MicroRNA regulation of mitochondrial and ER stress signaling pathways: implications for lipoprotein metabolism in metabolic syndrome

Patricia Christian and Qiaozhu Su
Department of Nutrition and Health Sciences, University of Nebraska-Lincoln, Lincoln, Nebraska

Submitted 23 April 2014; accepted in final form 31 August 2014

Christian P, Su Q. MicroRNA regulation of mitochondrial and ER stress signaling pathways: implications for lipoprotein metabolism in metabolic syndrome. Am J Physiol Endocrinol Metab 307: E729–E737, 2014. First published September 2, 2014; doi:10.1152/ajpendo.00194.2014.—The development of metabolic syndrome is closely associated with the deregulation of lipid metabolism. Emerging evidence has demonstrated that microRNAs (miRNAs) are intensively engaged in lipid and lipoprotein metabolism by regulating genes involved in control of intracellular lipid synthesis, mitochondrial fatty acid oxidation, and lipoprotein assembly. Mitochondrial dysfunction induced by altered miRNA expression has been proposed to be a contributing factor in the onset of metabolic diseases, while at the same time, aberrant expression of certain miRNAs is associated with the induction of endoplasmic reticulum (ER) stress induced by nutrient-surplus. These studies position miRNAs as a link between oxidative stress and ER stress, two cellular stress pathways that are deregulated in metabolic disease and are associated with very-low-density lipoprotein (VLDL) overproduction. Dyslipoproteinemia frequently accompanied with metabolic syndrome is initiated largely by the overproduction of VLDL and altered biogenesis of high-density lipoprotein (HDL). In this review, we highlight recent findings on the regulatory impact of miRNAs on the metabolic homeostasis of mitochondria and ER as well as their contribution to the aberrant biogenesis of both VLDL and HDL in the context of metabolic disorders, in an attempt to gain further insights into the molecular mechanisms of dyslipidemia in the metabolic syndrome.

microRNAs; fatty acid metabolism; ER stress; inflammation; lipoprotein metabolism

In 2011, the International Diabetes Federation estimated that 366 million people suffered from diabetes worldwide (97), with as much as 95% of cases attributed to type 2 diabetes (44). Several pathological conditions usually underlie type 2 diabetes, which together are known as the metabolic syndrome and include obesity, hypertension, glucose intolerance, insulin resistance, and dyslipidemia (62). Dyslipidemia is characterized by increased plasma triglyceride (TG) and free fatty acids (FAs), attributed to increased production of very-low-density lipoprotein (VLDL), as well as increased small dense low-density lipoprotein (LDL) and decreased high-density lipoprotein (HDL) (4). This lipid profile gives rise to an increased risk of cardiovascular disease (45), which is currently the leading cause of death in North America (68).

Metabolic Syndrome and Lipoprotein Deregulation

The link between insulin resistance and increased VLDL secretion has been thought to be derived from increased delivery of FAs to the liver secondarily to increased lipolysis within adipose tissue, as well as increased hepatic lipogenesis, increased levels and activity of microsomal triglyceride transfer protein (MTP), and loss of insulin’s ability to direct apolipoprotein B (apoB) toward degradation (2, 3, 67). Assembly and secretion of VLDL is a complicated process that takes place in the hepatic endoplasmic reticulum (ER) and is regulated by multiple factors. Co- and posttranslational lipidation of apoB, a key structural protein of VLDL, mediated by MTP is a critical step in VLDL assembly (17, 38, 48). In fact, mutations in the MTP gene result in a condition known as abetalipoproteinemia, characterized by an absence of apoB-containing lipoproteins in the plasma (81, 96). Intracellular abundance of apoB protein is also regulated by the availability of lipid substrates, including TG, cholesterol, and other lipid species. High levels of intracellular lipid stabilize apoB and stimulate the assembly and secretion of VLDL, whereas, when intracellular lipids are decreased, apoB is subjected to degradation through proteasomal and nonproteasomal pathways, reducing VLDL secretion (79). Thus, maintaining proper MTP activity and balancing the availability of lipids in hepatocytes are critical approaches in controlling VLDL secretion. Ample studies have reported that conditions that compromise MTP activity (38) and/or mitochondrial FA β-oxidation cause accumulation of lipid droplets in hepatocytes and muscle cells (66). Therefore, direct or indirect perturbations of mitochondrial function and rates of FA oxidation will increase the lipid availability within hepatocytes and contribute to VLDL overproduction.
A number of microRNAs (miRNAs) have been implicated in controlling cellular lipid and lipoprotein metabolism through cross-talk with metabolic signaling within mitochondria and the ER. In an attempt to provide further insights into the molecular mechanism of the development of hyperlipoproteinemia observed in metabolic syndrome, we review here how aberrant expression of miRNAs disturbs mitochondrial and ER metabolic homeostasis and the impacts of miRNAs on lipid and lipoprotein metabolism.

miRNA Structure and Function

miRNAs are a class of small noncoding RNAs, ~22 nucleotides in length. Although their discovery is relatively recent, they are now regarded as key participants in the regulation of a wide array of cellular processes, including cell proliferation, differentiation, apoptosis, and lipid metabolism. miRNAs have been identified in both plants and animals and appear to be conserved among closely related species. Furthermore, in humans and several other species, miRNA genes account for about 1% of the genome (6, 89). miRNA regulation depends on complementarity between the miRNA and the mRNA of its target gene, with the complementary sites usually found within the 3' UTR of the mRNA for animal genes. Notably, a target gene often contains complementary sites for several different miRNAs; at the same time, a single miRNA can regulate several different genes. Overall, it has been suggested that as much as 60% of all human genes are controlled by miRNAs (25). About one-quarter of human miRNAs are found within the introns of other protein-coding genes, allowing for coordinated transcription of the miRNA and the mRNA. This also lends to the conservation of miRNAs that are found within genes that are conserved among animal species. Many miRNAs display both temporal and tissue-specific expression, but miRNAs tend to be expressed at levels much higher than those typically seen for mRNAs (6).

Primary miRNA transcripts, referred to as pri-miRNAs, are first generated in the nucleus as long strands and then cleaved within the nucleus to release an ~70-nucleotide stem-loop intermediate, referred to as the pre-miRNA (10, 53). The pre-miRNA is exported into the cytoplasm where it is recognized and processed by Dicer, an RNase III endonuclease, to remove the looped portion (52, 60). The resulting small miRNA duplex is unwound, and one strand is incorporated into the RNA-induced silencing complex (RISC) (6, 60, 80). In most cases, one particular strand of the duplex is predominantly incorporated into the RISC and is known as miRNA; the opposing strand is referred to as miRNA* (43, 47, 80). However, several lines of evidence now show that miRNA*s are also loaded onto RISC and that strand selection is tissue dependent (47, 58). Given the fact that both strands can give rise to a functional miRNA, the 5' and 3' arms of the stem-loop precursor are also designated as 5p and 3p, respectively (29). After miRNA incorporation into RISC, the target mRNA transcripts are recognized due to their complementarity to the loaded miRNA and are cleaved by the endonuclease component of RISC. The miRNA remains incorporated in the RISC to direct the cleavage of additional mRNA transcripts, thereby blocking expression of the target gene (28). miRNAs may also downregulate gene expression through translational repression either by delaying or stopping ribosomal progression or by inducing degradation of the protein. Although the mechanisms of these proposed models have yet to be fully elucidated, including identification of concrete examples where this may be occurring, it is believed that RISC is still involved and that the process requires some degree of complementarity between the miRNA and the target mRNA (6, 24). Another proposed mechanism for the action of miRNAs predicts that mRNA targets may be sequestered into processing bodies (P bodies) within the cytoplasm, where they are not accessible to the translational machinery and may undergo degradation. This model is supported by studies that have found miRNAs, mRNA targets, and components of RISC associated with P bodies (24, 69). In addition, there have been several examples where miRNAs can positively regulate gene transcription (20, 37, 59, 70, 94). This process, referred to as RNA activation, requires complementarity between small double-stranded RNAs and noncoding sequences within the promoters of target genes and involves epigenetic mechanisms (39, 55, 56).

miRNAs in Mitochondrial Energy Metabolism

The majority of miRNAs are nuclear derived and mature and function in the cytosol. However, recent studies have unveiled that cytosolic miRNAs are capable of relocating into mitochondria to affect mitochondrial metabolic processes. Cytosolic miRNAs have been found relocated in the mitochondria of heart and liver cells and myotubes (5, 8, 16). In addition, mitochondrial genome-derived miRNAs have also been reported (5). Using a miRBase search, Bian et al. identified 33 pre-miRNA and 25 miRNA candidates within the human mitochondrial genome that were predicted to target 169 potential sites within the mitochondrial genome (5). Interestingly, the miRNAs found in the mitochondria of liver cells have been identified as a unique population from those found in the rest of the liver tissue (8). Accumulating evidence has further demonstrated that these miRNAs are involved in regulation of mitochondrial gene expression. For example, Das et al. (16) reported that miR-181c appears to translocate into the mitochondria and suppress the expression of cytochrome c oxidase subunit I (mt-COX1), one of three mitochondrial DNA encoded subunits of respiratory complex IV. In primary cultures of neonatal rat ventricular myocytes overexpressing miR-181c, mt-COX1 protein expression was decreased relative to control cells, suggesting that cytosolic miR-181c is able to regulate mt-COX1 translation (16) and affect subsequent mitochondrial energy metabolism. Another study reported that liver mitochondrial miRNA expression is altered in STZ-induced diabetic mice (8). This study showed that expression of miR-494, miR-202–5p, miR-134, and miR-155 were increased in the hepatic mitochondria of STZ mice, whereas miR-122 was decreased. Alteration of mitochondrial miRNA expression might exert impacts on mitochondrial energy metabolism in the diabetic state and indirectly affect lipid metabolism. A recent study conducted by Carrer et al. has provided further insights on the effect of miRNAs on mitochondrial lipid metabolism by investigating the impact of two miRNAs, miR-378 and miR-378*, derived from an intron of the PPARGC1b gene (12, 21).
miR-378 and -378* were coexpressed with PGC-1β and proposed to counter its biological activity. Specifically, the authors found that depletion of miR-378/378* protected mice from high-fat diet (HFD)-induced obesity. miR-378/378* knockout (KO) prevented hepatic lipid accumulation in mice and reduced plasma triglyceride (TG) levels by increasing energy expenditure. Through further investigation of the underlying molecular mechanism responsible for this phenotype, the authors found that HFD induced expression of miR-378/378* in the liver of WT mice and that miR-378/378* targeted a conserved seed region in the 3'-UTR of hepatic carnitine O-acetyltransferase (CRAT), a mitochondrial enzyme involved in directing the products of FA oxidation into the TCA cycle (12, 40), and reduced its mRNA level. As such, mitochondria from the livers of the KO mice had significantly increased oxidative capacity and FA β-oxidation rates compared with mitochondria isolated from the livers of WT mice (12). This study provides evidence that lipid overload (e.g., HFD) drives the upregulation and relocation of specific miRNAs from the cytosol into mitochondria to disturb mitochondrial energy metabolism. Not only could this be a mechanism for mitochondrial dysfunction in metabolic disease states, but consequently could also be a contributing factor for VLDL overproduction due to redirection of lipids away from mitochondrial FA oxidation toward de novo lipogenesis, thus increasing intracellular lipid availability for VLDL assembly (64, 84). It is therefore very likely that inhibiting the expression and activity of certain miRNAs, such as miR-378 and miR-378*, will be able to stimulate mitochondrial oxidation of FAs and decrease VLDL secretion.

miRNAs in Metabolic Inflammatory Stress and ER Stress

The development of metabolic syndrome is usually accompanied by the activation of metaflammation, a low-grade, chronic inflammation orchestrated by metabolic cells. Metaflammation is triggered by excess nutrients or other metabolites, which results in increased secretion of inflammatory cytokines such as tumor necrosis factor-α (TNFα) and IL-6, and activation of inflammatory pathways, including c-Jun NH2-terminal kinases (JNK), IkB kinase (IKK), and double-stranded RNA-activated protein kinase (PKR) signaling (34). Inflammatory stress induced in metabolic syndrome is involved in the dysregulation of VLDL metabolism. In a TNFα-induced insulin resistance hamster model, increased secretion of VLDL-apoB was directly associated with the dose and time duration of TNFα perfusion (71). In humans, plasma TNFα concentrations were positively correlated with plasma VLDL levels at the fasting and postprandial stages, and with a number of risk factors for coronary artery disease (82). The positive correlation between inflammatory factors and VLDL overproduction suggests that inhibiting inflammation may improve dyslipidemia in subjects with metabolic disease. Emerging evidence has shown that inflammatory responses are regulated by certain miRNAs, such as miR-223, miR-15a, and miR-16 (41, 57). In the adipose tissues of miR-223 KO mice, activation of inflammatory pathways was significantly increased relative to WT mice when both groups were fed a HFD (98). This was coupled with increased macrophage infiltration of the adipose tissues in the KO mice. WT mice that received bone marrow cells from miR-223 KO mice and were fed a HFD for 8 wk developed significant insulin resistance compared with those that received bone marrow cells from WT mice. A mechanistic study revealed that miR-223 targets the mRNA of Pknox1, a protein involved in the regulation of macrophage polarization, and reduces Pknox1 expression (98). Moreover, miR-223 was found to directly inhibit NLRP3 inflammasome activity (7, 30). Thus, increased expression of miR-223 upon HFD may be a feedback response of the host immune system to ameliorate metabolic inflammation induced by HFD. This evidence indicates that manipulating the abundance of certain miRNAs could be a potential strategy for combating nutrient surplus-induced inflammation.

Induction of ER stress and the unfolded protein response (UPR) is an essential component of the major inflammatory and stress signaling networks (34), which is also associated with the dysregulation of lipid and lipoprotein metabolism in metabolic syndrome (67). ER stress triggers the activation of three ER transmembrane sensors, IRE1α, β, and PERK, and ATF6, whose functions are to facilitate adaptation of the cells to the stress (31). Biosynthesis of apoB is modulated by both insulin and ER stress (67, 72). Modest ER stress induced by free FAs increases apoB secretion, whereas greater lipid loading results in severe ER stress and inhibits apoB production in McA-RH777 cells (67). Furthermore, constant overproduction of apoB in hepatocytes stimulated by an atherogenic diet has been reported to directly provoke hepatic ER stress and may contribute to the onset of hepatic insulin resistance (85). Of note, emerging evidence has demonstrated that expression of many miRNAs is regulated by ER stress signaling, and certain miRNAs have been shown to regulate factors involved in UPR signaling and influence either proadaptive or proapototic pathways (11, 61). Activation of PERK induces expression of miR-211, which in turn targets the promoter of chop/gadd153 and attenuates its expression. This action provides a window of opportunity for the cell to reestablish homeostasis prior to apoptotic commitment (15). More intriguingly, activation of PERK upon ER stress, coupled with the inflammatory transcription factor NF-κB, targets an enhancer site upstream of miR-30c-2* and induces its expression. miR-30c-2* subsequently targets a seed sequence within the XBPI 3'-UTR and promotes degradation and/or impedes translation of XBPI transcripts (9). Given that increased expression of XBPI promotes hepatic VLDL secretion and depletion of XBPI markedly decreases plasma TG, cholesterol, and free FAs due to a reduced VLDL secretion rate (51), reduced XBPI expression by miR-30c-2* in the context of ER stress may be a metabolic response to maintain the homeostasis of lipid metabolism.

Activation of IRE1α, another ER stress sensor, cleaves pre-miR-17, leading to the reduced expression of miR-17, which normally suppresses expression of thioredoxin-interacting protein (TXNIP) (54, 91). As a result, the increased expression of TXNIP activates the NLRP3 inflammasome and induces apoptosis in pancreatic β-cells, leading to the development of insulin resistance (54). Together, these studies indicate that miRNAs such as miR-223 and miR-30c-2* are involved in regulating lipid metabolism in mitochondria and ER as well as the subsequent development of hyperlipidemia observed in metabolic syndrome.
miR-122 treatment in mice (23, 46). Specifically, inhibition of saturase-1 (SCD1), ATP citrate lyase (ACLY), and phospho-
rates of FA and sterol synthesis and increased rates of FA reduces plasma TG and cholesterol levels due to decreased metabolism (90). Antisense knockdown of miR-122 in mice in regulating several processes involved in fetal liver develop-
transporter protein CAT-1. However, miR-122 may play a role in the specific gene target predicted for miR-122 is cationic acid

miR-30c in the control of VLDL secretion. As discussed above, assembly and secretion of VLDL are regulated by multiple factors, including the availability of lipid substrates, the activity of MTP, and the stability of apoB (38). Emerging evidence has demonstrated that each of these steps is vulnerable to miRNA regulation. Recently, an elegant study reported by Soh et al. (83) has demonstrated that miR-30c-5p (miR-30c) is capable of not only directly targeting the 3'-UTR of MTP mRNA to control plasma apoB-containing lipoprotein levels, but also of inhibiting hepatic lipid synthesis by reducing mRNA levels of lysophosphatidylglycerol acyltransferase 1 (LPGAT1), an enzyme involved in phospholipid synthesis. The study showed that overexpression of miR-30c in Huh7 hepatoma cells inhibited both mRNA and protein expression of MTP, which led to decreased MTP activity and apoB secretion. Specifically, the reduced expression of MTP hindered the proper lipidation of apoB, reducing secretion of apoB-containing lipoproteins, VLDL, and LDL. On the other hand, MTP mRNA and protein expression, as well as activity, were increased or decreased by hepatic delivery of anti-miR-30c or miR-30c, respectively. Lentiviral-mediated miR-30c expres-
sion in C57BL/6 WT mice fed a Western diet for 3 wk reduced plasma TG, total plasma cholesterol, and non-HDL cholesterol, whereas no significant changes were observed in HDL-choles-
terol levels. The authors further demonstrated that miR-30c inhibition or overexpression positively or negatively regulated LPGAT1 activity, respectively. In vivo studies in apoE KO mice further supported the in vitro observation by showing that lentiviral overexpression of miR-30c decreased plasma lipid levels and improved atherosclerotic plaque burden in both the aorta and the aortic root compared with treatment with a scrambled control. Overexpression of anti-miR-30c increased plasma TG and cholesterol levels in apoE KO mice fed a Western diet. Collectively, these data strongly suggest that miR-30c is directly involved in regulating VLDL biogenesis by targeting MTP mRNA and modulating lipid substrate availability for VLDL assembly.

miR-122 in the control of VLDL secretion. One of the best-studied miRNAs involved in lipid metabolism is miR-122. Not only is it exclusively expressed in the liver, but it accounts for 72% of all miRNA expressed in this organ (50). miR-122 is produced from the noncoding mRNA for the gene hcr, and the specific gene target predicted for miR-122 is cationic acid transporter protein CAT-1. However, miR-122 may play a role in regulating several processes involved in fetal liver development (13) and maintaining liver homeostasis, including lipid metabolism (90). Antisense knockdown of miR-122 in mice reduces plasma TG and cholesterol levels due to decreased rates of FA and sterol synthesis and increased rates of FA oxidation in hepatocytes (23). Several genes involved in FA and cholesterol synthesis, such as fatty acid synthase (FASN), acetyl-CoA carboxylase-1 (ACAC1), ACC2, stearoyl-CoA desaturase-1 (SCD1), ATP citrate lyase (ACLY), and phospho-

miR-33a/b in the control of VLDL secretion. In humans, miR-33a is located within an intron of the sterol-regulatory element-binding protein (SREBP)-2 gene, while miR-33b is located within the SREBP-1 gene, and each is coordinately expressed with their respective host gene. Mice, however, express only miR-33a, found within the SREBP-1 gene (26). In addition to the intensive studies that have showed the positive role of miR-33a/b in free FA and cholesterol synthesis, emerging studies have suggested that miR-33 is also indirectly involved in lipoprotein metabolism. Studies show that miR-

miR-155 in the control of VLDL secretion. Although miR-

Downloaded from http://ajpendo.physiology.org/ by 10.220.32.246 on June 4, 2017
developed more severe hepatic steatosis and increased serum VLDL/LDL-cholesterol levels relative to WT mice on the same diet. Microarray analysis showed that the liver X receptor/retinoid X receptor (LXR/RXR) pathway was upregulated in miR-155 KO mice. Furthermore, miR-155 appears to regulate cholesterol and FA metabolism pathways in liver by directly targeting LXRα (LXRA) (63), a transcriptional regulator of many genes involved in liver lipid metabolism (11). The binding site for miR-155 in the LXR gene is evolutionally conserved between mice and humans (63). Therefore, induced expression of miR-155 by HFD has been proposed to be a feedback mechanism that counters the action of LXR/RXR signaling, preventing the activation of hepatic de novo lipogenesis and possibly reducing hepatic VLDL secretion. Taken together, these studies indicate that miRNAs exert their regulatory effects at different levels of lipoprotein biosynthesis. Manipulating the expression of miRNAs that are engaged in VLDL metabolism, such as those regulating lipid biosynthesis, MTP-mediated apoB lipidation, or FA oxidation, could serve as pharmaceutical approaches for the prevention and treatment of VLDL overproduction in metabolic syndrome.

miRNAs in the Control of HDL-Cholesterol

The metabolism of HDL-cholesterol is closely associated with reverse cholesterol transport (RCT). RCT begins with hydrolysis of intracellular cholesterol esters, liberating free cholesterol for efflux from cells onto apolipoprotein AI (apoAI) to form HDL particles within the plasma. This efflux is primarily mediated by the cholesterol transporter adenosine triphosphate-binding cassette transporter A1 (ABCA1). HDL-cholesterol can then be transported to and be taken up by the liver for excretion. Cholesterol can also be removed in this way from macrophage foam cells in arterial walls to protect from atherosclerosis (27). Therefore, reduced plasma HDL-cholesterol levels is a contributing risk factor in the development of cardiovascular disease (45). Interestingly, recent studies have demonstrated that miRNAs are engaged in regulating the abundance of HDL-cholesterol (19, 26, 33, 42, 65, 73–75). These studies reveal that miRNAs are capable of targeting different steps of RCT and HDL metabolism, including HDL maturation, cholesterol efflux, and uptake of HDL cholesterol.

miR-33a/b in HDL maturation. Beyond its role in FA metabolism, many groups have shown that miR-33 is involved in control of cholesterol homeostasis. Studies using human cells (26, 33, 65), mice (33, 65, 77), and African green monkeys (75) have all demonstrated that miR-33 downregulates ABCA1, a target gene of SREBP-2, which subsequently reduces cholesterol efflux to form HDL (33, 65). miR-33a/b inhibition or deletion in mice results in increased plasma HDL-cholesterol levels (33, 65), allowing for increased rates of RCT and ameliorated atherosclerosis (32, 76, 78). In contrast, increased expression of miR-33 is capable of inhibiting efflux of cholesterol when cellular cholesterol levels are low. For instance, depleting cellular cholesterol in mouse J774 macrophages by treating the cells with lovastatin/β-cyclodextrin (65) or culturing human THP-1 macrophages in sterol-free conditions (33) increased miR-33 expression and decreased protein levels of ABCA1. In contrast, miR-33 expression was reduced in cholesterol-loaded mouse macrophages and mice fed a HFD (77). Given that SREBP-2 is negatively regulated by intracellular cholesterol levels in a feedback mechanism, and thusLovastatin/β-cyclodextrin treatment increased SREBP-2 expression, it has been proposed that miR-33 and SREBP-2 work together to tightly control cholesterol levels within cells (65), which is feasible due to their coordinated expression. These studies unveil that miR-33 not only controls cholesterol synthesis but is also involved in metabolism of HDL-cholesterol through the modulation of ABCA1 expression in RCT. These findings reveal great potential for using anti-miR-33 as a pharmaceutical approach to elevate the “good cholesterol” (HDL-cholesterol) in the treatment of hypercholesterolemia and atherosclerosis.

Other miRNAs involved in the control of cholesterol efflux. Like miR-33, miR-144 also targets ABCA1 and regulates HDL-cholesterol efflux. Furthermore, cotransfection of both miR-33 and miR-144 appeared to have an additive effect to decrease ABCA1 expression in Huh7 cells (74). Additionally, studies from Ramirez et al. (74) have also revealed that there is a regulatory circuit between LXR, ABCA1 and miR-144. ABCA1 expression is upregulated by HFD in response to excess cholesterol. miR-144 expression was also upregulated by LXR agonists in several cell culture models and by oral gavage in mice, which resulted in significantly reduced plasma cholesterol levels. Thus, the concomitant increased expression of both ABCA1 and miR-144 upon LXR activation may be a physiological response to fine-tune cholesterol efflux. Similar results were observed with agonists of farnesoid X receptor (FXR), which was shown to directly regulate miR-144 (19). Additionally, miR-145 also downregulated ABCA1 expression in another human hepatoma cell line, HepG2 cells, and mouse pancreatic islet cells to regulate cholesterol efflux (42). ABCA1 expression is also regulated by miR-758, which is highly expressed in the brain, heart, and aorta of mice. In mice, HFD suppressed miR-758 expression in the liver and macrophages, along with a consequent increase in plasma HDL-cholesterol levels, compared with chow-fed mice (73). These studies demonstrated that miRNAs exert their regulatory effects on HDL-cholesterol metabolism by coupling with ABCA1 and other regulatory factors in the RCT pathway.

miRNAs that control HDL-cholesterol uptake. Hepatic scavenger receptor class B type I (SR-BI) mediates selective HDL-cholesterol uptake, which is a pivotal step of RCT (1). Hepatic SR-BI expression was found to be downregulated by several miRNAs: miR-185, miR-96, and miR-223 (95). Overexpression of these miRNAs in HepG2 cells thus reduced intracellular and cell surface levels of SR-BI, resulting in a significant decrease in HDL uptake and reduced lipid accumulation within the cells. The authors further found that human SR-BI harbors two putative miR-185 target sites, a miR-96 site and a miR-223 site, within its 3′-UTR. These three miRNAs worked in a synergistic manner to decrease SR-BI levels. Inhibition of these miRNAs by their antisense miRNAs was able to reverse the suppressive effect of these miRNAs on SR-BI expression. Furthermore, HFD induced hepatic SR-BI expression, which was accompanied by reduced expression of miR-96 and miR-185 in the livers of apoE KO mice. These data suggest that miR-185, miR-96, and miR-223 negatively regulate HDL-cholesterol uptake by coordinately targeting SR-BI mRNA and modulating cholesterol metabolism (95). Another study, using steroidogenic cells, further showed that miR-125a and miR-455 directly target and downregulate expression of SR-BI in these cells, resulting in reduced uptake of...
HDL-cholesterol and reduced HDL-cholesterol-supported steroid synthesis and hormone production (36). The authors further found that miR-125a, but not miR-455, is highly expressed in mouse and rat livers, where it downregulates SR-BI levels, reduces selective HDL-cholesterol uptake into hepatocytes, and indirectly regulates plasma HDL-cholesterol level. (36).

Taken together, these studies demonstrated that miRNAs exert their impacts on HDL-cholesterol metabolism through multiple aspects, from cholesterol efflux to HDL-cholesterol uptake. These impacts could be synergistic and/or tissue specific. Given the critical role of HDL-cholesterol in the prevention of cardiovascular diseases, the diverse biological impacts of miRNAs on HDL metabolism provide an attractive potential for developing pharmaceutical approaches to control dyslipidemia.

Concluding Remarks

As shown in Table 1 and Fig. 1, miRNAs are clearly key regulators of various cellular pathways (Table 1) and physiological processes, including lipid and lipoprotein metabolism (Fig. 1). The evidence presented here demonstrates that a large number of miRNAs exert their biological impacts on host cells by physically relocating to specific cellular compartments.

Table 1. miRNAs involved in lipid and lipoprotein metabolism

<table>
<thead>
<tr>
<th>miRNA</th>
<th>Lipid-Related Gene</th>
<th>Function of Gene</th>
<th>Effects on Lipids</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>miR-30c</td>
<td>MTP</td>
<td>Lipidate apoB</td>
<td>↓ Plasma lipids</td>
<td>(83)</td>
</tr>
<tr>
<td>miR-155</td>
<td>LPGAT1</td>
<td>Lipid synthesis</td>
<td>↓ Hepatic lipids</td>
<td>(63)</td>
</tr>
<tr>
<td>miR-122</td>
<td>PMVK</td>
<td>Cholesterol synthesis</td>
<td>↑ Plasma cholesterol</td>
<td>(23)</td>
</tr>
<tr>
<td></td>
<td>FASN</td>
<td>FA synthesis</td>
<td>↑ Plasma TG</td>
<td></td>
</tr>
<tr>
<td></td>
<td>ACC2</td>
<td>FA synthesis</td>
<td>↑ Lipid synthesis</td>
<td></td>
</tr>
<tr>
<td></td>
<td>SCD1</td>
<td>FA synthesis</td>
<td>↓ FA oxidation</td>
<td></td>
</tr>
<tr>
<td></td>
<td>AMPKα</td>
<td>FA oxidation</td>
<td>↓ FA oxidation</td>
<td></td>
</tr>
<tr>
<td>miR-33a/b</td>
<td>HADHB</td>
<td>FA oxidation</td>
<td>↓ FA oxidation</td>
<td>(18, 26, 33, 61, 65)</td>
</tr>
<tr>
<td></td>
<td>CPT1α</td>
<td>FA oxidation</td>
<td>↑ Hepatic lipids</td>
<td></td>
</tr>
<tr>
<td></td>
<td>CROT</td>
<td>FA oxidation</td>
<td>↓ Plasma cholesterol</td>
<td></td>
</tr>
<tr>
<td></td>
<td>AMPKα</td>
<td>FA oxidation</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>ABCA1</td>
<td>Cholesterol efflux</td>
<td></td>
<td></td>
</tr>
<tr>
<td>miR-144</td>
<td>ABCA1</td>
<td>Cholesterol efflux</td>
<td>↓ Plasma cholesterol</td>
<td>(19, 74)</td>
</tr>
<tr>
<td>miR-145</td>
<td>ABCA1</td>
<td>Cholesterol efflux</td>
<td>↓ Plasma cholesterol</td>
<td>(42)</td>
</tr>
<tr>
<td>miR-758</td>
<td>ABCA1</td>
<td>Cholesterol efflux</td>
<td>↓ Plasma cholesterol</td>
<td>(73)</td>
</tr>
<tr>
<td>miR-378/378*</td>
<td>CRAT</td>
<td>FA oxidation</td>
<td>↑ Hepatic lipids</td>
<td>(12)</td>
</tr>
<tr>
<td>miR-185</td>
<td>SR-BI</td>
<td>Cholesterol uptake</td>
<td>↓ Plasma TG</td>
<td>(95)</td>
</tr>
<tr>
<td>miR-96</td>
<td>SR-BI</td>
<td>Cholesterol uptake</td>
<td>↓ Cholesterol uptake</td>
<td>(95)</td>
</tr>
<tr>
<td>miR-223</td>
<td>SR-BI</td>
<td>Cholesterol uptake</td>
<td>↓ Cholesterol uptake</td>
<td>(95)</td>
</tr>
<tr>
<td>miR-125a</td>
<td>SR-BI</td>
<td>Cholesterol uptake</td>
<td>↓ Cholesterol uptake</td>
<td>(36)</td>
</tr>
</tbody>
</table>

Fig. 1. MicroRNA (miRNA) regulation of lipoprotein metabolism. Schematic overview of miRNAs involved in metabolism of VLDL and HDL. Select hepatic miRNAs that regulate genes involved in either lipid biosynthesis or mitochondrial fatty acid (FA) oxidation influence lipid availability within hepatocytes and increase or decrease VLDL secretion. miRNAs that downregulate ATP-binding cassette transporter A1 (ABCA1) expression decrease cholesterol efflux from both the liver and extrahepatic tissues, such as the heart, brain, and macrophages, and decrease HDL production. Hepatic miRNAs that downregulate hepatic scavenger receptor class B type I (SR-BI) expression decrease selective HDL-cholesterol uptake into the liver.
pable of directly activating an eIF2α kinase, PKR, which is a double-stranded RNA-activated kinase involved in controlling translational initiation (86, 87). This may serve as an example that miRNAs are capable of directly targeting cellular proteins in order to regulate their activities. In addition, recent studies on the regulatory roles of miRNAs in the modulation of other long noncoding RNAs (88, 99) also greatly advance our knowledge of miRNA function and provide further insights into using miRNA mimics or antisense miRNAs as therapeutic approaches to combat dyslipidemia and metabolic syndrome.

**AUTHOR CONTRIBUTIONS**

Author contributions: P.C. and Q.S. analyzed data; P.C. and Q.S. interpreted results of experiments; P.C. and Q.S. prepared figures; P.C. and Q.S. drafted manuscript; P.C. and Q.S. edited and revised manuscript; Q.S. conception and design of research; Q.S. approved final version of manuscript.

**DISCLOSURES**

No conflicts of interest, financial or otherwise, are declared by the author(s).

**REFERENCES**


AJP-Endocrinol Metab. doi:10.1152/ajpendo.00194.2014 • www.ajpendo.org


