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# Epigenetic regulation of genes involved in the reverse cholesterol transport through interaction with miRNAs

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## TABLE OF CONTENTS

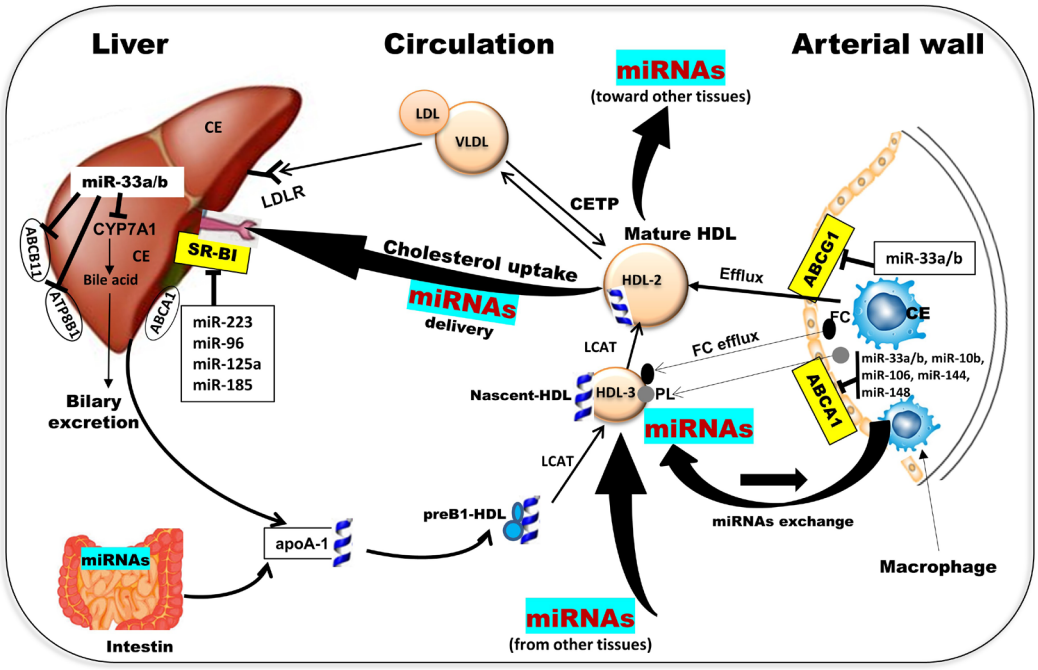


## 1. ABSTRACT

microRNAs (miRNAs) are a group of small non-coding RNA molecules known to regulate target genes at the post-transcriptional level. miRNAs are implicated in the regulation of multiple pathophysiological processes including dyslipidemia, a major risk factor for atherosclerosis. Emerging evidence suggests that miRNAs act as a novel class of epigenetic regulators of high-density lipoproteins cholesterol (HDL-C) from synthesis to clearance contributing remarkably to the pathogenesis of atherosclerosis. Accumulating studies have revealed that miRNAs such as miR-33, miR-27, miR-144, miR-758 and miR-20 are involved in the post-transcriptional control of *ABCA1, ABCG1* and *SCARB1* genes regulatory network of the reverse cholesterol transport (RCT). These miRNAs have been shown to be central players in the impairment of RCT pathway leading to the development of atherosclerosis. In this article, we present most recent understanding of involvement of relevant miRNAs in different steps of HDL metabolism and RCT pathway. We also discuss some of the actual limitations to the promise of these miRNAs and perspectives on their translation to clinical settings.

## 2. INTRODUCTION

Epidemiological studies have consistently identified low high-density lipoprotein cholesterol (HDL-C) as a strong and an independent predictor for coronary heart disease (CHD) (1). High-density lipoproteins (HDLs) exhibit a broad spectrum of anti-atherogenic properties including antioxidant, anti-inflammatory, nitric oxide–inducing mechanisms and antithrombotic effects (2, 3). The most plausible explanation for the atheroprotective role of HDLs has been that they act as an acceptor of free cholesterol removed from macrophage-derived foam cells present in the atherosclerotic plaque, returning it to the liver for excretion. This process was described earlier by Glomset in 1968 (4) and known as “reverse cholesterol




**Abbreviations: ABCA1**, ATP-binding cassette transporter A1; **apoA-I**, apolipoprotein A-I; **ABCB11**, ATP-binding cassette, subfamily B, member 11; **ABCG1**, ATP binding cassette, subfamily G, member 1; **ATP8B1**, amino-phospholipid transporter, class I, type 8B, member 1; **CE**, cholesteryl ester; **CETP**, cholesteryl ester transfer protein; **CYP7A1**, cholesterol 7a-hydroxylase 1; **HDL**, high-density lipoprotein cholesterol; **LCAT**, lecithin-cholesterol acyltransferase; **LDL**, low-density lipoprotein cholesterol; **LDLR**, LDL-receptor; **RCT**, reverse cholesterol transport; **PL**, phospholipids; **SR-BI**, scavenger receptor class B type I; **TG**, triglyceride; **VLDL**, very low-density lipoprotein cholesterol.

**Figure 1.** Schematic overview of RCT from peripheral tissues to the liver and regulatory role of miRNAs in HDL metabolism. Lipid-poor apoA-I interacts with ABCA1 to generate nascent HDL. The mature HDL particles can then serve as acceptors for cholesterol that is effluxed from cells in an ABCG1-dependent process. Next, HDL cholesteryl esters are next selectively taken up into the liver via SR-BI. Finally, excess of cholesterol is excreted from the liver into bile acid through ABCB11 and ATP8B1 transporters. miRNAs are shown to be involved in the regulation of different steps of RCT pathway. Figure shows representative miRNAs that have been shown to regulate post-transcriptionally target genes including *ABCA1, ABCG1* in the macrophages accumulated in atherosclerotic plaques and *SCARB1*, *CYP7A1, ABCB11* and *ATP8B1* in the liver. HDLs are also known to transport miRNAs from macrophages and other tissues to the liver and most likely to neighboring cells. Bold arrows indicate possible trafficking and exchange of miRNAs between tissues and cells. Abbreviations: ABCA1: ATP-binding cassette transporter A1, apoA-I: apoprotein A-I, ABCB11: ATP-binding cassette, subfamily B, member 11, ABCG1: ATP binding cassette, subfamily G, member 1, ATP8B1: amino-phospholipid transporter, class I, type 8B, CE: Cholestryl ester, CETP: cholesteryl ester transfer protein, CYP7A1: cholestrol 7a-hydroxylase 1, HDL: high-density lipoprotein cholesterol, LCAT: lecithin-cholesterol acyltransferase: LDL: low-density lipoprotein cholesterol, LDLR: LDL-receptor, RCT: reverse cholesterol transport, PL: phospholipids, SR-BI, scavenger receptor class B type I, TG: triglyceride, VLDL: very low-density lipoprotein cholesterol.

transport" (RCT) (reviewed in 5, 6) and summarized in Figure 1. During the first step of RCT, lipid-poor apolipoprotein A-I (ApoA-I) interacts with membrane lipid transporters such as ATP-binding cassette transporter A1 (ABCA1) to mediate cellular efflux of both cholesterol in the unesterified or free form and phospholipids (7) to generate nascent HDL particles (8). As a clear evidence for the role of ABCA1 in HDL formation, patients with Tangier disease (also known as familial alpha-lipoprotein deficiency); a condition characterized by a deficiency in plasma HDL-C and a severe reduction in cholesterol efflux, have mutations in *ABCA1* gene (9, 10). The mature HDL particles can then serve as acceptors for cholesterol that is effluxed from cells in an ABCG1-dependent process (8, 11). Furthermore, cholesterol in HDLs may be esterified by the enzymatic activity of lecithin-cholesterol acyltransferase (LCAT) to generate cholesteryl esters (CEs). It is noteworthy to mention that in humans, CEs can be transferred to apoB-containing lipoprotein particles, a process that is catalyzed enzymatically by the cholesteryl ester transfer protein (CETP) and ultimately transported to the liver. HDL cholesteryl esters are next selectively taken up into the liver via scavenger receptor class B type 1 (SR-BI) (12, 13). Within the liver, part of the cholesterol is converted into bile acid salts in a multistep process initiated by enzyme cholesterol 7a-hydroxylase (CYP7A1) (14). Finally, biliary lipids are secreted across the apical (canalicular) membrane of hepatocytes by three different transmembrane transporters: ABCB11, ABCG5/ABCG8, and ABCB4 (15) (Figure 1).

Despite the tremendous advance in biochemical and genetic characterization of RCT process and CVD biology, there remain significant

proportions of this disease's risk still not explained and effective therapies are limited. In fact, drugs raising HDL-C including niacin, fibrates and CETP inhibitors (torcetrapib, dalcetrapib, and anacetrapib), have failed to show clinical benefit especially in high-risk patients receiving intensive statin treatment (16, 17, 18). Moreover, some existing therapeutics such as statins are known to result in various side effects including muscle pain, stiffness and cramp (referred as myalgia) (19). Taken together, these studies underscore the intense interest in better understanding of regulatory mechanisms by which HDLs exert their atheroprotective effects, which may lead to the development of novel strategies to treat and prevent the progression of atherosclerosis.

At the transcriptional level, preclinical studies have shown that several nuclear receptors including peroxisome proliferator-activated receptors (PPARs), liver X-receptor (LXR), and farnesoid X receptor (FXR), influence lipid metabolism and genes involved in RCT pathway, therefore agonists have been proposed to raise HDL-C and reduce atherosclerosis (20). Unfortunately, despite the effectiveness of these nuclear receptors in preclinical studies, their translation to human clinical trials is still facing many challenges. For instance, administration of currently available LXR agonists has been shown to stimulate lipogenesis via sterol regulatory element binding protein-1c (SREBP1), leading to hepatic-specific side-effects like steatosis and hypertriglyceridemia (21, 22, 23). Thus, further research will be necessary to improve the efficacy and clinical safety of these transcription factor agonists as a drug for the treatment of CVD.

To pursue search for novel alternatives to target the residual risk of CVD, researchers have focused on investigating the regulation of RCT pathway at the post-transcriptional level. Emerging evidence indicates that; in addition to the more traditional regulatory schemes; RCT pathway is also under the control of epigenetic mechanisms. Epigenetics modifications are defined as heritable changes in gene expression that are not caused by DNA sequence alterations (24). They include changes in DNA methylation, histone modification and alteration in the expression of small non-coding RNA including microRNAs (miRNAs). In the past decades, miRNAs have received much attention due to their effects in a variety of disease states such as cancers and atherosclerotic cardiovascular diseases. Accumulating evidence is now connecting alteration in miRNAs expression with dysregulated lipid metabolism leading to atherosclerosis and its related disorders. In fact, available data support the role of miRNAs in directly targeting genes involved the regulation of different steps of HDL-C metabolism from synthesis to clearance (25). Additional experimental evidence indicates that miRNAs disrupt gene regulatory network of molecular processes associated to the initiation and progression of atherosclerosis (26). These findings have prompted scientists to reassess therapeutical trials that have failed previously to reduce CVD while attempting to raise HDL-C levels. Thus, the focus of this article is to provide an update on the emerging role of miRNAs in the control of RCT pathway and their contribution to the molecular mechanisms underlying atherosclerosis. Furthermore, the potential benefit and limitations of miRNA-based therapeutics to reach the clinical application in CVD are discussed.

## 3. miRNAs REGULATION OF REVERSE CHOLESTEROL TRANSPORT PATHWAY

### 3.1. miRNAs

miRNAs are endogenous small RNAs of approximately 18–24 nucleotides long that are transcribed from DNA but not translated into proteins (27). They were first discovered by Lee *et al.* (28) in 1993 and their biogenesis and regulation have been well described (29). Data on miRNAs has tremendously increased in the last decades due to significant progress in high-throughput technology and bioinformatic methods. Moreover, the computational prediction of miRNA target genes has greatly contributed to the understanding of miRNA-mediated gene regulation and their biological roles. As of 2017, over 2,600 different mature miRNAs have been identified in the human body, thereby demonstrating wide-ranging biological activity and therapeutic potential. The discovered miRNAs have been registered in different databases including mirBase (www.mirbase.org), miRDB (www.mirdb.org), miRWALK (www.mirwalk.uni-hd.de), and miRTarBase (mirtarbase.mbc.nctu.edu.tw) (30). In addition, there are two manually curated databases: the human microRNA-associated disease database (HMDD) available at http://cmbi.bjmu.edu.cn/hmdd and http://202.3.8.1.26.1.51/hmdd/tools/hmdd2.html (31, 32) and the miR2Disease database available at http://www.miR2Disease.org (33) for the study of miRNA-disease relationship.

miRNAs are important elements of the cell epigenetic machinery. They play a major role in fine-tuning the expression of target genes at the post-transcriptional level by binding to 3′-UTR of target mRNAs promoting their degradation and/or inhibiting their translation (34, 35). miRNAs are known to associate with the RNA-Induced Silencing Complex (RISC) and subsequently promote translational suppression and/or RNA degradation of their targets (27, 36). More interesting, miRNAs have been found to be stably transported through the blood stream in exosomes (37), apoptotic bodies (38), HDL particles (39), and in complexes with Ago2 (40). These circulating miRNAs have been demonstrated to regulate expression of target mRNAs following uptake

**Table 1.** miRNAs targeting main genes involved in reverse cholesterol transport pathway.

| miRNA species | Target Genes | Target Functions | References |
|---|---|---|---|
| miR-10b, miR-19, miR-27a/b miR-33a/b, miR-101, miR-106b, miR-128-2, miR-130, miR-144, miR-145, miR-148a, miR-301b, miR-302a, miR-613 | ABCA1 | HDL biogenesis, RCT, Cholesterol efflux | (46, 53, 78, 81-83, 116, 117) |
| miR-10b, miR-33a/b, miR-128, miR-378 | ABCG1 | Cholesterol efflux | (46, 59, 104) |
| miR-27a/b, miR-96, miR-125a, miR-185, miR-223, miR-455 | SRB1 | Cholesterol uptake | (39, 99, 100, 117) |
| miR-33a/b | CYP7A1<br>ABCB11<br>ATP8B1 | Bile acid synthesis and secretion | (64, 103) |
| miR-122 | CYP7A1 | Bile acid production<br>Absorption of cholesterol | (118) |

Abbreviations: **ABCA1**, ATP-binding cassette transporter A1; **ABCG1,** ATP-binding cassette transporter G1; **SR-BI**, scavenger receptor class B type I; **CYP7A1**, cholesterol 7α-hydroxylase; **ABCB11**, ATP-binding cassette subfamily B member 11 gene encoding bile salt export pump; **ATP8B1**, ATPase class I type 8b member 1; **RCT**, reverse cholesterol transport.

by recipient cells (41, 42) a process by which miRNAs may allow communication between different organs.

## 3.2. miRNAs and lipid metabolism

In the past few years, several studies have reported that miRNAs are critical regulators of lipid synthesis, fatty acid oxidation and lipoprotein formation and secretion (43). In this regard, several studies have showed that miRNAs including miR-33, miR-122, miR-378/378*, miR-125 and miR-30c are involved in lipoprotein metabolism (44-48). For instance, the liver-specific miR-122 has been shown to play a critical role in the regulation of cholesterol and triglyceride metabolism as demonstrated by its genetic deletion in mice and African green monkeys (49-51). Such a deletion has resulted in reduced: cholesterol synthesis, hepatic fatty acids and plasma cholesterol levels, and increased liver fatty acid β-oxidation (49-51). Additionally, several representative miRNAs with a significant impact on lipoprotein metabolism and metabolic diseases have been proposed as therapeutic targets for CVD (52, 53). Mechanistically, the dysregulation of miRNAs can disrupt several gene networks leading to metabolic disorders including metabolic syndrome, diabetes, obesity and atherosclerosis (25, 54, 55). With respect to atherosclerosis, it has increasingly becoming recognized that miRNAs play a prominent role in regulating biological pathways leading to the formation of atherosclerotic plaques (56, 57). Because of the strong inverse correlation between HDL and CHD and to better understand the atheroprotective role of HDL, we will focus mainly on relevant miRNAs involved in 1) the regulation of genes associated with HDL metabolism and 2) different steps of RCT from HDL biogenesis (ABCA1), cholesterol efflux (ABCA1 and ABCG1) and uptake of HDL-C (SR-BI) to bile acid secretion via ABCB11 and ATP8B1 transporters (Table 1).

## 3.3. miRNAs regulation of genes involved in reverse cholesterol transport

### 3.3.1. miRNAs-mediated *ABCA1* and *ABCG1* genes expression

#### 3.3.1.1. miR-33a/b

miR-33 family comprises miR-33a and miR-33b; (miR-33a/b), which are encoded in the introns of sterol-regulatory element-binding proteins (*SREBP*) 2 and *SREBP*1, respectively (45). In mice however, miR-33b is not expressed because of a deletion in part of the miR-33b encoding region. miR-33 is expressed in various cell types and tissues and considered as a key regulator of cholesterol homeostasis by controlling cholesterol efflux and HDL function in concert with their host genes, the *SREBP* transcription factors (45, 58). miR-33a is transcribed concomitantly with its host gene *SREBP2* under low sterol conditions resulting in an increase in cholesterol biosynthesis and uptake as well as a reduction in cholesterol efflux and excretion (25, 44, 46, 59, 60). On the other hand, miR-33b is co-transcribed with *SREBP1* under conditions such as insulin and liver X receptor (LXR) activation leading to an increase in intracellular cholesterol levels (25, 59, 60).

Numerous studies have reported that miR-33a and miR-33b downregulate *ABCA1* and *ABCG1* genes expression and cholesterol efflux in human and mouse liver cells and macrophages (44, 45, 52, 61-63) (Table 1). Silencing of miR-33 in mice; using

modified anti-sense oligonucleotides or viral delivery of hairpin inhibitors; has resulted in increased hepatic *ABCA1* and *ABCG1*expression associated with parallel increase in plasma HDL-C levels (44, 45, 61). Similarly, experimental studies have shown that miR-33 antagomirs enhance RCT (61, 64). Additional evidence to support the role of miR-33 in the regulation of *ABCA1* gene expression and HDL biogenesis *in vivo* was brought by a study using miR-33$^{-/-}$ mice (52). In this work, authors showed that liver ABCA1 protein and serum HDL-C levels were higher in miR-33-deficient mice than in control mice (52). Since mice are lacking miR-33b, studies have been extended to non-human primates' model to confirm the potential of anti-miR-33 therapy. In this regard, studies have shown that systemic delivery of anti-miRNA molecules that target both miR-33a and miR-33b results in increased hepatic expression of *ABCA1* and plasma HDL levels in African green monkeys (62, 65). In line with this, a recent study reported that healthy individuals with high HDL-C levels overexpress ABCA1 and ABCG1 and show a decrease of miR-33a in their peripheral blood mononuclear cells (66).

The aforementioned studies have prompted scientists to assess the therapeutic potential of miR-33 in the setting of atherosclerosis. Horie *et al.* (67) have demonstrated that inactivation of miR-33 in *ApoE*$^{-/-}$ mice results in an increase in ABCA1 expression, HDL-C levels, cholesterol efflux and prevention of the progression of atherosclerosis. Another study reported that treatment of *LDLR*$^{-/-}$ mice fed previously a western diet for 14 weeks with 2′-fluoro/methoxylethyl (2′F/MOE) anti-miR-33 oligonucleotides increased plasma HDL-C levels and enhanced the regression of atherosclerosis (61). Further studies have indicated that anti-miR-33 oligonucleotides reduce significantly atherogenesis in *LDLR*$^{-/-}$ mice fed a western diet for 12 weeks and improve HDL functionality (68, 69). In contrast, other investigators reported conflicting data showing that prolonged anti-miR-33 therapy failed to raise plasma HDL-C and did not prevent the progression of atherosclerosis (70). In addition, studies have suggested that anti-miR-33 therapies may cause unwanted effects, such as hypertriglyceridemia, obesity, hepatosteatosis, and insulin resistance (70-73), which imply that miR-33 members may have other functions than those identified previously. Overall, results from most of these studies suggest that inhibiting miR-33 may held great promise for the prevention and treatment of cardiovascular disease. Thus, understanding the exact mechanism by which miR-33 regulate ABCA1 and HDL biology is central to meet this promise.

#### 3.3.1.2. miR-27a/b

In addition to miR-33, the miR-27 family members; miR-27a and miR-27b, (miR-27a/b), have also been shown to play a role in the physiological processes of atherosclerosis and CVD (74, 75). miR-27a is an intergenic miRNA, while miR-27b is an intronic miRNA located within the fourteenth intron of the *C9orf3* host gene on human chromosome 9. miR-27 has been demonstrated to play a role in several cellular processes that are associated with atherosclerosis, including lipid metabolism, inflammation, oxidative stress, insulin resistance and type 2 diabetes (76, 77). Additionally, bioinformatics-based in silico analyses of putative miRNA binding sites have suggested that miR-27a/b have binding sites in the 3'-UTR of several lipid metabolism associated-genes such as *SREBP1, SREBP2, PPARα, and PPARγ, HMGCR, apoB100, apoE3, C/EBPa, GPAM* and *ANGPTL3* (74, 78, 79).

The role of miR-27 in the regulation of HDL biogenesis and cholesterol efflux has been investigated lately. Several studies have demonstrated that miR-27 significantly reduces ABCA1 activity (79, 80). Furthermore, Zhang *et al.* (81) have reported that miR-27 regulates cellular cholesterol efflux, influx and esterification/hydrolysis by repressing the expression of LPL, CD36, and ACAT1 in THP-1 macrophages and apoA-I in HepG2 cells. Subsequently, authors have demonstrated that miR-27a/b members target the 3'-UTR of *ABCA1* to regulate its mRNA and protein levels in several types of cells including THP-1, RAW264.7 and HepG2. In agreement with these *in vitro* reports, a subsequent study by another group has confirmed that miR-27a/b regulate *ABCA1* expression and cholesterol efflux to apoA-I in hepatocytes and macrophages (82). Moreover, recent studies have indicated the functional role of miR-27b in human liver is to regulate lipid metabolism via controlling the expression of several key lipid metabolism-related genes, including *ABCA1* and *LDLR*, (82, 83). However, these studies have also shown that miR-27b do not influence HDL-C levels in mice fed with chow high fat diet (82, 83). More interesting, miR-27 implication in atherosclerosis has also been investigated in several studies. In this respect, Boon *et al.* (84) have shown that miR-27 can have a proatherogenic role in endothelial cells activated by laminar shear stress. In contrary, a recent study revealed that promoting miR-27 function reduced the development of atherosclerosis in apoE$^{-/-}$ mice by direct targeting of LPL expression and attenuated lipid accumulation and secretion of the pro-inflammatory cytokines, while the inhibition of miR-27 had the opposite effects (75). LPL is known to have either a proatherogenic or an anti-atherogenic role and that may be upon its tissue localization. That could also be true for miRNAs which raises a very good question regarding the origin, localization and regulation of such molecules. With respect to miR-27, additional mechanistic studies are required to clarify these discrepancies to determine the context in which miR-27 could act as an anti-atherogenic or proatherogenic regulator. This might be relevant from

a clinical perspective since, at this time, major clinical trials have been unable to always find a correlation between increased HDL-C levels and decreased risk for CVD (85).

#### 3.3.1.3. miR-144

Of the other miRNAs found to be involved in the regulation of the RCT pathways; miR-144 has previously been shown to target ABCA1 and regulate HDL-cholesterol efflux. By using a luciferase reporter assay, Kang *et al.* (80) have demonstrated that miR-144 significantly reduces ABCA1 3'-UTR activity in U343 cell lines. *In vitro*, overexpression of miR-144 has been reported to decrease both cellular ABCA1 protein and cholesterol efflux to lipid-poor apoA-I protein (86). Similarly, two independent studies have revealed that silencing miR-144 in mice increases hepatic ABCA1 protein and HDL-C levels (86, 87). Subsequent studies by de Aguiar Vallim *et al.* (86) have identified a mechanism linking the activation of the nuclear receptor FXR *in vivo* to increased hepatic levels of miR-144, which in turn reduces hepatic ABCA1 and plasma HDL levels. Subsequently, it has been shown that administration of miR-144-3p mimics to apoE$^{-/-}$ mice on proatherogenic diet decreases ABCA1 expression and plasma HDL levels and effectively accelerates atheromatous plaque by impairing RCT and promoting pro-inflammatory cytokine production (88). This seems to be interesting as the mechanism by which HDL protects against atherosclerosis was questioned lately favoring the anti-inflammatory function of HDL (85). In this sense, the anti-inflammatory activity of HDL has also been described as an emerging biomarker, being superior to HDL-C levels, in assessing CVD risk (89). Together, these studies present evidence that ABCA1 is post-transcriptionally regulated by miR-144 *in vitro* and *in vivo*.

#### 3.3.1.4. miR-758

In vitro and in vivo data have suggested that miR-758 plays a key role in the regulation of atherosclerosis process. In this context, the expression level of *ABCA1* has been shown to be post-transcriptionally regulated by miR-758. In mouse and human cells *in vitro*, miR-758-3p repressed the expression of *ABCA1* gene through direct targeting of its 3'-UTR and conversely, anti-miR-758-3p inhibitors increased *ABCA1* expression (53). Subsequent experiments revealed that miR-758-3p reduced cholesterol efflux to apoA-I, whereas anti-miR-758-3p increased it (53). Furthermore, the function of miR-758-3p in atherosclerosis progression has been identified (90). In this regard, a recent study has reported that concomitant increase of miR-758 and miR-33b can modulate ABCAI and ABCG1 expression levels in in atherosclerotic plaques from hypercholesterolemic patients (91). Lately, another study has revealed that miR-758-5p reduced total cholesterol accumulation in THP-1 macrophage derived foam cells through markedly reducing cholesterol uptake without any significant effect on cholesterol efflux (92); most probably through targeting *CD36* gene. Ramirez *et al.* (53) have found that miR-758 levels were reduced in peritoneal macrophages from hypercholesterolemic LDLr-/- mice that were fed a high-fat diet. Other findings revealed low levels of circulating miR-758 along with other miRNAs could be responsible for elevated triglycerides and LDL-C levels, and low level of HDL-C in obese children (93). Based on these studies, one can assume that modulating miR-758 expression could represent a novel innovative therapeutic strategy to treat atherosclerosis via regulating the cholesterol efflux.

#### 3.3.1.5. miR-20a/b

The potential contribution of miR-20a/b members in post-transcriptional regulation of *ABCA1* gene, cholesterol efflux and atherosclerosis has been investigated lately. One study has looked at the role of miR-20a/b in the regulation of genes involved in the RCT pathway in THP-1 and RAW264.7. macrophage-derived foam cells (94). Authors found that miR-20a/b suppressed ABCA1 expression, which, in turn, decreased cholesterol efflux and increased cholesterol content. In contrary, miR-20a/b inhibitors increased ABCA1 expression and cholesterol efflux, decreased cholesterol content, and inhibited foam-cell formation. These results were corroborated by *in vivo* data using miR-20a/b-treated ApoE$^{-/-}$ mice. Authors showed decreased ABCA1 expression in the liver and reduction of RCT in mice model (94). Subsequently, miR-20a/b were shown to regulate the formation of nascent-HDL and promote atherosclerotic development, whereas miR-20a/b knockdown attenuated atherosclerotic formation. Together, these findings suggest that miR-20 could be a potential target for alleviating the development of atherosclerosis. However, the mechanisms by which miR-20 family members contribute to the regulation of ABCA1 and therefore to the alteration of RCT are still poorly known and represent an important developing field of research.

### 3.3.2. miRNAs and SR-BI expression

Scavenger receptor class B type 1 (SR-BI) is known to mediate selective uptake of HDL-C in the liver for bile acid synthesis and cholesterol excretion (12, 95). In fact, lack of SR-BI in mice impairs RCT and causes massive atherosclerosis (96). These findings suggest that SR-BI could be a potential target for blocking the development of atherosclerosis.

SR-BI is encoded by *SCARB1* gene. The regulation of *SCARB1* transcription and that of SR-BI expression levels are quite complex. At the level of

gene, *SCARB1* expression is controlled by different transcription factors such as PPAR, SREBP, LXR and liver receptor homologue (LRH-1) (97). *SCARB1* has also been shown to be controlled at the post-transcriptional level by alternative splicing and its physical interaction with PDX domain containing 1 (PDZK1) (95, 98). miRNAs, other emerging post-transcriptional regulators, have been shown to control hepatic SR-BI and thereby affect the uptake of HDL-C by the liver (99, 100). In this regard, several studies have provided evidence that miRNAs regulate post-transcriptionally *SCARB1*. Silencing of Drosha and Dicer resulted in an increase in *SCARB1*expression in HepG2 cells (99). *In vivo* treatment of rat adrenals with adrenocorticotropic hormone (ACTH) decreased the expression of miRNA-125a, miR-125b, and miR-455 and reciprocally increased SR-BI expression (99). Subsequently, experiments with luciferase constructs containing the 3′ UTR of *SCARB1* provided evidence that steroidogenic *SCARB1*is a direct target of miRNA-125a and miR-455. To confirm these observations, authors also used the transfection of Leydig tumor and liver Hepa1-6 cells with miR-125a and miR-455 precursors to demonstrate that miR-125a and miR-455 can significantly reduce SR-BI expression and HDL-C uptake in these cells (99). Similarly, other miRNAs including miR-185, miR-96, and miR-223 have been shown to decrease SR-BI expression along with a repression of HDL-C uptake in THP-1 cells and in the liver of ApoE-null mice on high fat diet (100), while antagomirs of these miRNAs revealed the opposite effects. The list of miRNAs affecting *SCARB1* gene expression is continuing to grow. Lately, Mysore *et al.* (101) reported that miR-192-3p altered the expression profile of many lipogenic genes including *SCARB1* in adipocytes. Vickers *et al.* (39) demonstrated that miR-223 regulated SR-BI in the ApoE-null mice fed high fat diet for 16 weeks. The decrease in SR-B1 and increase in miR-223 expression were totally reversed by intravenous injection of human recombinant HDL (150 µg) collected from patients at the NIH Clinical Center using cholate dialysis method (39). Finally, these finding suggest that promoting the expression of SR-BI through modulation of miRNAs may have a protective effect against atherosclerosis.

#### 3.3.3. miRNAs-mediated *CYP7A1, ABCB11, ATP8B1* genes expression

miRNAs have been also reported to be involved in the final step of RCT, by regulating genes involved in bile acid synthesis and secretion. In the liver, a portion of cholesterol is converted to bile acid salts by cholesterol 7a-hydroxilase (CYP7A1) (14). Next, biliary lipids are secreted from the liver to cells via three different transporters; ATP-binding cassette, subfamily B, member 11 (ABCB11), ATP-binding cassette, subfamily G, member 5/8 (ABCG5/8), and ATP Binding Cassette Subfamily B Member 4 (ABCB4) (102). Previously, Allen *et al.* (64) have reported that miR-33 regulates the expression of many bile acid transporters, including ABCB11 and ATP8B1 that control bile secretion. These authors have confirmed that systemic inhibition of miR-33 causes an increase in sterols in bile and enhanced RCT *in vivo*. Additional studies have shown that miR-33 can target *CYP7A1* gene to coordinate the regulation of hepatic bile acid synthesis and to maintain hepatic lipid homeostasis (103). In addition to its role in lipid metabolism, miR-33 has been shown to regulate other cellular processes such as innate immune system and inflammation (104). These observations suggest that miR-33 is a multifunctional miRNA and its role, particularly in inflammation, needs to be deeply addressed since this may be relevant to lipid metabolism and atherosclerosis. This could also be true for the most abundant HDL-derived miRNA, miR-223, that can be transferred to endothelial cells and inhibit intracellular adhesion molecule 1 (ICAM-1), thereby reducing monocyte adhesion and inflammation (105).

## 4. OPEN QUESTIONS

Although the existing *in vitro* and *in vivo* data provide convincing evidence that specific miRNAs control HDL metabolism and RCT pathway, several questions remain to be fully addressed before moving toward the development of miRNA-based therapeutics for CVD.

1. A great deal of work has suggested that HDL particles capacity to promote cholesterol efflux from cells vary upon their composition in lipids and proteins as well as certain pathological conditions (106, 107). To gain new insights into the complexity of HDL composition and function, research has lately focused on numerous miRNAs shown to regulate gene networks that control HDL biogenesis and RCT process. Even though most of these studies have suggested that HDL-bound miRNAs directly alter their targets gene expression (39), many open questions are left unanswered regarding miRNAs/HDL complex formation and function. For instance, do miRNAs that regulate HDLs biogenesis control also the shape, size, composition and functional properties of these particles? How and at what stage during HDL synthesis miRNAs are loaded onto these particles? Is miRNAs/HDL complex stability influenced by types of diet, HDL modifying drugs or risk factors such as oxidative stress and metabolic disorders?

2. The origin and the nature of miRNAs that contribute to the control of genes involved in the removal and transport of cholesterol between different tissues and cells are not yet well described. Many studies have clearly shown that carriers including vesicular, nonvesicular and lipoprotein particles might exchange and transfer miRNAs to regulate gene expression

in target cells and alter phenotypes. In addition, dysregulated miRNAs in different tissues including circulating miRNAs, may also have the capability of regulating gene expression either directly or indirectly under various conditions (108, 109). In this context, a recent study by Thomou *et al.* (110) clearly showed that adipose-derived circulating miRNAs can regulate gene expression in distal tissues such as liver, offering a good illustration of how fat organ may constitute an important source of *circulating* exosomal *miRNAs*, which can impact gene expression in *other peripheral tissues*. Nonetheless, an outstanding question remains: What is the origin of HDL-associated miRNAs?

3. A more pertinent question relates to the mechanisms of miRNAs-mediated repression of genes involved in the RCT. At present, it is still unclear whether these miRNAs act in response to the same metabolic cues and atherogenic signals such as inflammation, cellular stress, overnutrition and disease state or if each specific miRNA has its own stimulus-response connection. Answering this question would certainly help designing a better strategy to target miRNAs affecting RCT gene network and therefore raising HDL-C.

4. With respect to HDL and metabolic diseases, is there cross-talk between miRNAs controlling HDL metabolism and those involved in various metabolic disorders including metabolic syndrome, obesity, diabetes and hypertension? This is certainly a very complex question that will probably require implementation of systems biology approaches combined with functional studies to validate current data.

5. The diversity of post-transcriptional regulators including DNA and RNA methylation, RNA-binding proteins (RBPs) and their interactions have uncovered a new level of complexity of gene expression regulation in many physiological process and diseases. DNA methylation has been shown to be associated with atherosclerosis (111), coronary heart disease (112) and cancer (113). So, these regulators lead to asking the following questions; what is the impact of miRNAs/methylated-RNA/RBPs regulatory axis on HDL metabolism and RCT regulatory genes expression? How miRNAs/RBPs interaction (cooperation/competition) orchestrates their target genes expression program?

6. Another type of RNA has recently emerged and attracted much attention; circular RNAs (circRNAs), which are a novel class of long noncoding RNAs. Evidences are arising that circRNAs might regulate miRNA function as microRNA sponges and play a significant role in transcriptional control (114). In addition, circRNAs have been shown to play a role in atherosclerosis (115). Thus, the crucial question, however, is: what is the functional relevance of circRNAs in human health and disease particularly in lipid homeostasis, HDL metabolism/RCT and atherosclerosis? Finally, answering all these questions may lay a strong foundation for implementation of new relevant research with respect to the functionality of HDLs in the removal of cholesterol and therefore discovery of novel useful biomarkers for the risk of CVD.

## 5. CONCLUSIONS AND FUTURE PERSPECTIVES

Although our current knowledge of miRNAs and their relationship with genes involved in the RCT pathway is still in its infancy, we believe that there are sufficient evidences that these molecules contribute to the control of HDL biogenesis and function. Consequently, miRNAs may have the ability to regulate cholesterol removal and thus the development of atherosclerosis. This suggests that understanding exact mechanism(s) for miRNAs function can help develop novel strategies to promote RCT and alleviate atherosclerosis and related diseases. However, there is still a long way to go before we can benefit from the use of miRNAs as alternative therapeutics due to significant number of limitations including: (i) The exhaustive repertoire of miRNAs (MiRNome) involved in RCT pathway is still undefined. Most of the known miRNAs associated with this process have a predominantly negative effect on target genes, thus, a better search of novel miRNAs that specifically promote RCT gene network will be necessary. (ii) So far, few strategies have been employed to test the combinatorial effect of multiple miRNAs. Studying miRNA individually might be an oversimplification of the RNA biology, as it is known that a single miRNA may interact with several targets, and conversely a target mRNA is subject to regulation by several miRNAs. Therefore, modeling combinatorial (e.g. ComiR) of miRNAs regulation might be more accurate and efficient in predicting outcome than just focusing on study of a miRNA in isolation.

In sum, despite the enthusiasm raised by the association between HDL and miRNAs and the increasing interest in studying the epigenetic fine-tuning mechanisms of RCT gene network, data are still lacking on the exact role of these miRNAs *in vivo*. Well focused study design may certainly increase our knowledge in this promising area and will provide novel therapeutic avenues to better combat the burden of cardiovascular and metabolic diseases.

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**Abbreviations:** **ABCA1**, ATP-binding cassette transporter A1; **apoA-I**, apolipoprotein A-I; **ABCB11**, ATP-binding cassette, subfamily B, member 11; **ABCG1**, ATP binding cassette, subfamily G, member 1; **ATP8B1**, amino-phospholipid transporter, class I, type 8B, member 1; **CE**, cholesteryl ester; **CETP**, cholesteryl ester transfer protein; **CYP7A1**, cholesterol 7a-hydroxylase 1; **HD**L, high-density lipoprotein cholesterol; **LCAT**, lecithin-cholesterol acyltransferase; **LDL**, low-density lipoprotein cholesterol; **LDLR**, LDL-receptor; **RCT**, reverse cholesterol transport; **PL**, phospholipids; **SR-BI**, scavenger receptor class B type I; **TG**, triglyceride; **VLDL**, very low-density lipoprotein cholesterol.

**Key Words:** miRNA; ABCA1, HDL; SR-BI, Reverse cholesterol transport, Review

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