Noncoding RNAs in Cholesterol Metabolism and Atherosclerosis

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Abstract

Proper maintenance of cellular and plasma cholesterol levels is critical for proper metabolic function and as such is regulated through tightly controlled mechanisms at both the transcriptional and posttranscriptional level. Cardiometabolic diseases, including atherosclerosis, a prominent cause of human morbidity and mortality in western societies (Glass and Witztum 2001; Lusis 2000), are caused in large part by dysregulation of cholesterol and lipid homeostasis. Although

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many environmental and genetic factors are known to contribute to atherogenesis, elevated levels of low-density lipoprotein cholesterol (LDL-C) are the primary risk factors for atherosclerosis and are sufficient to drive the progression of this disease. For this reason, the pathways governing plasma LDL-C levels have been extensively studied, and their modulation has led to effective therapies for the treatment of atherosclerosis.

2.1 Introduction

Proper maintenance of cellular and plasma cholesterol levels is critical for proper metabolic function and as such is regulated through tightly controlled mechanisms at both the transcriptional and posttranscriptional level. Cardiometabolic diseases, including atherosclerosis, a prominent cause of human morbidity and mortality in western societies (Glass and Witztum 2001; Lusis 2000), are caused in large part by dysregulation of cholesterol and lipid homeostasis. Although many environmental and genetic factors are known to contribute to atherogenesis, elevated levels of low-density lipoprotein cholesterol (LDL-C) are the primary risk factors for atherosclerosis and are sufficient to drive the progression of this disease. For this reason, the pathways governing plasma LDL-C levels have been extensively studied, and their modulation has led to effective therapies for the treatment of atherosclerosis.

The primary treatment option for patients with hypercholesterolemia is statins, a class of drugs which competitively inhibit 3-hydroxy-3methylgutaryl-CoA reductase (HMGCR), the rate-limiting enzyme in the cholesterol biosynthesis pathway (Steinberg 2008; Steinberg et al. 2008). Reduced intracellular cholesterol synthesis in the liver, in response to statins, leads to activation of the sterol regulatory element-binding protein (SREBP), which increases the expression of the LDL receptor (LDLR). This in turn promotes LDL-C uptake from the blood and reduces levels of pro-atherogenic lipoproteins in circulation (Brown and Goldstein 1976). Although statin therapies have proven to be effective at reducing cholesterol levels and limiting cardiovascular-related deaths, some patients have poor tolerance for statin therapies, and the majority of patients still experience adverse coronary events despite treatment with statins (Steinberg 2008; Steinberg et al. 2008). As a result, developing novel approaches for lowering cholesterol that can be used alone or in combination with statins has become a major goal for cardiovascular research. Among these, approaches that modulate noncoding RNAs, including microRNAs (miRNAs) and long noncoding RNAs (lncRNAs), have generated a great deal of interest due to their ability to regulate key pathways in cholesterol metabolism and their dysregulation in different disease states (Grundy 2008; Grundy et al. 2004). In this review, we highlight recent work demonstrating the importance of miRNAs and lncRNAs in regulation of cholesterol metabolism and discuss the potential of noncoding RNA-based therapeutic approaches for the treatment of atherosclerosis.

2.2 miRNAs Regulating HDL-C Metabolism and Reverse Cholesterol Transport

In addition to lowering levels of circulating pro-atherogenic lipids, much interest has recently been focused upon developing strategies to promote the removal of cholesterol from arterial macrophages, as a means to limit plaque progression and promote plaque regression. This process, known as reverse cholesterol transport, involves efflux of cholesterol esters from arterial macrophages by the lipid transporters ATP-binding cassette transporter A1 (ABCA1) and ABCG1 onto circulating high-density lipoprotein cholesterol (HDL-C) molecules (Brooks-Wilson et al. 1999; Gelissen et al. 2006; Oram and Vaughan 2000). These cholesterol esters are then transported to the liver where they can be converted into bile acids and removed from the body (Brooks-Wilson et al. 1999; Gelissen et al. 2006; Oram and Vaughan 2000). In recent years, numerous miRNAs have been shown to control numerous aspects of the reverse cholesterol transport pathway, including HDL biogenesis and uptake, cellular cholesterol efflux, and bile acid synthesis and secretion (Fig. 2.1). These include miR-10b, miR-27b, miR-33, miR-96, miR-125a, miR-128a, miR-144, miR-148a, miR-185, miR-223, miR-302a, and miR-455 (De Aguiar Vallim et al. 2013; Goedeke et al. 2015a, b; Ramirez et al. 2013b; Vickers et al. 2014; Wagschal et al. 2015; Wang et al. 2012, 2013). While all of these miRNAs have the

Fig. 2.1 miRNA regulation of HDL-C metabolism. Schematic overview of miRNAs involved in the regulation of HDL-C metabolism. *Blue boxes* highlight miRNAs, which regulate genes that control HDL-C. *ABC* indicates ATP-binding cassette, *SR-BI* scavenger receptor B1, *FXR* farnesoid X receptor, and *LCAT* lecithin–cholesterol acyltransferase (Figure was created using the Servier Medical Art illustration resources (http://www.servier.com))

potential to be important regulators of reverse cholesterol transport and atherogenesis, only a few have thus far been shown to have a significant impact on atherosclerotic plaque progression. Two independent groups have identified miR-144 as an important regulator of ABCA1 expression in both monocyte/macrophages and the liver (De Aguiar Vallim et al. 2013; Ramirez et al. 2013b). These studies further demonstrate that overexpression of miR-144 decreases circulating HDL-C, while inhibition of miR-144 was found to increase circulating HDL-C. Importantly, further work has since demonstrated that administering miR-144 mimics to *ApoE*$^{-/-}$ mice on a pro-atherogenic diet reduces hepatic ABCA1 expression and plasma HDL-C levels leading to increased atherosclerotic plaque formation (Hu et al. 2014). More recently, miR-302a has also been found to control the expression of ABCA1, thereby influencing HDL-C levels. Treatment with inhibitors of miR-302a in vivo was demonstrated to increase hepatic ABCA1 and plasma HDL-C leading to reduce atherosclerosis (Meiler et al. 2015). While most of these miRNAs have not been extensively studied for their role in regulating atherosclerosis, a great deal of work has been done exploring the role of the miR-33 family of miRNAs in regulation of this disease.

2.2.1 miR-33a/miR-33b

The miR-33 family consists of two members, miR-33a and miR-33b. These are intronic miRNAs, which are encoded within the *SREBP2* and *SREBP1 genes*, respectively (Najafi-Shoushtari et al. 2010; Rayner et al. 2010; Marquart et al. 2010; Gerin et al. 2010; Horie et al. 2010). The SREBP1 transcription factor is regulated in response to factors such as insulin and LXR ligands and is primarily responsible for the induction of genes involved in fatty acid synthesis (Horton et al. 2002). SREBP2, on the other hand, is regulated by changes in sterol levels and is the primary factor involved in the induction of genes regulating cholesterol biosynthesis and uptake, thereby allowing cells to carefully regulate their intracellular cholesterol levels (Horton et al. 2002). miR-33a and miR-33b have been demonstrated to be co-transcribed along with their host genes, so conditions that modulate the expression of the *SREBP* genes result in concomitant changes in miR-33 levels (Rayner et al. 2010; Najafi-Shoushtari et al. 2010; Marquart et al. 2010). While the SREBP transcription factors have different target gene specificities, the miR-33 isotypes share the same seed sequence and are therefore believed to target the same set of genes. However, it is not clear whether slight differences in the mature sequence of these miRNAs may alter their affinity for certain targets.

The important role of miR-33 in regulating HDL-C metabolism was first revealed in studies showing that miR-33 could target the cholesterol transporters ABCA1 and ABCG1, both in hepatocytes and macrophages (Najafi-Shoushtari et al. 2010; Rayner et al. 2010; Marquart et al. 2010; Gerin et al. 2010; Horie et al. 2010). Overexpression of miR-33 reduced the expression of ABCA1 and ABCG1 in the liver of mice and decreased plasma HDL-C (Marquart et al. 2010; Najafi-Shoushtari et al. 2010; Rayner et al. 2010; Gerin et al. 2010). Conversely, antisense

oligonucleotides (ASOs) that reduced miR-33 level increased the expression of ABCA1 and ABCG1 and raised plasma HDL-C (Rayner et al. 2010; Najafi-Shoushtari et al. 2010; Marquart et al. 2010). Similarly, genetic loss of miR-33 in mice was reported to increase liver ABCA1 expression and plasma HDL-C (Horie et al. 2010). Importantly, inhibition of miR-33 in nonhuman primates was also demonstrated to significantly increase plasma HDL-C (Rayner et al. 2011a; Rottiers et al. 2013). Additionally, miR-33 was found to regulate hepatic bile acid synthesis through targeting of CYP7a1 (Li et al. *Hepatology* 2013) and secretion via modulation of ABCB11 and ATP8B1 (Allen et al. 2012). Due to its key role in regulating the removal of cholesterol from macrophages, HDL-C biogenesis in the liver, and bile acid metabolism, miR-33 was proposed to be an important regulator of reverse cholesterol transport and the progression of atherosclerosis (Allen et al. 2012; Rayner et al. 2011b). Collectively, these data indicated that ASOs against miR-33 might be a promising therapeutic option to raise HDL-C and treat patients with atherosclerosis.

2.2.2 miR-33a/miR-33b and Atherosclerosis

In response to these initial findings, numerous studies have been performed assessing the impact of miR-33 antagonism on atherosclerosis in mice. The first study of this sort demonstrated that short-term (4-week) administration of miR-33 inhibitors to $Ldlr^{-/-}$ mice with established plaques was capable of raising HDL-C levels and promoting atherosclerotic plaque regression (Rayner et al. 2011b). However, another study found that treatment with anti-miR-33 ASOs did not sustain elevated plasma HDL-C levels during a 12-week atherosclerosis progression study, raised plasma triglycerides, and did not have any effect on plaque development (Marquart et al. 2013). Three additional atherosclerosis progression studies using $Ldlr^{-/-}$ (Rotllan et al. 2013; Ouimet et al. 2015) and $ApoE^{-/-}$ mice (Karunakaran et al. 2015b) reported a decrease in plaque burden in animals treated with miR-33 inhibitors. Surprisingly, although the expression of ABCA1 in the liver and/or macrophages was elevated in mice treated with miR-33 ASOs, HDL-C remained unchanged in these progression studies (Rotllan et al. 2013; Ouimet et al. 2015; Karunakaran et al. 2015b). Together, these findings suggest that the anti-atherosclerotic effects of the ASOs may be the result of direct effects on the plaque rather than alterations in circulating lipids. Consistent with this theory, anti-miR-33 ASOs were found to be efficiently taken up by macrophages in the aortic root of $Ldlr^{-/-}$ mice, which could promote ABCA1-/ABCG1-mediated cholesterol efflux. Additionally, it has been reported that anti-miR-33 ASOs decreased inflammatory burden and promoted the recruitment of M2-like macrophages (Rayner et al. 2011b; Ouimet et al. 2015) and T_{reg} cells (Ouimet et al. 2015). Furthermore, bone marrow transplant experiments in which $ApoE^{-/-}$ mice were reconstituted with $ApoE^{-/-} \times miR\text{-}33^{-/-}$ bone marrow demonstrated that loss of miR-33 in hematopoietic cells caused reduced lipid accumulation in atherosclerotic plaques but did not affect HDL-C levels or total plaque size (Horie et al. 2012). However, whole-body

loss of miR-33 in *ApoE*$^{-/-}$ mice did increase plasma HDL-C and reduce total plaque size (Horie et al. 2012). While the low levels of HDL-C and impaired macrophage cholesterol efflux of *ApoE*$^{-/-}$ mice make this a poor model in which to study effects on reverse cholesterol transport, these findings do indicate that both the liver and macrophages are likely involved in mediating the effects of miR-33 on atherosclerotic plaque formation, although the specific contributions are still not entirely clear.

In addition to its role in regulating lipid metabolism, further studies have showed that miR-33 can also target a number of genes involved in other metabolic processes including fatty acid β-oxidation, insulin signaling, and mitochondrial function (Karunakaran et al. 2015b; Gerin et al. 2010; Davalos et al. 2011; Ramirez et al. 2013a). These findings highlight the complex role of miR-33 in regulating metabolic function and indicate that it may have more diverse functions than have previously been elucidated. Consistent with this, *miR-33*$^{-/-}$ mice on a high-fat diet were found to gain more weight than control animals, leading to more rapid development of hepatic steatosis and insulin resistance (Horie et al. 2013). Similarly, some studies have indicated that prolonged anti-miR-33 therapy may cause unwanted effects such as hypertriglyceridemia (Marquart et al. 2013; Allen et al. 2014; Goedeke et al. 2014) and hepatic steatosis (Goedeke et al. 2014). In contrast, similar long-term miR-33 inhibitor studies showed no change (Rotllan et al. 2013; Ouimet et al. 2015; Horie et al. 2012; Rottiers et al. 2013) or even a decrease (Karunakaran et al. 2015a; Rayner et al. 2011a) in plasma triacylglycerides (TAGs) in mice and nonhuman primates.

Overall, these studies indicate that anti-miR-33 therapies may be a promising approach for developing novel therapies for atheroprotection. However, the multitude of miR-33 targets involved in many different metabolic functions, and the potential for adverse effects of long-term miR-33 inhibition warrant further exploration. Additionally, because there are two isoforms of miR-33 present in humans, but mice and other rodents do not express miR-33b, little is known about the role of this miRNA in regulating atherosclerotic plaque development and overall metabolic function. This is especially important because the host genes for miR-33a and miR-33b (*SREBP2* and *SREBP1*) are regulated by different nutritional and hormonal stimuli and miR-33b is more likely to be altered under conditions of metabolic dysfunction such as diabetes and obesity. Recently, a miR-33b knock-in model was reported (Horie et al. 2014), which should allow further exploration of the role of miR-33b in atherosclerosis and other metabolic diseases.

2.3 miRNAs and LDL-C Metabolism

While the role of miRNAs in regulating HDL-C metabolism has been extensively studied, the importance of miRNAs in controlling LDL-C remains to be fully elucidated. Nevertheless, a number of studies have reported the importance of miRNAs in controlling circulating LDL-C by regulating cholesterol biosynthesis, very-low-density lipoprotein (VLDL) secretion, and LDLR activity (Fig. 2.2).

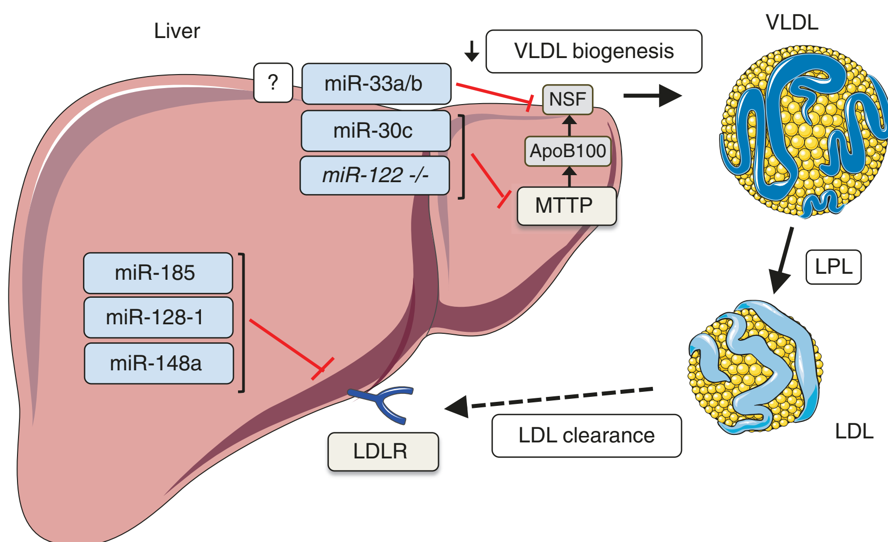

Fig. 2.2 miRNA regulation of LDL-C metabolism. Schematic overview of miRNAs involved in the regulation of LDL-C metabolism. *Blue boxes* highlight miRNAs, which regulate genes that control LDL-C. LDLR indicates low-density lipoprotein receptor, *MTTP* microsomal triglyceride transfer protein, *LPL* lipoprotein lipase, and *NSF* N-ethylmaleimide-sensitive factor (Figure was created using the Servier Medical Art illustration resources (http://www.servier.com))

2.3.1 miRNAs That Regulate Cholesterol Biosynthesis and VLDL Secretion

2.3.1.1 miR-122

The first miRNA identified as a major regulator of cholesterol metabolism was miR-122 (Elmen et al. 2008; Esau et al. 2006; Krutzfeldt et al. 2005). This miRNA is the most abundant miRNA in the liver, accounting for at least 80% of the total miRNA content. Importantly, pharmacological inhibition of miR-122 in mice and nonhuman primates using ASOs or genetic ablation of miR-122 in mice results in a marked reduction of plasma cholesterol levels (Elmen et al. 2008; Esau et al. 2006; Krutzfeldt et al. 2005). Despite these intriguing findings, the molecular mechanisms by which miR-122 controls lipoprotein metabolism remain largely unknown. It is thought that the reduced circulating cholesterol observed in mice treated with miR-122 antagonists is mediated by a combination of different metabolic processes including inhibition of cholesterol and fatty acid biosynthesis, enhanced fatty acid oxidation, and decreased VLDL secretion (Elmen et al. 2008; Esau et al. 2006; Krutzfeldt et al. 2005). However, the direct miR-122 target genes that influence lipid metabolism remain largely unknown. While these findings suggest that the use of miR-122 inhibitors might represent a useful approach to treat dyslipidemia, two recent studies have shown that miR-122 deficiency in mice results in hepatic steatosis, fibrosis, and hepatocellular carcinoma, raising concerns about the potential therapeutic value of miR-122 inhibitors.

2.3.1.2 miR-30c in Lipid Metabolism and Atherosclerosis

In addition to miR-122, miR-30c levels also regulate circulating lipids by regulating the expression of microsomal triglyceride transfer protein (MTTP), an enzyme that lipidates Apo-B and triglycerides and is required for the secretion of plasma lipoproteins that contain Apo-B (Irani et al. 2016; Soh et al. 2013). Moreover, miR-30c inhibits hepatic lipid synthesis. As a result, overexpression of miR-30c using lentiviral constructs or miRNA mimics significantly reduces plasma cholesterol levels and attenuates the progression of atherosclerosis (Irani et al. 2016; Soh et al. 2013). Interestingly the inhibition of MTTP expression and VLDL secretion does not influence the accumulation of lipids in the liver, suggesting that miR-30c therapies might be an interesting approach for treating patients with homozygous hypercholesterolemia.

2.3.1.3 miR-33a/miR-33b

Besides the abovementioned role of miR-33a/miR-33b in regulating HDL-C metabolism and the progression and regression of atherosclerosis, several reports have shown that miR-33 might also control VLDL metabolism (Allen et al. 2014; Goedeke et al. 2014). In this regard, Baldán and colleagues found that chronic inhibition of miR-33 in rodents enhances hepatic VLDL-C. The authors showed that targeting miR-33 in vivo increases the expression of N-ethylmaleimide-sensitive factor (NSF), an ATPase enzyme involved in intracellular trafficking and membrane fusion (Allen et al. 2014). These findings correlate with another study that shows a marked increase in circulating TAG (Goedeke et al. 2014). In agreement with these observations, genetic ablation of miR-33 in mice results in obesity, insulin resistance, hepatosteatosis, and dyslipidemia (Horie et al. 2013). Despite these results, other similar studies in mice and nonhuman primates showed no differences or even a decrease in circulating TAG (Rayner et al. 2011a). The factors that might explain these discrepancies need further investigation.

2.3.1.4 miR-96/miR-182/miR-183

miR-96/miR-182/miR-183 was identified as the most upregulated miRNA in mice fed a chow diet supplemented with a mixture of lovastatin plus ezetimibe (to deplete hepatic cholesterol and enhance cholesterol biosynthesis) compared to mice fed a chow diet supplemented with cholesterol (to increase hepatic cholesterol content and inhibit cholesterol synthesis) (Jeon et al. 2013). The miR-96/miR-182/miR-183 locus is regulated by SREBP and regulates the expression of key proteins that control the maturation and activation of SREBP. miR-96 and miR-182 inhibit the expression of INSIG and FBXW7, proteins that control the retention of the SREBP/SCAP complex in the ER and the degradation nuclear SREBP, respectively (Jeon et al. 2013). As a consequence, overexpression of all three miRNAs enhances cholesterol and fatty acid synthesis in HeLa cells. Further experiments in vivo will be important to define the specific contribution of these miRNAs in regulating hepatic lipid homeostasis and lipoprotein metabolism.

2.3.2 miRNAs That Regulate LDLR Expression and LDL-C Clearance

Several studies have recently identified miRNAs that regulate LDL-C metabolism via posttranscriptional regulation of LDLR. Notably, miR-27a/miR-27b, miR-128-1, miR-130b, miR-148a, miR-185, miR-199a, and miR-301 were shown to directly target the 3'UTR of *LDLR* and modulate LDLR expression in human and mouse hepatic cells (Alvarez et al. 2015; Goedeke et al. 2015b; Jiang et al. 2015; Wagschal et al. 2015). Of these miRNAs, only miR-128-1, miR-148a, and miR-185 significantly altered plasma LDL-C in vivo (Goedeke et al. 2015a; Jiang et al. 2015; Wagschal et al. 2015; Yang et al. 2014). Thus, we will only discuss in greater detail how these miRNAs regulate hepatic lipid metabolism and circulating LDL-C.

2.3.2.1 miR-148a

miR-148a has been recently identified by two independent studies as an important regulator of hepatic LDLR expression and lipoprotein metabolism in a number of mouse models (Goedeke et al. 2015a; Wagschal et al. 2015). In the first study, Goedeke and colleagues developed a high-throughput genome-wide screening assay to systematically identify miRNAs that regulate LDLR activity in human hepatic cells (Goedeke et al. 2015a). From this screen, the authors identified and characterized miR-148a as a negative regulator of LDLR expression and activity. miR-148a is highly expressed in the liver, and its expression is regulated by dietary lipids and SREBP1. Of note, pharmacological inhibition of miR-148a using ASOs lowered plasma LDL-C levels in two different mouse models of hypercholesterolemia (Goedeke et al. 2015a; Wagschal et al. 2015). Surprisingly, targeting miR-148a in vivo also increases hepatic ABCA1 expression and circulating HDL-C. Further experiments demonstrated that *ABCA1* is also a miR-148a target gene. Collectively these studies underscore the therapeutic potential of modulating miR-148a expression to treat dyslipidemias (high plasma LDL-C and low circulating HDL-C).

Several labs independently identified SNPs (rs4722551, rs4719841, and rs6951827) in the promoter region of miR-148a associated with altered plasma TC, LDL-C, and TAG levels (Do et al. 2013; Global Lipids Genetics Consortium et al. 2013; Huan et al. 2015). In particular, a miR-eQTL analysis performed in human livers revealed a strong correlation between SNP status and miR-148a expression (Wagschal et al. 2015). Although the exact mechanism by which these SNPs contribute to altered plasma lipids remains unknown, it could be possible that these genetic variations might influence the regulation of miR-148a expression via SREBP1. However, whether these SNPs affect SREBP1-induced transcription requires further investigation. The role of miR-148a in regulating lipid metabolism is likely to be more complex and not only mediated by its targeting on LDLR and ABCA1. In particular, miR-148a was demonstrated to directly target the 3'UTR of other genes involved in lipid metabolism, including *PGC1a*, *AMPK*, and *INSIG1* (Wagschal et al. 2015; Goedeke et al. 2015a). Taken together, these findings

highlight the importance of miR-148a in regulating lipid metabolism in mice and humans and underscore the therapeutic potential of modulating miR-148a expression to treat dyslipidemias.

2.3.2.2 miR-128-1

Besides miR-148a, Wagschal and colleagues also found a strong association of a number of SNPs in the miR-128-1 gene locus and altered plasma lipid levels (Wagschal et al. 2015). miR-128a regulates the expression of numerous genes associated with multiple metabolic pathways. miR-128a directly targets the 3'UTR of the *LDLR* and *ABCA1*, and its inhibition in mice results in a significant decrease in circulating cholesterol and TAG. In addition to its role in controlling lipoprotein metabolism, targeting miR-128-1 in vivo also improved glucose tolerance and insulin sensitivity. Mechanistically, the authors found that miR-128-1 regulates the expression of the insulin receptor (INSR) and insulin receptor substrate 1 (IRS-1) and downstream phosphorylation levels of Akt. Finally, it was also found that miR-128-1 controls the expression of genes associated with fatty acid synthesis, including the fatty acid synthase (FASN) and SIRT1, an NAD^+-dependent energy sensor and deacetylase that can directly deacetylate and inactivate SREBP1 and thus modulate SREBP1-dependent lipogenesis. Altogether, these observations suggest that miR-128a expression might influence plasma lipid levels by regulating the expression of genes associated with lipid and glucose metabolism. Further studies are necessary to define the specific contribution of miR-128a target genes in different tissues and their impact on regulating lipid and glucose metabolism.

2.3.2.3 miR-185

Several reports have recently shown that miR-185 regulates cholesterol metabolism in vitro and in vivo by regulating the expression of LDLR and SRBI, a hepatic HDL-C receptor that facilitates the uptake of cholesteryl esters from HDL in the liver (Jiang et al. 2015; Wang et al. 2013; Yang et al. 2014). Interestingly, miR-185 also targets KH-type splicing regulatory protein (KSRP), a RNA-binding protein that negatively regulates the expression of the human LDLR (Jiang et al. 2015). These findings suggest that inhibiting miR-185 in vivo might attenuate atherosclerosis by reducing circulating LDL-C and promoting reverse cholesterol transport. Indeed, a recent study demonstrated that inhibition of miR-185 in the atheroprone $ApoE^{-/-}$ mice significantly reduced plasma cholesterol levels and attenuated the progression of atherosclerosis (Jiang et al. 2015).

2.4 LncRNAs and Cholesterol Metabolism

While the contribution of miRNAs in regulating lipoprotein metabolism has been deeply studied in the last few years, the role of lncRNAs in controlling cholesterol homeostasis has just started to emerge. Recent studies have demonstrated the important contribution of lncRNAs as key regulators of cholesterol metabolism (Fig. 2.3).

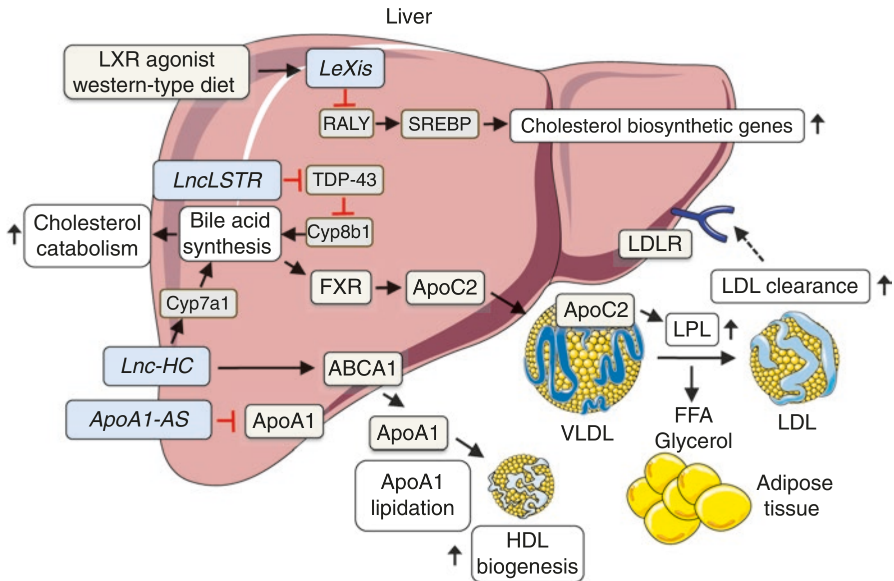

Fig. 2.3 LncRNA regulation of cholesterol metabolism. Schematic overview of lncRNAs involved in the regulation of hepatic cholesterol homeostasis and lipoprotein metabolism. *Blue boxes* highlight lncRNAs, which regulate genes that control cholesterol metabolism. *FXR* indicates farnesoid X receptor, *LeXis* liver-expressed LXR-induced sequence, *LncLSTR* liver-specific triglyceride regulator, *ApoA1-AS* ApoA1 antisense, *LPL* lipoprotein lipase, and *TDP-43* TAR DNA-binding protein 43 (Figure was created using the Servier Medical Art illustration resources (http://www.servier.com))

2.4.1 LeXis

Tontonoz's group has recently identified liver-expressed LXR-induced sequence (*LeXis*), a liver noncoding RNA that is markedly induced in response to LXR agonists and high-fat diet (Sallam et al. 2016). Hepatic *LeXis* overexpression reduces circulating cholesterol, attenuates cholesterol biosynthesis, and inhibits the expression of cholesterol biosynthetic genes. Conversely, genetic ablation of *LeXis* or acute pharmacological inhibition using ASOs enhances the hepatic expression of genes associated with cholesterol biosynthesis, leading to a significant accumulation of cholesterol in the liver. Mechanistically, the authors found that *LeXis* interacts with and influences the binding of RALY to DNA. The authors hypothesize that RALY might cooperate with SREBP2 to control the expression of cholesterol biosynthetic genes. However, further studies are needed to support this hypothesis. Another important question that remains to be addressed is the study of the contribution of *LeXis* in regulating cholesterol metabolism in humans. Importantly, mouse and human genomic comparison revealed a moderate conservation on the *LeXis* genetic locus in a region adjacent to the human *ABCA1* gene.

Further experiments are warranted to determine whether the putative lncRNA annotated in this region (TCONS_00016452) regulates cholesterol metabolism in humans.

2.4.2 Lnc-HC and ApoA1-AS

Other lncRNAs that have been associated to the regulation of cholesterol metabolism include *Lnc-HC* and *ApoA1-AS* (Halley et al. 2014; Lan et al. 2016). *Lnc-HC* is highly expressed in the liver and interacts with hnRNPA2B1 forming a RNA-protein complex, which can then bind to the target mRNAs, *Cyp7a1* and *Abca1* (Lan et al. 2016). Inhibition of *Lnc-HC* increased *Cyp7a1* and *Abca1* expression in hepatocytes, thus promoting cholesterol catabolism. *Lnc-HC* is conserved in humans and rodents and appears to be highly expressed in the liver and fat (Lan et al. 2016). These observations suggest that *Lnc-HC* might play a role in regulating lipid metabolism. However, additional studies in vivo are needed to define the role of *Lnc-HC* in regulating hepatic lipid homeostasis and lipoprotein metabolism. Another interesting finding is the identification of an antisense LncRNA (*ApoA1-AS*) encoded in the apolipoprotein gene cluster that contains four different transcripts including *ApoA1*, *ApoA4*, *ApoA5*, and *ApoC3*. *ApoA1-AS* controls the expression of the apolipoprotein gene cluster epigenetically by recruiting histone-modifying enzymes (Halley et al. 2014). Targeting *ApoA1-AS* using ASOs increases ApoA1 expression in both monkey and human cells and enhances hepatic RNA and protein expression in African green monkeys. While these results are of interest, it is still not known whether the increase in circulating ApoA1 influences plasma lipid levels and/or lipoprotein metabolism.

2.4.3 LncLSTR

Liver-specific triglyceride regulator (LncLSTR) was identified using an unbiased screen aimed to determine lncRNAs highly expressed in the liver (Li et al. 2015). Of note, specific inhibition of LncLSTR leads to a marked reduction in circulating TAG. Mechanistically, LncLSTR depletion increases apoC2 levels, an activator of the lipoprotein lipase (LPL), thus enhancing VLDL and chylomicron catabolism, leading to an increase in plasma TAG clearance (Li et al. 2015). Hepatic LncLSTR expression is regulated by FXR and forms a complex with TDP-43, thus regulating Cyp8b1 expression, a critical enzyme involved in bile acid synthesis.

2.5 Concluding Remarks

Work in recent years has clearly established both miRNAs and lncRNAs as important regulators of lipid metabolism. Since the human genome encodes thousands of lncRNAs, it is expected that in the near future, other lncRNAs will also be identified

as major regulators of lipid metabolism. Despite this exciting future, the analysis and the identification of functional lncRNAs will be challenging because of the modest conservation of these RNA molecules between species. Additionally, a more complete understanding of the mechanisms by which these lncRNAs excerpt their effects and additional studies directly assessing the impact of the lncRNAs on atherosclerotic plaque formation will be needed to properly assess the therapeutic potential of lncRNA-based therapeutic approaches.

On the other hand, a large amount of work has been done demonstrating that miRNAs are capable of targeting key factors regulating lipid metabolism and can have an important impact on the development and progression of atherosclerosis in animal models. As such, miRNA-based therapies may provide useful complimentary approaches for the treatment of atherosclerosis in humans. However, the complicated role of many miRNAs in targeting numerous different genes in different tissues and under different physiologic conditions raises concerns that these therapeutic approaches may result in unintended and possibly detrimental outcomes. These risks are highlighted by the adverse outcomes apparent in some experiments examining inhibition/ablation of miR-33 and miR-122. As such, additional studies including tissue-specific knockouts and careful target gene assessment will be important to understand the full impact of miRNA alterations prior to developing treatment strategies for human patients.

Conflict of Interest The authors declare that they have no conflict of interest.

Human and Animal Rights N/A

References

Allen RM, Marquart TJ, Albert CJ, Suchy FJ, Wang DQ, Ananthanarayanan M, Ford DA, Baldan A. miR-33 controls the expression of biliary transporters, and mediates statin- and diet-induced hepatotoxicity. EMBO Mol Med. 2012;4:882–95.

Allen RM, Marquart TJ, Jesse JJ, Baldan A. Control of very low-density lipoprotein secretion by N-ethylmaleimide-sensitive factor and miR-33. Circ Res. 2014;115:10–22.

Alvarez ML, Khosroheidari M, Eddy E, Done SC. MicroRNA-27a decreases the level and efficiency of the LDL receptor and contributes to the dysregulation of cholesterol homeostasis. Atherosclerosis. 2015;242:595–604.

Brooks-Wilson A, Marcil M, Clee SM, Zhang LH, Roomp K, Van Dam M, Yu L, Brewer C, Collins JA, Molhuizen HO, Loubser O, Ouelette BF, Fichter K, Ashbourne-Excoffon KJ, Sensen CW, Scherer S, Mott S, Denis M, Martindale D, Frohlich J, Morgan K, Koop B, Pimstone S, Kastelein JJ, Genest Jr J, Hayden MR. Mutations in ABC1 in Tangier disease and familial high-density lipoprotein deficiency. Nat Genet. 1999;22:336–45.

Brown MS, Goldstein JL. Receptor-mediated control of cholesterol metabolism. Science. 1976;191:150–4.

Davalos A, Goedeke L, Smibert P, Ramirez CM, Warrier NP, Andreo U, Cirera-Salinas D, Rayner K, Suresh U, Pastor-Pareja JC, ESPLUGUES E, Fisher EA, Penalva LO, Moore KJ, Suarez Y, Lai EC, Fernandez-Hernando C. miR-33a/b contribute to the regulation of fatty acid metabolism and insulin signaling. Proc Natl Acad Sci U S A. 2011;108:9232–7.

De Aguiar Vallim T, Tarling E, Kim T, Civelek M, Baldan A, Esau C, Edwards P. MicroRNA-144 regulates hepatic ABCA1 and plasma HDL following activation of the nuclear receptor FXR. Circ Res. 2013;112(12):1602–12.

Do R, Willer CJ, Schmidt EM, Sengupta S, Gao C, Peloso GM, Gustafsson S, Kanoni S, Ganna A, Chen J, Buchkovich ML, Mora S, Beckmann JS, Bragg-Gresham JL, Chang HY, Demirkan A, Den Hertog HM, Donnelly LA, Ehret GB, Esko T, Feitosa MF, Ferreira T, Fischer K, Fontanillas P, Fraser RM, Freitag DF, Gurdasani D, Heikkila K, Hypponen E, Isaacs A, Jackson AU, Johansson A, Johnson T, Kaakinen M, Kettunen J, Kleber ME, Li X, Luan J, Lyytikainen LP, Magnusson PK, Mangino M, Mihailov E, Montasser ME, Muller-Nurasyid M, Nolte IM, O'Connell JR, Palmer CD, Perola M, Petersen AK, Sanna S, Saxena R, Service SK, Shah S, Shungin D, Sidore C, Song C, Strawbridge RJ, Surakka I, Tanaka T, Teslovich TM, Thorleifsson G, Van Den Herik EG, Voight BF, Volcik KA, Waite LL, Wong A, Wu Y, Zhang W, Absher D, Asiki G, Barroso I, Been LF, Bolton JL, Bonnycastle LL, Brambilla P, Burnett MS, Cesana G, Dimitriou M, Doney AS, Doring A, Elliott P, Epstein SE, Eyjolfsson GI, Gigante B, Goodarzi MO, Grallert H, Gravito ML, Groves CJ, Hallmans G, Hartikainen AL, Hayward C, Hernandez D, Hicks AA, Holm H, Hung YJ, Illig T, Jones MR, Kaleebu P, Kastelein JJ, Khaw KT, et al. Common variants associated with plasma triglycerides and risk for coronary artery disease. Nat Genet. 2013;45:1345–52.

Elmen J, Lindow M, Silahtaroglu A, Bak M, Christensen M, Lind-Thomsen A, Hedtjarn M, Hansen JB, Hansen HF, Straarup EM, Mccullagh K, Kearney P, Kauppinen S. Antagonism of microRNA-122 in mice by systemically administered LNA-antimiR leads to up-regulation of a large set of predicted target mRNAs in the liver. Nucleic Acids Res. 2008;36:1153–62.

Esau C, Davis S, Murray SF, Yu XX, Pandey SK, Pear M, Watts L, Booten SL, Graham M, Mckay R, Subramaniam A, Propp S, Lollo BA, Freier S, Bennett CF, Bhanot S, Monia BP. miR-122 regulation of lipid metabolism revealed by in vivo antisense targeting. Cell Metab. 2006;3:87–98.

Gelissen IC, Harris M, Rye KA, Quinn C, Brown AJ, Kockx M, Cartland S, Packianathan M, Kritharides L, Jessup W. ABCA1 and ABCG1 synergize to mediate cholesterol export to apoA-I. Arterioscler Thromb Vasc Biol. 2006;26:534–40.

Gerin I, Clerbaux LA, Haumont O, Lanthier N, DAS AK, Burant CF, Leclercq IA, Macdougald OA, Bommer GT. Expression of miR-33 from an SREBP2 intron inhibits cholesterol export and fatty acid oxidation. J Biol Chem. 2010;285:33652–61.

Glass CK, Witztum JL. Atherosclerosis. the road ahead. Cell. 2001;104:503–16.

Global Lipids Genetics Consortium, Willer CJ, Schmidt EM, Sengupta S, Peloso GM, Gustafsson S, Kanoni S, Ganna A, Chen J, Buchkovich ML, Mora S, Beckmann JS, Bragg-Gresham JL, Chang HY, Demirkan A, Den Hertog HM, Do R, Donnelly LA, Ehret GB, Esko T, Feitosa MF, Ferreira T, Fischer K, Fontanillas P, Fraser RM, Freitag DF, Gurdasani D, Heikkila K, Hypponen E, Isaacs A, Jackson AU, Johansson A, Johnson T, Kaakinen M, Kettunen J, Kleber ME, Li X, Luan J, Lyytikainen LP, Magnusson PK, Mangino M, Mihailov E, Montasser ME, Muller-Nurasyid M, Nolte IM, O'Connell JR, Palmer CD, Perola M, Petersen AK, Sanna S, Saxena R, Service SK, Shah S, Shungin D, Sidore C, Song C, Strawbridge RJ, Surakka I, Tanaka T, Teslovich TM, Thorleifsson G, Van Den Herik EG, Voight BF, Volcik KA, Waite LL, Wong A, Wu Y, Zhang W, Absher D, Asiki G, Barroso I, Been LF, Bolton JL, Bonnycastle LL, Brambilla P, Burnett MS, Cesana G, Dimitriou M, Doney AS, Doring A, Elliott P, Epstein SE, Eyjolfsson GI, Gigante B, Goodarzi MO, Grallert H, Gravito ML, Groves CJ, Hallmans G, Hartikainen AL, Hayward C, Hernandez D, Hicks AA, Holm H, Hung YJ, Illig T, Jones MR, Kaleebu P, Kastelein JJ, Khaw KT, et al. Discovery and refinement of loci associated with lipid levels. Nat Genet. 2013;45:1274–83.

Goedeke L, Rotllan N, Canfran-Duque A, Aranda JF, Ramirez CM, Araldi E, Lin CS, Anderson NN, Wagschal A, De Cabo R, Horton JD, Lasuncion MA, Naar AM, Suarez Y, Fernandez-Hernando C. MicroRNA-148a regulates LDL receptor and ABCA1 expression to control circulating lipoprotein levels. Nat Med. 2015a;21:1280–9.

Goedeke L, Rotllan N, Ramirez CM, Aranda JF, Canfran-Duque A, Araldi E, Fernandez-Hernando A, Langhi C, De Cabo R, Baldan A, Suarez Y, Fernandez-Hernando C. miR-27b inhibits LDLR and ABCA1 expression but does not influence plasma and hepatic lipid levels in mice. Atherosclerosis. 2015b;243:499–509.

Goedeke L, Salerno A, Ramirez CM, Guo L, Allen RM, Yin X, Langley SR, Esau C, Wanschel A, Fisher EA, Suarez Y, Baldan A, Mayr M, Fernandez-Hernando C. Long-term therapeutic

silencing of miR-33 increases circulating triglyceride levels and hepatic lipid accumulation in mice. EMBO Mol Med. 2014;6:1133–41.

Grundy SM. Promise of low-density lipoprotein-lowering therapy for primary and secondary prevention. Circulation. 2008:117;569–73; discussion 573.

Grundy SM, Cleeman JI, Merz CN, Brewer Jr HB, Clark LT, Hunninghake DB, Pasternak RC, Smith Jr SC, Stone NJ, Coordinating Committee of the National Cholesterol Education Program. Implications of recent clinical trials for the National Cholesterol Education Program Adult Treatment Panel III Guidelines. J Am Coll Cardiol. 2004;44:720–32.

Halley P, Kadakkuzha BM, Faghihi MA, Magistri M, Zeier Z, Khorkova O, Coito C, Hsiao J, Lawrence M, Wahlestedt C. Regulation of the apolipoprotein gene cluster by a long noncoding RNA. Cell Rep. 2014;6:222–30.

Horie T, Baba O, Kuwabara Y, Chujo Y, Watanabe S, Kinoshita M, Horiguchi M, Nakamura T, Chonabayashi K, Hishizawa M, Hasegawa K, Kume N, Yokode M, Kita T, Kimura T, Ono K. MicroRNA-33 deficiency reduces the progression of atherosclerotic plaque in ApoE−/− mice. J Am Heart Assoc. 2012;1:e003376.

Horie T, Nishino T, BABA O, Kuwabara Y, NAKAO T, NISHIGA M, Usami S, Izuhara M, Nakazeki F, Ide Y, Koyama S, Sowa N, Yahagi N, Shimano H, Nakamura T, Hasegawa K, Kume N, Yokode M, Kita T, Kimura T, Ono K. MicroRNA-33b knock-in mice for an intron of sterol regulatory element-binding factor 1 (Srebf1) exhibit reduced HDL-C in vivo. Sci Rep. 2014;4:5312.

Horie T, Nishino T, Baba O, Kuwabara Y, Nakao T, Nishiga M, Usami S, Izuhara M, Sowa N, Yahagi N, Shimano H, Matsumura S, Inoue K, Marusawa H, Nakamura T, Hasegawa K, Kume N, Yokode M, Kita T, kimura T, Ono K. MicroRNA-33 regulates sterol regulatory element-binding protein 1 expression in mice. Nat Commun. 2013;4:2883.

Horie T, Ono K, Horiguchi M, Nishi H, Nakamura T, Nagao K, Kinoshita M, Kuwabara Y, Marusawa H, Iwanaga Y, Hasegawa K, Yokode M, Kimura T, Kita T. MicroRNA-33 encoded by an intron of sterol regulatory element-binding protein 2 (Srebp2) regulates HDL in vivo. Proc Natl Acad Sci U S A. 2010;107:17321–6.

Horton JD, Goldstein JL, Brown MS. SREBPs: activators of the complete program of cholesterol and fatty acid synthesis in the liver. J Clin Invest. 2002;109:1125–31.

Hu YW, Hu YR, Zhao JY, Li SF, Ma X, Wu SG, Lu JB, Qiu YR, Sha YH, Wang YC, Gao JJ, Zheng L, Wang Q. An agomir of miR-144-3p accelerates plaque formation through impairing reverse cholesterol transport and promoting pro-inflammatory cytokine production. PLoS One. 2014;9:e94997.

Huan T, Rong J, Liu C, Zhang X, Tanriverdi K, Joehanes R, Chen BH, Murabito JM, Yao C, Courchesne P, Munson PJ, O'Donnell CJ, Cox N, Johnson AD, Larson MG, Levy D, Freedman JE. Genome-wide identification of microRNA expression quantitative trait loci. Nat Commun. 2015;6:6601.

Irani S, Pan X, Peck BC, Iqbal J, Sethupathy P, Hussain MM. MicroRNA-30c mimic mitigates hypercholesterolemia and atherosclerosis in mice. J Biol Chem. 2016;291:18397–409.

Jeon TI, Esquejo RM, Roqueta-Rivera M, Phelan PE, Moon YA, Govindarajan SS, Esau CC, Osborne TF. An SREBP-responsive microRNA operon contributes to a regulatory loop for intracellular lipid homeostasis. Cell Metab. 2013;18:51–61.

Jiang H, Zhang J, Du Y, Jia X, Yang F, Si S, Wang L, Hong B. microRNA-185 modulates low density lipoprotein receptor expression as a key posttranscriptional regulator. Atherosclerosis. 2015;243:523–32.

Karunakaran D, Richards L, Geoffrion M, Barrette D, Gotfrit RJ, Harper ME, Rayner KJ. Therapeutic inhibition of miR-33 promotes fatty acid oxidation but does not ameliorate metabolic dysfunction in diet-induced obesity. Arterioscler Thromb Vasc Biol. 2015a;35(12):2536–43.

Karunakaran D, Thrush AB, Nguyen MA, Richards L, Geoffrion M, Singaravelu R, Ramphos E, Shangari P, Ouimet M, Pezacki JP, Moore KJ, Perisic L, Maegdefessel L, Hedin U, Harper ME, Rayner KJ. Macrophage mitochondrial energy status regulates cholesterol efflux and is enhanced by anti-miR33 in atherosclerosis. Circ Res. 2015b;117:266–78.

Krutzfeldt J, Rajewsky N, Braich R, Rajeev KG, Tuschl T, Manoharan M, Stoffel M. Silencing of microRNAs in vivo with 'antagomirs'. Nature. 2005;438:685–9.

Lan X, Yan J, Ren J, Zhong B, Li J, Li Y, Liu L, Yi J, Sun Q, Yang X, Sun J, Meng L, Zhu W, Holmdahl R, Li D, Lu S. A novel long noncoding RNA Lnc-HC binds hnRNPA2B1 to regulate expressions of Cyp7a1 and Abca1 in hepatocytic cholesterol metabolism. Hepatology. 2016;64:58–72.

Li T, Francl JM, Boehme S, Chiang JY. Regulation of cholesterol and bile acid homeostasis by the cholesterol 7α-hydroxylase/steroid response element-binding protein 2/microRNA-33a axis in mice. Hepatology. 2013;58(3):1111–21. doi: 10.1002/hep.26427.

Li P, Ruan X, Yang L, Kiesewetter K, Zhao Y, Luo H, Chen Y, Gucek M, Zhu J, Cao H. A liver-enriched long non-coding RNA, lncLSTR, regulates systemic lipid metabolism in mice. Cell Metab. 2015;21:455–67.

Lusis AJ. Atherosclerosis. Nature. 2000;407:233–41.

Marquart TJ, Allen RM, Ory DS, Baldan A. miR-33 links SREBP-2 induction to repression of sterol transporters. Proc Natl Acad Sci U S A. 2010;107:12228–32.

Marquart TJ, Wu J, Lusis AJ, Baldan A. Anti-miR-33 therapy does not alter the progression of atherosclerosis in low-density lipoprotein receptor-deficient mice. Arterioscler Thromb Vasc Biol. 2013;33:455–8.

Meiler S, Baumer Y, Toulmin E, SENG K, Boisvert WA. MicroRNA 302a is a novel modulator of cholesterol homeostasis and atherosclerosis. Arterioscler Thromb Vasc Biol. 2015;35:323–31.

Najafi-Shoushtari SH, Kristo F, Li Y, Shioda T, Cohen DE, Gerszten RE, Naar AM. MicroRNA-33 and the SREBP host genes cooperate to control cholesterol homeostasis. Science. 2010; 238:1566–9.

Oram JF, Vaughan AM. ABCA1-mediated transport of cellular cholesterol and phospholipids to HDL apolipoproteins. Curr Opin Lipidol. 2000;11:253–60.

Ouimet M, Ediriweera HN, Gundra UM, Sheedy FJ, Ramkhelawon B, Hutchison SB, Rinehold K, Van Solingen C, Fullerton MD, Cecchini K, Rayner KJ, Steinberg GR, Zamore PD, Fisher EA, Loke P, Moore KJ. MicroRNA-33-dependent regulation of macrophage metabolism directs immune cell polarization in atherosclerosis. J Clin Invest. 2015;125:4334–48.

Ramirez CM, Goedeke L, Rotllan N, Yoon JH, Cirera-Salinas D, Mattison JA, Suarez Y, De cabo R, Gorospe M, Fernandez-Hernando C. MicroRNA 33 regulates glucose metabolism. Mol Cell Biol. 2013a;33:2891–902.

Ramirez CM, Rotllan N, Vlassov AV, Davalos A, Li M, Goedeke L, Aranda JF, Cirera-Salinas D, Araldi E, Salerno A, Wanschel AC, Zavadil J, Castrillo A, Jungsu K, Suarez Y, Fernandez-Hernando C. Control of cholesterol metabolism and plasma HDL levels by miRNA-144. Circ Res. 2013b;112(12):1592–601.

Rayner KJ, Esau CC, Hussain FN, Mcdaniel AL, Marshall SM, Van Gils JM, Ray TD, Sheedy FJ, Goedeke L, Liu X, khatsenko OG, Kaimal V, Lees CJ, Fernandez-Hernando C, Fisher EA, Temel RE, Moore KJ. Inhibition of miR-33a/b in non-human primates raises plasma HDL and lowers VLDL triglycerides. Nature. 2011a;478:404–7.

Rayner KJ, Sheedy FJ, Esau CC, Hussain FN, Temel RE, Parathath S, Van Gils JM, Rayner AJ, Chang AN, Suarez Y, Fernandez-Hernando C, Fisher EA, Moore KJ. Antagonism of miR-33 in mice promotes reverse cholesterol transport and regression of atherosclerosis. J Clin Invest. 2011b;121:2921–31.

Rayner KJ, Suarez Y, Davalos A, Parathath S, Fitzgerald ML, Tamehiro N, Fisher EA, Moore KJ, Fernandez-Hernando C. miR-33 Contributes to the regulation of cholesterol homeostasis. Science. 2010;238:1570–3.

Rotllan N, Ramirez CM, Aryal B, Esau CC, Fernandez-Hernando C. Therapeutic silencing of microRNA-33 inhibits the progression of atherosclerosis in Ldlr−/− mice--brief report. Arterioscler Thromb Vasc Biol. 2013;33:1973–7.

Rottiers V, Obad S, Petri A, Mcgarrah R, Lindholm MW, Black JC, Sinha S, Goody RJ, Lawrence MS, Delemos AS, Hansen HF, Whittaker S, Henry S, Brookes R, Najafi-Shoushtari SH, Chung RT, Whetstine JR, Gerszten RE, Kauppinen S, Naar AM. Pharmacological inhibition of a

microRNA family in nonhuman primates by a seed-targeting 8-mer antimiR. Sci Transl Med. 2013;5:212ra162.
Sallam T, Jones MC, Gilliland T, Zhang L, Wu X, Eskin A, Sandhu J, Casero D, Vallim TQ, Hong C, Katz M, Lee R, Whitelegge J, Tontonoz P. Feedback modulation of cholesterol metabolism by the lipid-responsive non-coding RNA LeXis. Nature. 2016;534:124–8.
Soh J, IqbAL J, Queiroz J, Fernandez-Hernando C, Hussain MM. MicroRNA-30c reduces hyperlipidemia and atherosclerosis in mice by decreasing lipid synthesis and lipoprotein secretion. Nat Med. 2013;19:892–900.
Steinberg D. The statins in preventive cardiology. N Engl J Med. 2008;359:1426–7.
Steinberg D, Glass CK, Witztum JL. Evidence mandating earlier and more aggressive treatment of hypercholesterolemia. Circulation. 2008;118:672–7.
Vickers KC, Landstreet SR, Levin MG, Shoucri BM, Toth CL, Taylor RC, Palmisano BT, Tabet F, Cui HL, Rye KA, Sethupathy P, Remaley AT. MicroRNA-223 coordinates cholesterol homeostasis. Proc Natl Acad Sci U S A. 2014;111:14518–23.
Wagschal A, Najafi-Shoushtari SH, Wang L, Goedeke L, Sinha S, Delemos AS, Black JC, Ramirez CM, Li Y, Tewhey R, Hatoum I, Shah N, Lu Y, Kristo F, Psychogios N, Vrbanac V, Lu YC, Hla T, De Cabo R, Tsang JS, Schadt E, Sabeti PC, Kathiresan S, Cohen DE, Whetstine J, Chung RT, Fernandez-Hernando C, Kaplan LM, Bernards A, Gerszten RE, Naar AM. Genome-wide identification of microRNAs regulating cholesterol and triglyceride homeostasis. Nat Med. 2015;21:1290–7.
Wang D, Xia M, Yan X, Li D, Wang L, Xu Y, Jin T, Ling W. Gut microbiota metabolism of anthocyanin promotes reverse cholesterol transport in mice via repressing miRNA-10b. Circ Res. 2012;111:967–81.
Wang L, Jia XJ, Jiang HJ, Du Y, Yang F, Si SY, Hong B. MicroRNAs 185, 96, and 223 repress selective high-density lipoprotein cholesterol uptake through posttranscriptional inhibition. Mol Cell Biol. 2013;33:1956–64.
Yang M, Liu W, Pellicane C, Sahyoun C, Joseph BK, Gallo-Ebert C, Donigan M, Pandya D, Giordano C, Bata A, Nickels Jr JT. Identification of miR-185 as a regulator of de novo cholesterol biosynthesis and low density lipoprotein uptake. J Lipid Res. 2014;55:226–38.