miRNA Biogenesis
Micro-RNAs (miRNAs) are short, evolutionarily conserved, noncoding RNAs (18–25 nucleotides) that have emerged as important regulators of gene expression. miRNAs are encoded in the genome as intergenic sequences or within the introns of protein-coding genes and transcribed to a primary transcript (pri–miRNA) by RNA polymerase II. Pri–miRNAs are processed by the endonuclease Drosha–DGCR8 complex to generate a precursor miRNA (pre–miRNA). Then the pre–miRNA is transported from the nucleus to the cytoplasm where it is further processed by the endonuclease DICER to generate the mature form. Finally, the mature miRNA is incorporated into the RNA-induced silencing complex where it binds to the 3′-untranslated region (3′-UTR) of its target genes, promoting translational repression and mRNA degradation.1–3 Each miRNA can regulate the expression of numerous mRNAs involved in the same cellular pathway or physiological process,4,5 thus making miRNAs fine-tune regulators of multistep biological processes. Interestingly, recent studies have also demonstrated that miRNAs can participate in the communication between different organs and are stably transported in blood.6 Moreover, these circulating miRNAs have been shown to be potential biomarkers during the progression of pathophysiological processes.7

miRNA Regulation of High-Density Lipoprotein Metabolism
Deregulation of cholesterol homeostasis can trigger numerous cardiometabolic diseases such as atherosclerosis and type-II diabetes mellitus. High-density lipoproteins (HDLs) transport excess cholesterol from the peripheral tissues into the liver for reutilization and secretion to feces in a process known as reverse cholesterol transport (RCT). During the first step of HDL biogenesis, HDL particles are synthesized in the liver and intestine by the incorporation of cholesterol and phospholipids into lipid-poor ApoA1 particles. ATP-binding cassette (ABC) A1, a member of the ABC membrane transporter family, plays a key role in nascent HDL formation.8 The importance of ABCA1 in HDL biogenesis was illustrated when Tangier disease, a condition characterized by the near-absence of HDL, was attributed to a mutation in this gene.9 In addition to ABCA1, other
proteins participate in HDL metabolism, including ABCG1 and scavenger receptor class B type 1 (SR-BI). ABCG1 mediates cholesterol efflux to HDL as shown by in vivo studies that demonstrate that nascent HDL formed from the interaction of ApoA1/ABCA1 serves as an acceptor for ABCG1-mediated cholesterol efflux.\(^10,11\) SR-BI contributes to HDL maturation and also mediates the delivery of cholesteryl ester into the liver, among other tissues.\(^12,13\) When cholesterol arrives to the liver, a part is enzymatically transformed to bile salt acids in a multistep process initiated by the action of cholesterol 7α-hydroxylase.\(^14\) Cholesterol conversion into highly soluble bile acids allows the transport of cholesterol in the digestive system for its excretion. The final step in RCT is the secretion of biliary lipids from the hepatocytes. There are 3 different transmembrane transporters along the apical membrane of the hepatocyte that actively promotes this process: the heterodimer ABCG5/ABCG8 (which facilitates cholesterol efflux), ABCB11 and the phospholipid pump, ABCB4.\(^15\) Moreover, another transporter, ATP8B1, is also necessary for correct secretion of bile. ATP8B1 moves phosphatidylserine in the opposite direction to the transport of phosphatidylcholine by ABCB4 to maintain the asymmetry of phospholipids required for proper membrane function.\(^16,17\)

Because cholesterol cannot be degraded in the cell, RCT is an essential process to ensure that cholesterol levels are balanced within the body. Work over the past years has identified miRNAs as important regulators of HDL-cholesterol (HDL-C) metabolism. miRNAs control most of the steps of RCT including HDL biogenesis, cellular cholesterol efflux, hepatic HDL-C uptake, and bile acid synthesis and secretion.\(^18\) In addition to their role in regulating HDL-C metabolism, HDL-enriched miRNAs regulate gene expression in recipient cells, thus providing an exciting novel mechanism that could explain a part of the antiatherogenic effect of HDL.\(^19\) In the following sections, we will discuss how specific miRNAs control HDL metabolism (Figure), the physiological relevance of HDL-mediated transport of miRNAs and the clinical relevance of HDL-derived miRNAs as cardiovascular disease (CVD) biomarkers.

**miR-33a/b**

Work from our group and from others identified miR-33a/b as essential regulators of lipid metabolism. The miR-33 family consists of 2 members, miR-33a and miR-33b, which are encoded within the introns of the sterol response element–binding protein genes, SREBP2 and SREBP1, respectively.\(^20,21\) SREBPs are transcription factors that control the expression of genes involved in lipid biosynthesis and uptake of lipoproteins. SREBP2 activates the transcription of genes that participate in the cholesterol biosynthetic pathway, as well as the low-density lipoprotein receptor (LDLR). The SREBP1 gene generates 2 different isoforms, SREBP1a and SREBP1c, that participate in the regulation of lipid metabolism. Although SREBP1c activates the expression of genes associated with fatty acid synthesis, SREBP1a promotes the expression of genes that control cholesterol metabolism and fatty acid synthesis. Although both SREBP1 isoforms are expressed in the liver, SREBP1c is the most abundant in the adult liver and is activated in response to insulin.\(^22,23\) Intriguingly, metabolic stimuli that activate the SREBP transcription factors lead to increased expression of miR-33a/b, suggesting that these miRNAs are coregulated with their host genes. Indeed, several studies have demonstrated that both miR-33a/b work with their respective SREBP host genes to tightly control intracellular levels of cholesterol and fatty acids.\(^24,25\) Interestingly, the passenger strand of miR-33, miR-33*, has also been shown to accumulate to steady state levels during SREBP activation, suggesting that miR-33 may regulate lipid metabolism through both arms of the miR-33/miR-33* duplex.\(^26\)

**Figure.** Micro-RNA (miRNA) regulation of high-density lipoprotein (HDL) metabolism and reverse cholesterol transport. Schematic overview of miRNAs involved in the regulation of HDL metabolism and reverse cholesterol transport (RCT). Gray boxes highlight miRNAs that regulate genes that control HDL metabolism and RCT (red boxes). Note that only miRNAs highlighted in red have been demonstrated to influence HDL metabolism in vivo. Figure was created using the Servier Medical Art illustration resources (http://www.servier.com). ABC indicates ATP-binding cassette; CYP7A1, cholesterol 7α-hydroxylase; and SR-BI, scavenger receptor B1.
The 3′-UTR of ABCA1 contains 3 highly conserved binding sites for miR-33. All of these sites are required for miR-33 repression of ABCA1 expression. Functional assays overexpressing miR-33 in human and mouse hepatocytes and macrophages demonstrated a decrease in ABCA1 mRNA and protein levels, as well as an inhibition in cholesterol efflux to ApoA1 and nascent HDL.20,21,25,27,28 More importantly, antagonists of miR-33 in vivo increased hepatic ABCA1 expression and elevated plasma HDL-C levels.7,20,27 Similar results were observed in miR-33-deficient mice.24 These findings indicate a physiologically relevant role for miR-33 in regulating hepatic ABCA1 expression and HDL biogenesis. miR-33 also controls the expression of other important cholesterol transporters such as ABCG1 (only in rodents) and NPC1.20,27 Interestingly, it has been shown that miR-33 influences cholesterol efflux independently of its ability to repress ABCA1 and ABCG1 expression.

More specifically, Karunakaran et al29 have recently shown that miR-33 inhibition increases mitochondrial respiration and ATP production through the upregulation of many miR-33 direct targets including peroxisome proliferator-activated receptor γ coactivator 1-α, pyruvate dehydrogenase kinase isozyme 4, and solute carrier family 25, which favors ABCA1-mediated cholesterol efflux. In addition to the role of miR-33 in regulating cellular cholesterol efflux and HDL biogenesis, several studies have also revealed a role for miR-33 in controlling bile acid synthesis and secretion. In this regard, Allen et al30 identified that miR-33 regulates the expression of different bile acid transporters including ABCB11 and ATP8B1. As such, inhibition of miR-33 lead to increased sterol accumulation in the bile and enhanced RCT in vivo.31,32 Furthermore, Li et al32 reported that cholesterol 7α-hydroxylase, the rate-limiting enzyme in bile acid synthesis, is a direct target gene of miR-33. Altogether, these studies demonstrate the essential role of miR-33 in controlling the expression of genes associated with different steps of HDL metabolism and RCT.

Many studies have shown that therapeutic silencing of miR-33 results in a marked increase in circulating HDL-C levels. However, most of these studies have been performed in mouse models where miR-33b is absent. In contrast with humans and other mammals, rodents only have 1 copy of miR-33 (miR-33a in humans) encoded within the SREBP2 gene. Even though miR-33a and miR-33b have the same seed sequence and bioinformatic analyses have shown that both miRNAs are predicted to target the same genes, anti-miR-33 therapy has been performed in nonhuman primates to address the role of both isoforms in HDL metabolism. In 2 independent studies, treatment of nonhuman primates with antisense inhibitors against miR-33 resulted in increased plasma HDL-C levels.33,34 In addition, Horie et al35 recently developed miR-33b knockin mice for an intron of the SREBP1 gene. In vitro studies using macrophages from these mice showed reduced ABCA1 and ABCG1 expression and cholesterol efflux capacity to ApoA1 and HDL. Moreover, miR-33b knockin heterozygous mice exhibited a significant decrease in circulating HDL-C compared with wild-type mice. Taken together, these studies suggest an important role for both miR-33 family members in regulating HDL-C metabolism.

miR-33a/b, HDL-C Metabolism and Atherosclerosis

Accumulation of cholesterol into the intima layer of large arteries causes CVD. Complications of CVD are the most common causes of death in Western societies. Early epidemiological data demonstrated a strong inverse association of plasma levels of HDL-C with CVD,36–39 which led to the development of the HDL-C hypothesis, which posits the intervention to raise circulating HDL-C.40,41 However, recent findings have challenged the validity of this hypothesis including clinical trials aimed to therapeutically elevate circulating HDL-C and additional epidemiological studies.42 These findings suggest that the cardioprotective effect of HDL is likely because of its ability to remove cholesterol from plaques in the artery wall for excretion (RCT). Because miR-33 regulates HDL-C levels and the RCT process at multiple steps, the therapeutic potential of miR-33 to treat CVD is promising. Indeed, several groups have developed studies in different mouse models to address the functional effect of miR-33 inhibition on the progression and regression of atherosclerosis. The first study, lead by Rayner et al43 studied the efficacy of anti-miR-33 therapy during the regression of atherosclerosis. To assess the effects of inhibiting miR-33 in a model of established atherosclerosis, Ldlr−/− mice were first fed a Western-type diet for 14 weeks (baseline), after which they were switched to a chow diet to avoid atherosclerosis progression and treated with 2′-fluoro/methoxyl methyl miR-33 antisense oligonucleotides. The authors found that antagonism of miR-33 significantly increased plasma HDL-C levels, directed macrophage polarization to an M2-like phenotype, and enhanced the regression of atherosclerosis. Further studies examining the effect of anti-miR-33 therapy during atherogenesis have been conflicting. Although 2 independent groups showed a beneficial effect of miR-33 inhibition in attenuating the progression of atherosclerosis,44,45 Marquardt et al46 did not observe any improvement in atherogenesis under similar conditions. Interestingly, the 3 studies demonstrated that antagonism of miR-33 in hypercholesterolemic mice fails to increase plasma HDL-C levels. These findings suggest that inhibition of miR-33 might promote atheroprotection through mechanisms independent of increasing circulating HDL-C. Indeed, Ouimet et al47 recently reported that anti-miR-33 therapy decreases inflammation in atherosclerotic plaques by promoting anti-inflammatory M2 macrophage polarization and induction of FOXP3+ T regs. The discrepancy between the studies could be explained by some experimental variations such as cholesterol content in the Western diet, length of treatment or oligonucleotide chemical modifications. The role of miR-33 during the progression of atherosclerosis has also been studied in a mouse models deficient for miR-33 expression. Of note, genetic ablation of miR-33 in apoE−/− mice attenuated the progression of atherosclerosis and increased circulating HDL-C levels.50

Whereas most of the aforementioned studies strongly suggest that anti-miR-33 therapy might be useful for the treatment of CVD, many recent reports have shown that chronic anti-miR-33 therapy might cause deleterious effects, such as hypertriglyceridemia48,49,50 and moderate hepatic steatosis.51 Furthermore, high-fat-diet–fed miR-33-deficient mice develop obesity, hepatosteatosis, and insulin resistance.53 In
contrast to the results obtained in mice, antagonism of miR-33 in nonhuman primates does not increase circulating triglyceride levels. In conclusion, these results demonstrate the key role of miR-33 in regulating HDL-C metabolism and atherogenesis (Table). However, the discrepancies found between animal models and the different antisense oligonucleotide chemistry warrant further investigation.

**Other miRNAs That Regulate HDL-C Metabolism**

In the past years, it has been demonstrated that ABCA1 expression is tightly regulated at the post-transcriptional level. A large number of miRNAs, including miR-758, miR-144, miR-26, miR-27a/b, miR-302a, miR-128-1, and miR-148a, have been shown to regulate ABCA1 expression. miR-758 is downregulated in macrophages loaded with cholesterol and in the liver of mice fed a high-fat diet. Cholesterol efflux is regulated in vitro by miR-758; however, its role in vivo has yet to be addressed. Two independent studies demonstrated the role of miR-144 in HDL-C metabolism. In one of these studies, Ramirez et al identified miR-144 using an unbiased genome-wide screen of miRNAs modulated by liver X receptor ligands. In the other study, de Aguiar Vallim et al identified miR-144 in a genome-wide screen aimed at identifying miRNAs modulated by Farnesoid X receptor. The in vivo experiments in both reports demonstrated, through gain- and loss-of-function experiments, that miR-144 levels influence hepatic ABCA1 expression in macrophages and hepatocytes and raise plasma HDL-C levels. Moreover, a third study demonstrated that overexpression of miR-144 impairs RCT and promotes proinflammatory cytokine production, thus accelerating atherosclerosis in apoE−/− mice. Multiple groups have shown that miR-27a/b and miR-26 also modulate cholesterol efflux in hepatocytes and macrophages by regulating ABCA1 expression. However, the manipulation of miR-27b expression in mice fed a chow or Western-type diet does not significantly influence circulating plasma HDL-C levels.

This finding might be explained by the potential effect of hepatic miR-27 levels in regulating the expression of numerous miR-27 target genes associated with lipid metabolism including, angioptel-like 3 (Angptl3) and glycerol-phosphate acyltransferase 1 (Gpam). Interestingly, ANGPTL3 inhibits the activity of lipoprotein lipase and endothelial lipase and increases circulating HDL-C levels. Thus, the effect of hepatic miR-27b overexpression or silencing in controlling plasma HDL-C levels might be influenced by its inhibitory effect on the expression of numerous mRNA targets. Recent work reveals that miR-302a also modulates HDL-C metabolism. Interestingly, long-term in vivo administration of anti--miR-302a raises HDL-C and ABCA1 expression in the liver and aorta of Ldlr−/− mice fed a high-fat diet. Finally, 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. Most importantly, antagonism of miR-148a in vivo markedly increased hepatic ABCA1 expression and plasma HDL-C levels. As both miR-148a and miR-128-1 also control circulating levels of LDL cholesterol by post-transcriptionally regulating hepatic LDLR expression, further studies are warranted to determine whether inhibition of these miRNAs can ameliorate the elevated LDL-cholesterol/HDL-C ratio and decrease atherosclerosis in a humanized mouse.

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**Table. miR-33 and Atherosclerosis**

<table>
<thead>
<tr>
<th>Anti–miRNA-33 Therapy</th>
<th>Animal Model</th>
<th>Effect on Plasma HDL</th>
<th>Effect on Atherosclerosis</th>
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<tr>
<td>2′F/MOE</td>
<td>Non-human primates</td>
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<tr>
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<tr>
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<td>miR-33−/− ApoE−/− mice</td>
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2′F/MOE indicates 2′-fluoro/methoxyethyl; HDL, high-density lipoprotein; and LNA, locked nucleic acid.

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2′F/MOE indicates 2′-fluoro/methoxyethyl; HDL, high-density lipoprotein; and LNA, locked nucleic acid.
model of heterozygous familial hypercholesterolemia (Ldlr+/−; ApoBec1+/−; hApoB5′). Apart from the miRNAs discussed above, numerous other miRNAs including miR-19b, miR-106b, miR-93, miR-101 have been described to have an impact on ABCA1 expression and HDL-C metabolism under different physiological stimuli.68–72 Interestingly, gut microbiota metabolism of anthocyanin modifies RCT by modifying miR-10b expression. Specifically, Wang et al.73 demonstrate that protocatechuic acid (a product of the anthocyanin Cy-3-G metabolism) boosts RCT in apoE−/− mice by increasing both ABCA1- and ABCG1-dependent cholesterol efflux from macrophages.

As explained above, HDL transports cholesterol from peripheral tissues to the liver for excretion. The selective uptake of HDL-C into the liver is mediated by SR-BI.74,75 Lack of SR-BI in mice impairs RCT and causes massive atherosclerosis.76 SR-BI expression is controlled by different transcription factors such as peroxisome proliferator-activated receptors, SREBP, liver X receptor, and liver receptor homologue.77 At the post-transcriptional level, SR-BI expression is controlled by alternative splicing, and its interaction with PDX domain containing 1,7,5,78 Moreover, many miRNAs have been shown to regulate SR-BI expression. In the hepatoma cell line, HepG2, the silencing of Drosha and DICER produce an increase in SR-BI expression,79 which confirms the influence of miRNAs in controlling SR-BI levels. Specifically, the 3′-UTR of SR-BI has conserved binding sites for miR-455, miR-125a, miR-185, miR-96, and miR-223,79–81 Overexpression of these miRNAs attenuates SR-BI protein expression and HDL-C uptake in the liver and some steroligenic cell lines and tissues. Conversely, inhibition of these miRNAs enhances SR-BI expression and HDL-C uptake.79,81 In addition to these in vitro studies, it has also been demonstrated that genetic ablation of miR-223 enhances hepatic SR-BI expression and increases circulating HDL-C levels.80 Interestingly, miR-223 also regulates cholesterol biosynthesis through the direct repression of the sterol enzyme, 3-hydroxy-3-methylglutaryl-CoA synthase 1, and HDL biogenesis via Sp3 inhibition. In contrast to miR-33, miR-223 expression is attenuated in cholesterol-depletion conditions (statin treatment), thus further preventing cellular cholesterol reduction via miR-223 downregulation of 3-hydroxy-3-methylglutaryl-CoA synthase 1 expression.80 miR-96 and miR-185 levels are also regulated in vivo. The expression of both miRNAs inversely correlates with the hepatic expression of SR-BI in apoE−/− mice fed a high-fat diet.81

Altogether, these studies suggest that a complex miRNA network controls HDL-C metabolism at different steps to maintain whole-body cholesterol homeostasis.

**HDL as Carriers of miRNAs**

Recent studies have suggested that circulating miRNAs could be transported from donor cells to recipient cells by exosomes, apoptotic bodies, microvesicles, ribonucleoproteins (nucleophosmin 1, argonaute 2), and lipoproteins.89,82,83 In contrast to rapid degradation of naked miRNAs in plasma, circulating miRNAs are remarkably stable,84 suggesting an endogenous protective mechanism against ribonuclease activity via incorporation with these carriers. It is well-known that exosomes transport miRNAs to recipient cells acting as mediators of cell-to-cell communications. In addition to exosomes, lipoproteins, especially HDL, play a critical role as the carriers of miRNAs in cardiometabolic disorders.85–87 The idea that HDL can bind and transport endogenous miRNAs was first suggested by Vickers et al,77 who profiled miRNAs in pure human HDL using real-time polymerase chain reaction arrays. Although HDL and LDL share many of the most abundant miRNAs, such as miR-223, their profiles are statistically different and HDL-miRNA transport seems to be more prominent than LDL.19,87 Moreover, mouse models of hypercholesterolemia and dyslipidemic patients exhibit a significantly distinct HDL-miRNA profile compared with healthy subjects, indicating that the miRNA cargo of HDL may be involved in the atherosclerotic disease process and cardiometabolic disorders.87

**Cellular Transportation of HDL-Bound miRNAs**

The actual mechanisms of cellular export of miRNAs to HDL remain unclear. Although previous studies suggest that the ceramide pathway triggers cellular export of miRNAs through exosomes, the cellular export of miRNAs to HDL is repressed by the ceramide pathway.87–89 Chemical or small interfering RNA inhibition of neutral sphingomyelinase 2, the key enzyme in ceramide synthesis, resulted in reduced exosomal-miRNA export.90 However, inhibition of neutral sphingomyelinase 2 by GW4869 increased the amounts of miR-223 exported to HDL from macrophages,91 indicating the distinct mechanisms of cellular export of a specific miRNA through the exosomal pathway and the HDL pathway. About the cellular uptake of exosomal miRNAs, there are 2 major hypotheses: endocytosis and membrane fusion.92 Although the detailed mechanisms of how HDL delivery of miRNAs to recipient cells are unclear, it is suggested to be mediated by SR-BI,77 which would probably deliver miRNAs into the cytoplasm instead of the endosomal-lysosomal pathway and make miRNAs become more stable with intact functional integrity.

**HDL-Bound miRNAs as Cardiovascular Biomarkers**

Circulating miRNAs have been suggested as potential biomarkers in the early detection of coronary artery disease and acute myocardial infarction. Although significantly altered levels of miR-126, miR-17, miR-92a, miR-155, miR-145, miR-133a, and miR-208a were found in patients with coronary artery disease, the levels of miR-208a, miR-1, miR-133a, and miR-499 were higher in patients with acute myocardial infarction than healthy subjects.91,92 Such evidence supports the potential role of miRNAs in the early detection of atherosclerotic CVD, which raises the possibility that HDL-bound miRNAs may be used as biomarkers in cardiometabolic disorders. Several vascular and inflammation-associated miRNAs, including miR-92a, miR-126, miR-150, and miR-378, that regulate angiogenesis were also found in HDL.85 The percentage of HDL-bound miR-155, a proinflammatory miRNA that is specifically expressed in atherosclerotic plaques and proinflammatory macrophages, is significantly higher in patients with acute coronary syndrome than in healthy subjects.93 Isolation of HDL from familial hypercholesterolemia patients suggested
the most abundant HDL-bound miRNAs were miR-223, miR-105, and miR-106a, which have a greater concentration than healthy subjects. Moreover, higher miR-486 and miR-92a levels in HDL subfractions were found in patients with acute coronary syndrome. However, a study from Wagner et al indicated that levels of HDL-bound miR-92a were significantly lower in patients with acute coronary syndrome than in healthy subjects. Possible explanations for these discrepancies include small patient populations, leading to slight alterations in patients with coronary artery disease compared with healthy subjects. Moreover, the amount of miRNAs associated with HDL could be underestimated because of an inefficient recovery of miRNAs during the lipoprotein isolation process. These results remind us to carefully interpret the results of biomarker studies using HDL isolation. Extensive testing and large-scale studies are necessary to verify the results of biomarker studies from HDL-bound miRNAs.

**Functional Importance of HDL-Bound miRNAs**

HDL-bound miRNAs have also been shown to have a physiological relevance. Incorporating exogenous miR-223 to native HDL in cultured human hepatic cells (Huh7) resulted in significantly increased intracellular miR-223 levels, which suppressed the mRNA expression of miR-223 targets including Rho-related GTP-binding protein Rhob (RHOB) and ephrin-A1 (EFNA1). Moreover, incubation of Huh7 cells with HDL isolated from familial hypercholesterolemia subjects, enriched in miR-105, significantly increased intracellular miR-105 levels and downregulated miR-105 mRNA targets. This effect was not observed when Huh7 cells were incubated with healthy HDL, suggesting that HDL transports endogenous miRNAs and directly alters miRNA-targeted gene expression. As for the anti-inflammatory properties, HDL-bound miR-223 could also be transferred to endothelial cells, resulting in suppression of intercellular adhesion molecule 1 and the expression of other proinflammatory genes. Importantly, HDL isolated from miR-223−/− mice or cellular antagonism of miR-223 in cells treated with HDL-containing miR-223 failed to inhibit intercellular adhesion molecule-1 expression in endothelial cells. In the same study, the authors demonstrated that intercellular adhesion molecule-1 is a direct target of HDL-bound miR-223 and regulates leukocyte adhesion. In addition to its anti-inflammatory role, miR-223 also enhances ABCA1 expression and promotes cholesterol efflux in Huh7 cells. These results indicated the cholesterol regulation and anti-inflammatory nature of HDL-bound miR-223, which might partially explain the protective functions of HDL from atherosclerosis. In addition to miR-223, other miRNAs are abundantly present in HDL including miR-92a and miR-155. However, their functional relevance has not been investigated so far. In summary, although strong in vitro evidence suggests the functional importance of HDL-bound miRNAs, further in vivo studies are critical to confirm these in vitro observations and demonstrate HDL-to-cell communication via miRNAs.

**Potential Challenges in the Study of HDL-Bound miRNAs**

Even though several studies have suggested the importance of HDL-bound miRNAs in cardiometabolic disorders, there are still some issues that should be overcome before clinical application. First, as mentioned above, the purification process of HDL-bound miRNAs is critical to obtain precise information. Second, establishing novel in vivo models are necessary to clarify all the in vitro evidence. Moreover, miRNAs might regulate networks of genes associated with the same physiological process, thus influencing the expression of numerous direct and indirect target genes related to the same pathway. This complexity makes their functional validation more difficult. Analysis of the miRNA target-mRNA interactome may provide a way to deal with such complicated interactions. Finally, the patient populations from current studies are indeed small in size, which may have potential biases when interpreting the results. Large-scale studies should be performed to verify the results.

**Concluding Remarks**

Work from the past decade has highlighted a prominent role for miRNAs in regulating cholesterol homeostasis. In particular, the role of miRNAs in regulating HDL-C metabolism has been extensively investigated, as evidenced by studies demonstrating the crucial role of miRNAs in post-transcriptionally regulating ABCA1 expression and multiple genes involved in the RCT pathway. In addition, interactions between HDL and miRNAs could be mediators of systemic gene expression, especially those of which are involved in atherosclerosis. Although the detailed transportation mechanisms need to be explored, the HDL-bound miRNAs may serve as useful clinical biomarkers. Furthermore, these HDL-bound miRNAs have been shown to have functional importance, with some of them exhibiting anti-inflammatory properties. Consequently, novel therapeutic approaches that exploit miRNA-dependent gene silencing offers a promising new future for the management of CVD. From a clinical perspective, this comes at a particularly crucial time, as clinical trials have been unable to significantly correlate increased HDL-C levels with decreased risk for CVD. As several miRNAs, including miR-33, target many regulatory points of HDL-C metabolism, inhibiting this subset of noncoding RNAs may represent a novel approach to increase HDL function and perhaps reduce the risk of atherosclerosis. This approach is not without caveats, however, as the regulatory nature of miRNAs that allow one to target an entire pathway may also lead to deleterious off-target effects. As such, future studies using tissue-specific knockout mice and antisense technologies are needed to fully assess the efficacy of inhibiting miRNA expression to control whole-body RCT and HDL function.

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C. Fernández-Hernando has patents on the use of miR-33 inhibitors and C. Fernández-Hernando and L. Goedeke have patents on the use of miR-27b and miR-148a inhibitors.

References


miRNAs are small noncoding RNAs that post-transcriptionally regulate gene expression. miRNAs have emerged as potential therapeutic targets to increase circulating high-density lipoprotein-cholesterol levels and enhancing reverse cholesterol transport. High-density lipoprotein particles serve as carriers of miRNAs and may be used as biomarkers of cardiovascular disease.
Figure 1