (*AMPK*), and insulin-induced gene 1 (*INSIG1*). Importantly, overexpression of miR-148a in mice reduces hepatic LDLR and ABCA1 expression, thus increasing circulating LDL-C and reducing plasma HDL-C levels [14▪▪,17▪▪]. Conversely, antagonism of miR-148a in $ApoB^{TG}Ldlr^{-/+}$ and in $Apoe^{-/-}$ mice decreases circulating LDL-C and increases plasma HDL-C [14▪▪,17▪▪]. In addition to the liver, miR-148a is also expressed in a number of other tissues, including adipose tissue and hematopoietic cells. Interestingly, GWAS have

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associated SNPs in the miR-148a locus to obesity [25]. Moreover, miR-148a is highly expressed in macrophages and controls ABCA1 expression and cholesterol efflux [17▪▪]. Taken together, these findings suggest that miR-148a antisense oligonucleotide therapy could be useful for treating dyslipidemia and CVD. Further studies should test this possibility.

mir-128-1

In humans, miR-128 is encoded within an intron of the *R3HDM1* gene on chromosome 2, and appears to be co-expressed with its host gene in numerous tissues [17▪▪]. Physiological studies have revealed that miR-128-1 plays a key role in regulating cholesterol/lipid and energy homeostasis. Long-term inhibition of miR-128-1 in hyperlipidemic $Apoe^{-/-}$ mice leads to a marked decrease in circulating VLDL-C/LDL-C, VLDL-associated TAGs, and hepatic steatosis [17▪▪]. Similar to miR-148a, miR-128-1 also controls circulating lipoprotein metabolism by directly targeting the 3′UTR of *LDLR* and *ABCA1* [17▪▪]. miR-128-1 antagonism also improves glucose clearance by enhancing hepatic insulin sensitivity. In addition to regulation of lipoprotein metabolism, miR-128-1 regulates ABCA-1 expression in macrophages, and its inhibition increases ABCA1 expression and macrophage cholesterol efflux. Whereas these findings suggest that miR-148 and miR-128-1 antagonism might be promising therapeutic approaches for treating dyslipidemia, obesity, and CVD, additional studies will be important to demonstrate efficacy in other animal models and examine the possible deleterious effects of long-term silencing.

miR-30c

miR-30c has been identified in association with altered levels of circulating TC, TAGs, and VLDL biogenesis [16]. miR-30c inhibits MTP, thus reducing VLDL-C production. Interestingly, miR-30c inhibits hepatic lipid synthesis by targeting lysophosphatidyl glycerol acyltransferase 1 (LPGAT1), which is involved in phospholipid synthesis. Importantly, miR-30c overexpression using lentivirus *in vivo* markedly reduces circulating VLDL-C and LDL-C, and improves atherosclerotic plaque burden in $Apoe^{-/-}$ mice. Similar results were obtained when miR-30c was overexpressed using miRNA mimics. As expected by its role in suppressing hepatic MTP expression, antagonism miR-30c increases plasma VLDL-C and LDL-C levels, and promotes atherogenesis. Of note, miR-30c inhibits VLDL production without causing hepatic steatosis because it also inhibits hepatic lipid synthesis, suggesting that miR-30c overexpression might be an attractive approach for treating patients with homozygous hypercholesterolemia.

MICRORNAS AND HDL-C METABOLISM

Because cholesterol cannot be degraded, mammalian cells orchestrate the removal of excess cholesterol from the peripheral tissues to liver for reutilization and secretion to feces through a process known as reverse cholesterol transport (RCT). As important step of RCT, nascent HDL particles are generated in the liver and intestine through the efflux of cholesterol and phospholipids to lipid poor apolipoprotein A-1 (ApoA1) particles. ABCA1 plays a key role in HDL formation as evidenced by a near-absence of plasma HDL in patients with Tangier disease, who have mutations in the *ABCA1* gene [26]. In addition to ABCA1, ABCG1 also facilitates cholesterol efflux to mature HDL particles, whereas scavenger receptor class B type 1 (SR-BI) contributes to HDL remodeling and mediates the delivery of cholesterol ester to the liver and other steroidogenic tissues [27,28]. Within the liver, part of the cholesterol is enzymatically converted into bile acid salts in a multistep process initiated by enzyme cholesterol 7α-hydroxylase (CYP7A1) [29]. As a final step of RCT, these highly soluble biliary lipids are secreted from the hepatocytes with the help of three different transmembrane transporters: ATP-binding cassette, subfamily B, member 11 (ABCB11), ATP-binding cassette, subfamily G, member 5/8 (ABCG5/ABCG8) and ATP-binding cassette, subfamily B, member 4 (ABCB4) [30]. In addition, another transporter – aminophospholipid transporter, class I, type 8B, member 1 (ATP8B1) – maintains the asymmetry of phospholipids required for proper membrane function [31]. Over the past decade, miRNAs have emerged as important regulators of HDL metabolism and RCT, including direct targeting of genes involved in cellular cholesterol efflux, HDL biogenesis, hepatic HDL uptake, and bile acid synthesis and secretion [32]. In addition to their role in regulating HDL metabolism, HDL-enriched miRNAs regulate gene expression in recipient cells, thus providing an exciting novel mechanism that could explain a part of the anti-atherogenic effect associated with HDL [33,34]. Our group and others have identified numerous miRNAs including miR-33a/b, miR-144, miR-148a, and miR-128, which are important regulators of HDL biology, particularly in the context of atherosclerosis [11–13,35–38].

MIR-33 AND HDL-C METABOLISM

The sterol response element-binding proteins are master regulators of sterol and fatty acid synthesis

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[39]. miR-33 family of microRNAs consists of *miR-33a* and *miR-33b,* which are encoded within the introns of the *SREBF2* and *SREBF1* genes, respectively, and are co-expressed in different stimulatory conditions [12,13,32]. Both miR-33 isoforms share the same seed sequence, but differ in two nucleotides in the 3′ region. The relevance of miR-33 on sterol metabolism was initially revealed in studies showing that miR-33 modulated the expression of *ABCA1* and *ABCG1* both in mouse hepatocytes and macrophages. Overexpression of miR-33 in mouse liver resulted in a decrease in *ABCA1* and *ABCG1* expression, and a parallel decrease in the plasma HDL-C levels. Alternatively, the antagonism of miR-33 expression using antisense oligonucleotides (ASO-33) resulted in an increase in hepatic *ABCA1/ABCG1* expression and plasma HDL-C levels [11–13]. Genetic ablation of *miR-33* recapitulated the results from ASO studies, as the *miR-33*$^{-/-}$ mice had increased liver *ABCA1* expression and elevated plasma HDL-C indicating the physiological relevance of miR-33 in regulation of HDL-C metabolism [10]. Most of these studies were performed through therapeutic and genetic modulation of miR-33 in mice [40]. However, rodents have only one isoform of miR-33 (equivalent of miR-33a in humans), but not miR-33b. Although miR-33a and miR-33b have same seed sequence and same predicted targets, studies have been done in non-human primates to address the role of both isoforms in HDL-C metabolism. Treatment of African green monkeys with antisense inhibitors against miR-33a and miR-33b resulted in an increase in plasma HDL-C levels (25–30%) [37,41]. In addition, Horie *et al.* [42] recently developed miR-33b knockin mice in an intron of *Srebp1* gene. Macrophages from these mice had reduced *ABCA1* and *ABCG1* expression and cholesterol efflux capacity to ApoA1 and HDL. In addition, knockin heterozygous mice had a 35% reduction in HDL-C levels compared to wild-type counterparts, confirming the physiological role of miR-33b in regulating HDL-C metabolism [42].

Remarkably, miR-33 has also been reported to influence cholesterol efflux independent of its ability to regulate *ABCA1/ABCG1* expression. Karunakaran *et al.* [43▪] have demonstrated that inhibition of miR-33 increases mitochondrial respiration and ATP production through up-regulation of miR-33 target genes including peroxisome proliferator-activated receptor γ coactivator 1-α (*PGC1-α*), pyruvate dehydrogenase kinase isozyme 4 (*PDK4*), and solute carrier family 25 (SLC25A25), and promotes ABCA1-mediated cholesterol efflux. In addition to regulating cholesterol efflux and HDL biogenesis, several studies have reported a role for miR-33 in regulating bile acid synthesis and secretion. Allen *et al.* [44] have shown that miR-33 regulates the expression of bile acid transporters including *ABCB11* and *ATP8B1*, and as such systemic silencing of miR-33 leads to increased sterols in bile and enhanced RCT *in vivo*. Taken together, these studies suggest an important role for both miR-33 family members in regulating HDL-C metabolism, and RCT and atherosclerosis progression. Additionally, miR-33 might also modulate the inflammatory status of atherosclerotic plaques independent of its regulation of HDL. It was shown that miR-33 inhibitors efficiently transduce macrophages accumulated in the subendothelial space of aortic root of *Ldlr*$^{-/-}$ mice and promote their polarization towards anti-inflammatory M2 phenotype by direct targeting of *AMPK*. Another target of miR-33 is *ALDH1A2*, a gene involved in retinoic acid metabolism, which is also de-repressed in these macrophages, resulting in the induction of regulatory T cells and atheroprotection [45▪].

In addition to regulating lipid accumulation through RCT, Ouimet *et al.* [46] has recently reported that miR-33 also promotes lipid accumulation in macrophages infected by *Mycobacterium tuberculosis* (Mtb) by suppressing lysosomal degradation and autophagy. In particular, overexpression of miR-33 in Mtb-infected macrophages repressed autophagy of Mtb, promoted lipid body formation, and repressed fatty acid oxidation by targeting genes that encode proteins involved in autophagosome formation [autophagy protein 5 (*ATG5*)], *ATG7, ATG12*, microtubule associated protein 1 light chain 3 beta (*MAP1LC3B*), lysosomal function [lysosomal-associated membrane protein 1 (*LAMP1*)], lysosomal acid lipase (*LIPA*), and the regulation of autophagy (*UVRAG*, *AMPK).* Conversely, inhibition of miR-33 promoted the targeting and killing of Mtb by autophagy machinery [46]. In another recent study, Lai *et al.* found that miR-33 regulates innate immune response via ABCA1 remodeling of membrane microdomains. miR-33 augments macrophage lipid raft content and enhances pro-inflammatory cytokine induction and NF-κB activation by lipopolysaccharide (LPS) [47]. In contrast to Ouimet *et al.*'s study, the authors found that LPS markedly reduces the expression of miR-33, which correlates with a significant down-regulation of *Srebp2*, the host gene for miR-33 [47]. In agreement with this observation, numerous studies have shown that SREBP2 expression is significantly reduced in macrophages stimulated with LPS [48,49].

In addition to the role of miR-33 in regulating lipid accumulation in macrophages and hepatocytes, Nishiga *et al.* [50▪] reported that miR-33 preserves lipid raft cholesterol content in cardiac fibroblasts through the regulation of *ABCA1* expression and maintains adaptive fibrotic response in the remodeling heart. Surprisingly, miR-33-deficient mice showed impaired

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systolic function after thoracic aortic constriction (TAC). Interestingly, another recent study has demonstrated that miR-33 protects against neointimal hyperplasia induced by arterial mechanical stretch in grafted veins [51▪]. Mechanistically, the authors found that miR-33 targets bone morphogenetic protein 3 (BMP3) and attenuates vascular smooth muscle cell (VSMC) proliferation and neointimal expansion. These findings correlate with a number of studies that have linked miR-33 expression and cellular proliferation. Together, these findings suggest that miR-33 might regulate other cellular functions apart from lipid metabolism.

A number of recent studies have shown that chronic antagonism or genetic ablation of miR-33 can cause adverse effects including dyslipidemia, obesity, hepatic steatosis, and insulin resistance [52–54]. Horie *et al.* [54] found that miR-33 regulates the expression of SREBP1 and its absence increases hepatic SREBP1 levels and activation. Although the authors suggest that the increased levels of SREBP1 contribute to the marked obesity, dyslipidemia, and insulin resistance observed in miR-33-deficient mice, early study from Brown and Goldstein's laboratory found a modest effect on body weight and circulating lipids in hepatic SREBP1a and

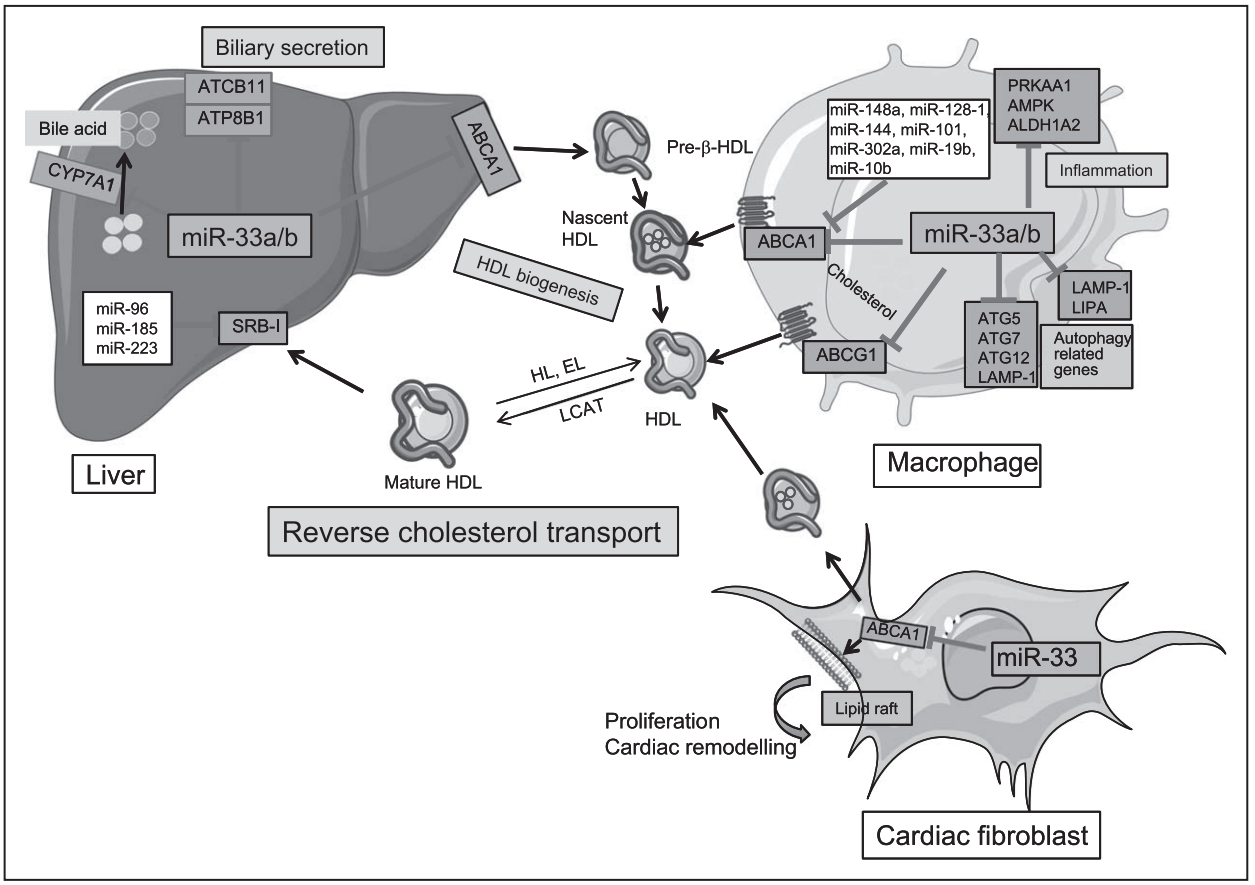

FIGURE 1. MicroRNA (miRNA) regulation of HDL-C metabolism. ABCA1, a major transporter that regulates HDL biogenesis and cholesterol efflux in macrophages accumulated in the artery wall, is regulated by a number of miRNAs including miR-33. miR-33 controls numerous steps of the reverse cholesterol transport pathway by regulating the expression of numerous genes associated with HDL biogenesis (ABCA1), cholesterol efflux in peripheral tissues including macrophages and cardiac fibroblasts [ABCA1 and ABCG1 (only in rodents)], and bile acid synthesis (CYP7A1) and secretion (ABCB11 and ATP8B1) in liver. In addition, miR-33 also promotes lipid accumulation in macrophages and favors Mtb survival by targeting the expression of key autophagy effectors ATG5, ATG7, ATG12, LAMP-1, and LIPA, and controls macrophage polarization by regulating the expression of PRKAA1, AMPK, and ALDH1A2. In addition to miR-33, ABCA1 is highly regulated at the post-transcriptional level in several tissues by numerous miRNAs including miR-148a, miR-144, miR-101, miR-128, miR-27a/b, miR-302a, and miR-10b. Free cholesterol in nascent HDL is further esterified to cholesteryl esters by lecithin-cholesterol acyltransferase (LCAT), leading to the formation of mature HDL particles. HDL particles deliver cholesterol to the liver via the SRB1 receptor, which is also regulated by several miRNAs including miR-185, miR-223, and miR-96. This figure was performed using the Servier Medical Art illustration resources. AMPK, AMP-activated protein kinase; ABCA1, ATP-binding cassette A1; HDL-C, high-density lipoprotein cholesterol; LAMP-1, Lysosomal-associated membrane protein 1; Mtb, *Mycobacterium tuberculosis*.

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SREBP1c transgenic mice [55,56]. These observations suggest that the obesity phenotype observed in the miR-33-deficient mice is likely mediated by its effect in several metabolic tissues and its regulation of other genes apart from *SREBF1*. Further studies using tissue specific miR-33 knockout mice will help to dissect the specific contribution of miR-33 in several metabolic tissues, and how this contributes to the marked obesity and insulin resistance phenotype observed in the global miR-33-deficient mice.

In addition to miR-33, a number of other miRNAs have been reported to regulate different aspects of HDL-C metabolism including cellular cholesterol efflux, HDL biogenesis, and HDL uptake. Some of these miRNAs including miR-758, miR-144, miR-26, miR-27a/b, miR302a, miR-148a, miR-128-1, and miR-19b have been shown to regulate ABCA1 expression in macrophages and hepatocytes [14▪▪,17▪▪,57–62]. A recent study has shown that inhibition of miR-302a in macrophages results in increased *ABCA1* expression and subsequent cholesterol efflux to ApoA1 particles *in vitro*, whereas long-term in-vivo antagonism of miR-302a led to an enhanced liver ABCA1 expression and plasma HDL levels, and decreased atherosclerosis [61]. Similarly, it has been shown that miR-128-1 and miR-148a also contribute to the post-transcriptional regulation of ABCA1. Overexpression of miR-128-1 and miR-148a in mice significantly lowered circulating HDL-C and hepatic ABCA1 levels [14▪▪,17▪▪]. Most importantly, antagonism of miR-148a *in vivo* markedly increased hepatic ABCA1 expression and plasma HDL-C levels [14▪▪]. Interestingly, not all miRNAs that regulate *ABCA1* expression and cholesterol efflux *in vitro* influence plasma HDL-C levels. For instance, miR-27a/b modulates cholesterol efflux from hepatocytes and macrophages through the regulation of *ABCA1*. However, in-vivo manipulation of miR-27b did not influence plasma HDL-C levels in chow or high-fat diet conditions, possibly because of miR-27 targeting other lipid-related genes angiopoietin like 3 (*ANGPTL3*) and glycerol-3-phosphate acyltransferase (*GPAM*), which counteract the influence on HDL-C levels through ABCA1 regulation [63,64]. HDL transports cholesterol esters from peripheral tissues to the liver, in which it is selectively taken up by SR-BI receptors – a critical step of RCT. A number of miRNAs regulate the expression of SR-BI and thereby affect the uptake of HDL by the liver. In particular, overexpression of miR-455, miR-125a, miR-185, miR-96, and miR-223 attenuates SR-BI expression and HDL uptake in liver cell lines [65,66]. Importantly, genetic ablation of miR-223 in mice enhances hepatic SR-BI and plasma HDL levels [67]. Due to space limitations, specific roles of other miRNAs are not discussed in this review. Altogether, progress made in this field has demonstrated that a complex network of miRNAs regulate different stages of HDL-C metabolism by coordinating post-transcriptional control of multiple genes (Fig. 1).

CONCLUSION

Over recent years, contributions of many research groups have made evident the important role of miRNAs in the regulation of lipid metabolism and CVD. In this review article, we have highlighted some of the most important and recent findings related to miRNA-mediated regulation of HDL-C, LDL-C, and the development of atherosclerosis. These findings indicate that miRNA-targeted therapies may serve as a novel approach for the treatment of CVD. However, chronic treatment or genetic ablation of some of these miRNAs (miR-122 and miR-33) has been found to result in adverse effects including dyslipidemia, obesity, hepatic steatosis, and hepatocellular carcinoma. These findings along with an inability to demonstrate the specific mechanisms by which miRNAs exert these phenotypic effects have raised concerns about the use of miRNA-based therapies. As such, future experiments will be important for elucidating the specific functions of miRNAs in different tissues and the contribution of individual miRNA targets toward mediating specific phenotypes. These steps will be necessary to facilitate the progress and assess the outcome of miRNA-based clinical trials for CVDs. Additional human genetic studies identifying genetic variations in miRNAs genetic locus associated with plasma lipid levels and cardiovascular risk will be important for defining the importance of miRNAs in regulating lipid metabolism and cardiovascular disease in humans.

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Conflicts of interest

There are not conflicts of interest.

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Papers of particular interest, published within the annual period of review, have been highlighted as:

- ▪ of special interest
- ▪▪ of outstanding interest

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