Published in final edited form as:

Curr Opin Lipidol. 2016 December ; 27(6): 623–629. doi:10.1097/MOL.0000000000000358.

Truths and Controversies Concerning the Role of miRNAs in Atherosclerosis and Lipid Metabolism

Ángel Baldán^{a,*} and Carlos Fernández-Hernando^{b,*}

^aEdward A. Doisy Department of Biochemistry & Molecular Biology, Center for Cardiovascular Research, and Liver Center, Saint Louis University, 1100 S. Grand Blvd., Saint Louis, MO 63104.

^bVascular Biology and Therapeutics Program, Integrative Cell Signaling and Neurobiology of Metabolism Program, Section of Comparative Medicine, and Department of Pathology, Yale University School of Medicine, 10 Amistad St., New Haven, CT 06510.

Abstract

Purpose of review—Better tools are sorely needed for both the prevention and treatment of cardiovascular diseases, which account for more than one third of the deaths in Western countries. MicroRNAs typically regulate the expression of several mRNAs involved in the same biological process. Therapeutic manipulation of miRNAs could restore the expression of multiple players within the same physiologic pathway, and ideally offer better curative outcomes than conventional approaches that target only one single player within the pathway. This review summarizes available studies on the prospective value of targeting miRNAs to prevent dyslipidemia and atherogenesis.

Recent findings—Silencing the expression of miRNAs that target key genes involved in lipoprotein metabolism *in vivo* with antisense oligonucleotides (ASOs) results in the expected de-repression of target mRNAs in liver and atherosclerotic plaques. However, the consequences of long-term anti-miRNA treatment on both circulating lipoproteins and athero-protection are yet to be established.

Summary—A number of studies have demonstrated the efficacy of miRNA mimics and inhibitors as novel therapeutic tools for treating dyslipidemia and cardiovascular diseases. Nevertheless, concerns over unanticipated side effects related to de-repression of additional targets should not be overlooked for miRNA-based therapies.

Keywords

miRNAs; miR-122; miR-148a; miR-33; atherosclerosis; dyslipidemia

1. INTRODUCTION

MicroRNAs (miRNA) are ~22 nt RNAs that associate to the RNA-Induced Silencing Complex (RISC) and bind to complementary sequences (generally in the 3'UTR) of target mRNAs, resulting in RNA degradation and/or translational repression of the target gene [1].

*co-corresponding authors.

In the last decade, miRNAs have been recognized as essential regulators of multiple (patho)-physiological processes. Atherosclerosis is a progressive disorder of the vascular wall, which can result in plaques containing extracellular lipid, a necrotic core, and inflammatory cells [2, 3]. Such plaques can eventually rupture leading to heart attacks, strokes, and peripheral artery disease, which collectively accounted for >30% of all deaths in the US during the last decade [4]. Dysregulation of lipid metabolism, especially hypercholesterolemia has long been recognized as a risk factor for the both atherosclerosis and the risk of cardiovascular disease [4, 5]. Statins are the most common pharmacologic avenue for hypercholesterolemic patients [6, 7]. The potential for miRNA-based therapies (alone or in combination with statins) have been recently proposed as alternatives for patients at risk of developing cardiovascular disease. This review summarizes recent studies on anti-miRNA-based therapies that exploit the ability of miRNAs to target key genes involved in hepatic and peripheral lipoprotein metabolism, with an emphasis on those miRNAs that influence both lipoprotein metabolism and atherosclerosis.

2. THE ROLE OF miR-33 ON REVERSE CHOLESTEROL TRANSPORT AND ATHEROGENESIS

SREBPs are the master regulators of sterol and fatty acid synthesis [8]. The miR-33 family consists of two members, miR-33a and miR-33b, expressed from within introns of *SREBP-2* and *SREBP-1*, respectively [9–13]. The host and its intragenic miRNA are co-expressed, so conditions that modulate the expression of the *SREBP* genes result in concomitant changes in miR-33 levels [9–11]. Both miR-33 isotypes share the same seed sequence, but differ in 2 nt in the 3' region. Whether these 2 nt confer target specificity is currently unknown.

The relevance of miR-33 on sterol metabolism was initially revealed in studies showing that miR-33 modulated the expression of the cholesterol transporters ATP-binding cassette transporter A1 (ABCA1) and ABCG1, both in hepatocytes and macrophages [9–13]. Overexpression of miR-33 in the mouse liver resulted in attenuated expression of ABCA1 and ABCG1, and led to a concomitant decrease in plasma high-density lipoprotein cholesterol (HDL-C) [9–12]. Conversely, antisense oligonucleotides (ASOs) that reduced miR-33 expression (ASO-33) resulted in increased hepatic amounts of these transporters, as well as elevated plasma HDL-C [9–11]. The phenotype of *miR-33*^{-/-} mice recapitulated the results with ASOs, with increased liver ABCA1 expression and elevated plasma HDL-C [13]. Hepatic bile secretion is also regulated by miR-33, via modulation of the canalicular transporters ABCB11 and ATP8B1 [14]. It was proposed that by controlling hepatic (and presumably also intestinal) HDL biogenesis (via ABCA1), peripheral HDL lipidation (via ABCA1 and ABCG1), and bile secretion (via ABCB11 and ATP8B1), miR-33 could modulate the flow of cholesterol through the reverse cholesterol transport (RCT) pathway [14, 15]. Importantly, studies in African green monkeys showed that the effect of ASO-33 on HDL-C was preserved in monkeys fed a variety of diets [16, 17]. Further studies in cells and in mice showed that miR-33 also coordinates the expression of genes involved in fatty acid β -oxidation and insulin signaling [12, 18, 19]. Collectively, these reports suggested miR-33 is a regulatory hub for several intracellular metabolic processes. The data also implied that

ASOs against miR-33 could be used therapeutically to raise HDL-C in patients and provide atheroprotection.

Further support for the proposal that miR-33 might be targeted to decrease cardiovascular risk came from population studies showing that the levels of miR-33a and/or miR-33b were increased in human atherosclerotic plaques, compared to normal artery walls [20, 21] and in the blood of hypercholesterolemic children, compared to healthy controls [22]. One should keep in mind, though, the small number of patients involved in these studies, and the very low levels of miR-33 in the circulation. Larger population studies will be necessary to firmly establish miR-33a/b as a bona fide biomarker of cardiovascular risk.

The consequences of loss of miR-33 on atherogenesis have been evaluated in several studies (Table 1). The first study in *Ldlr*^{-/-} mice showed that a 4-week treatment with 2'-fluoro/methoxyethyl (2'F/MOE) ASO-33 after 14-weeks of western-type diet (WD) feeding raised HDL-C in circulation and accelerated the regression of atheromata, compared to control ASOs [15]. Another study in WD-fed *Ldlr*^{-/-} mice using locked nucleic acid (LNA) ASOs showed that ASO-33 therapy did not sustain elevated plasma HDL-C levels over the course of 12 weeks, raised plasma triglycerides, and did not have an effect on the progression of atherosclerosis [23]. Three additional progression studies using 2'F/MOE ASO-33 in WD-fed *Ldlr*^{-/-} mice [24, 25] and in *ApoE*^{-/-} mice [20] reported unchanged plasma HDL-C and yet a decrease in atheromata. Paradoxically, although HDL-C remained unchanged in these four progression studies, hepatic and/or macrophage *Abca1* expression was still de-repressed in ASO-33-treated mice, compared to control ASOs. Perhaps the ASO-33-dependent atheroprotection in the context of unchanged HDL-C [20, 24, 25] may be the result of direct effects of the ASO in the plaque: either through accelerated ABCA1/ABCG1-dependent cholesterol efflux to HDL, or decreased inflammatory burden. Authors showed that ASO-33 efficiently transduced macrophages in the aortic root of *Ldlr*^{-/-} mice, and promoted the recruitment of M2 macrophages [15, 25] and T_{reg} cells [25]. These results suggest that anti-miR-33 therapy might exert its atheroprotective effects by promoting an anti-inflammatory phenotype directly in the plaque. Despite the low HDL-C levels and impaired cholesterol efflux previously demonstrated in *ApoE*^{-/-} mice, transplantation with *ApoE*^{-/-} × *miR-33*^{-/-} bone marrow showed a significant reduction in lipid content within atherosclerotic plaques compared to mice transplanted with *ApoE*^{-/-}, but no difference in circulating HDL-C or total plaque size [26]. Conversely, *ApoE*^{-/-} × *miR-33*^{-/-} mice had decreased atherosclerotic lesions, elevated plasma HDL-C, and decreased circulating monocytes, compared to *ApoE*^{-/-} mice [26]. Hence, the relative contributions of hepatic and plaque miR-33 to atheroprotection are still unknown and require further investigation.

A few recent reports have shown that chronic anti-miR-33 therapy might have unwanted effects that include hypertriglyceridemia [27–29] and hepatic steatosis [29]. Consistent with these latter observations, *miR-33*^{-/-} mice gained weight at an accelerated pace and developed obesity, hepatosteatosis, and insulin resistance [30]. In contrast, other similar ASO long-term studies in mice and non-human primates showed no change [17, 24–26] or even a decrease [16, 31] in plasma TAG. The factors that explain the discrepancies in circulating lipids and atherogenesis among these studies are yet to be revealed.

In conclusion, despite the early promising results of ASO-33 in short-term experiments (elevated HDL-C and enhanced RCT), more recent long-term studies have introduced more questions than answers. While some, but not all, studies suggest that anti-miR-33 therapy might offer atheroprotection, the potential risk for obesity, hypertriglyceridemia, and fatty liver disease cannot be overstated. Finally, a critical aspect in miR-33-related studies in mice is that the intron of *SREBP-1* containing miR-33b is altered in rodents, thus abrogating miR-33b expression. Given that the *SREBP-2* and *SREBP-1* genes are controlled by different nutritional and hormonal stimuli, murine studies are neglecting regulatory circuits controlling by miR-33b in the human liver, particularly in response to insulin. A miR-33b humanized transgenic mouse was recently reported [32], which will presumably provide a better model to study the role of human miR-33a/b in physiology and disease. Additional studies will be necessary to establish the viability of ASO-33-based therapies in patients.

3. OTHER miRNAs THAT REGULATES HDL-C METABOLISM

Besides miR-33, a number of other miRNAs have been shown to control numerous aspects of HDL metabolism including HDL biogenesis, HDL uptake and cellular cholesterol efflux. These include miR-144, miR-148a, miR-302a, miR-128a, miR-10b, miR-27b, miR-223, miR-185, miR-96, miR-455 and miR-125a. Because of space limitation, we are going to discuss only the recent findings of those miRNAs that influence both, HDL-C metabolism and atherosclerosis. Two independent reports have identified miR-144 as a regulator of ABCA1 expression in monocyte/macrophages and liver. The *in vivo* experiments in both reports demonstrated that overexpression and inhibition of miR-144 decreases and increases circulating HDL-C respectively. These results were further confirmed by a third study, that also demonstrated that systemic miR-144 overexpression diminishes hepatic ABCA1 expression, decreases plasma HDL-C levels and enhances atherogenesis. In addition to miR-144, miR-302a also controls the expression of ABCA1 and its expression influences HDL-C levels and the progression of atherosclerosis. Antagonism miR-302a *in vivo* increases hepatic ABCA1 and plasma LDL-C and attenuates atherosclerosis.

4. THE ROLE OF miR-122 AND miR-30c IN VLDL-C and LDL-C METABOLISM

A number of recent studies have reported the importance of miRNAs in controlling circulating LDL-C levels by regulating very-low density lipoprotein (VLDL) secretion, cholesterol biosynthesis and LDLR activity.

The liver-enriched miR-122 was the first miRNA shown to control plasma LDL-C levels. Antagonism miR-122 using antisense oligonucleotides markedly reduces circulating LDL-C in rodents and non-human primates. Similarly, genetic ablation of miR-122 in mice results in a significant decrease in plasma LDL-C. Even though numerous groups have demonstrated the hypolipemic effect of miR-122 on LDL-C levels, the molecular mechanisms by which miR-122 controls lipid metabolism remain poorly understood. Studies in *miR-122*^{-/-} mice and mice treated with miR-122 inhibitors have demonstrated that absence or inhibition of miR-122 results in a significant inhibition of lipid biosynthesis, enhanced fatty acid oxidation and reduced levels of hepatic microsomal triglyceride transfer protein (MTTP), an

enzyme that plays a central role in lipoprotein assembly and VLDL production. Despite these beneficial effects, studies in miR-122 deficient mice have shown that sustained loss of miR-122 *in vivo* result in hepatosteatosis, hepatitis and hepatocellular carcinoma. These observations damped the enthusiasm for developing anti-miR-122 therapies for treating dyslipidemias.

In addition to miR-122, miR-30c also controls plasma LDL-C by regulating the expression of MTP and numerous genes associated to hepatic lipid synthesis including phosphatidylglycerol acyltransferase 1 (LPGAT1), elongation of very long chain fatty acids protein 5 (ELOVL5), StAR-related lipid transfer domain protein 3 (STARD3) and membrane bound O-acyltransferase domain containing 1 (MBOAT1). As a result, overexpression of miR-30c using miR-30c mimics or lentivirus expressing miR-30c markedly inhibits hepatic MTP expression, decreases lipogenesis and reduces circulating LDL-C and VLDL-C. Most importantly, overexpression of miR-30c attenuates atherogenesis. These findings suggest that increasing hepatic miR-30c levels might be a valuable option for treating dyslipidemia and atherosclerosis.

5. IDENTIFICATION OF miR-148a AND OTHER miRNAs AS IMPORTANT REGULATORS OF LDL-C METABOLISM AND ATHEROSCLEROSIS

Two recent studies have identified miRNAs that regulate circulating lipids using two different experimental approaches. Interestingly, both studies uncovered the importance of miR-148a in regulating hepatic LDL receptor (LDLR) expression and activity. miR-148a is an intergenic miRNA encoded in chromosome 7 and highly expressed in the liver, adipose tissue and inflammatory cells (macrophages). Previous clinical data demonstrate an association with different SNPs in the miR-148a promoter region with altered plasma lipid (TC, LDL-C and triglyceride) levels. While the mechanism by which these genetic variations within the promoter region of miR-148a influences circulating lipids is unknown, the Näär and Fernández-Hernando laboratories have recently demonstrated that miR-148a levels affect the expression of LDLR and ABCA1 in human hepatic cells and in mouse liver. Importantly, antagonism miR-148a *in vivo* markedly increased hepatic LDLR expression, lowering circulating LDL-C in two different mouse models (*APOBtg;Ldlr^{-/+}* and *ApoE^{-/-}*). Interestingly, miR-148a also regulates the expression of hepatic ABCA1 and inhibition of miR-148a increased plasma HDL-C levels in mice. These observations suggest that antagonism miR-148a might represent an interesting option to simultaneously lower plasma LDL-C and raise circulating HDL-C. In addition to regulating genes associated with lipid metabolism in the liver, miR-148a is also highly expressed in macrophages and its inhibition increases ABCA1 expression and enhances cholesterol efflux, suggesting that miR-148a silencing might increase RCT, thereby attenuating atherogenesis. Further studies are necessary to determine whether absence of miR-148a or chronic miR-148a silencing influences the progression and regression of atherosclerosis.

In addition to miR-148a, other miRNAs that regulate LDLR expression have recently been identified, including miR-27a/b, miR-185, miR-128-1, miR-130b, and miR-301. Of these miRNAs, only miR-128-1, miR-185 and miR-27a/b significantly altered plasma LDL-C *in*

vivo. Most importantly, inhibition of miR-185 expression markedly reduces plasma TC and LDL-C levels and attenuates atherogenesis in ApoE deficient mice. The results obtained using miR-27a/b mimics and inhibitors are somehow controversial. In the first study, the authors overexpressed and inhibited miR-27b using AAV8-miR-27b and LNA anti-miR-27b respectively in C57BL6 mice. The results showed that circulating HDL-C and LDL-C were not altered despite the significant effect of miR-27 levels on hepatic LDLR and ABCA1 expression. In the second study, the authors assessed the effect of miR-27b overexpression and silencing in *ApoE*^{-/-} mice. Interestingly, the results showed that overexpression miR-27b increased plasma TAG levels, but decreased circulating total cholesterol (TC) and LDL-C. By contrast, antagonism miR-27b in *ApoE*^{-/-} mice increased plasma TAG levels, but decreased circulating TC and LDL-C. These results were explained by the direct inhibitory effect of miR-27b in regulating lipoprotein lipase (LPL), a key enzyme that controls TAG hydrolysis from apoB-containing lipoproteins. However, whether or not miR-27b controls lipoprotein metabolism by inhibiting LPL expression and activity is not known. Thus, additional experiments assessing VLDL production and clearance are critical to support this notion. In addition to its role in controlling lipoprotein metabolism, miR-27b is highly expressed in macrophages and controls the expression of numerous mRNA targets associated with lipid metabolism including ABCA1, scavenger receptor A1 (SR-A1), LOX-1, CD-36 and LPL. Nevertheless, the impact of miR-27b in regulating macrophage cholesterol metabolism and foam cell formation remains to be elucidated. These observations suggest that manipulating miR-27b *in vivo* could influence the progression of atherosclerosis. In fact, Xie and colleagues have recently reported that overexpression and inhibition of miR-27b attenuates and enhances atherogenesis respectively in *ApoE*^{-/-} mice.

6. CONCLUDING REMARKS

Recent studies have demonstrated the critical role of miRNAs in regulating lipid metabolism and related cardiovascular disorders. In particular, we discussed in this review article how overexpression or inhibition of miRNAs influences circulating lipids and atherogenesis in mice and non-human primates. Despite these exciting findings, chronic treatment or genetic ablation of some of these miRNAs (miR-122 and miR-33) results in adverse effects including hepatic steatosis, dyslipidemia, obesity and hepatocellular carcinoma. As such future experiments will be important for elucidating the molecular mechanism by which miRNAs regulates lipid homeostasis. A combination of tissue specific knockout mouse models and cutting edge molecular biology techniques that allow the identification of miRNAs target-selectivity in different metabolic tissues will help us to define the specific contribution of a single miRNA in controlling lipid metabolism. These steps are crucial if we want to translate these findings the clinic.

Acknowledgments

Á.B. is supported in part by NIH Grant R01HL107794 and American Heart Association (AHA) Grant GRNT20460189. C.F.-H. is supported in part by NIH Grants R01HL107953 and R01HL106063 and Foundation Leducq Transatlantic Network of Excellence in Cardiovascular Research and AHA Established Investigator Award.

References

1. Bartel DP. MicroRNAs: target recognition and regulatory functions. *Cell*. 2009; 136:215–233. [PubMed: 19167326]
2. Lusis AJ. Atherosclerosis. *Nature*. 2000; 407:233–241. [PubMed: 11001066]
3. Glass CK, Witztum JL. Atherosclerosis. the road ahead. *Cell*. 2001; 104:503–516. [PubMed: 11239408]
4. Rosamond W, Flegal K, Friday G, et al. Heart disease and stroke statistics–2007 update: a report from the American Heart Association Statistics Committee and Stroke Statistics Subcommittee. *Circulation*. 2007; 115:e69–171. [PubMed: 17194875]
5. Kannel WB, Castelli WP, Gordon T. Cholesterol in the prediction of atherosclerotic disease. New perspectives based on the Framingham study. *Ann Intern Med*. 1979; 90:85–91. [PubMed: 217290]
6. Baigent C, Keech A, Kearney PM, et al. Efficacy and safety of cholesterol-lowering treatment: prospective meta-analysis of data from 90,056 participants in 14 randomised trials of statins. *Lancet*. 2005; 366:1267–1278. [PubMed: 16214597]
7. Steinberg D. Thematic review series: the pathogenesis of atherosclerosis. An interpretive history of the cholesterol controversy, part V: the discovery of the statins and the end of the controversy. *J Lipid Res*. 2006; 47:1339–1351. [PubMed: 16585781]
8. 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; 109:1125–1131. [PubMed: 11994399]
9. Najafi-Shoushtari SH, Kristo F, Li Y, et al. MicroRNA-33 and the SREBP Host Genes Cooperate to Control Cholesterol Homeostasis. *Science*. 2010; 328:1566–1569.
10. Rayner KJ, Suarez Y, Davalos A, et al. miR-33 Contributes to the Regulation of Cholesterol Homeostasis. *Science*. 2010; 328:1570–1573.
11. 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; 107:12228–12232. [PubMed: 20566875]
12. Gerin I, Clerbaux LA, Haumont O, et al. Expression of miR-33 from an SREBP2 intron inhibits cholesterol export and fatty acid oxidation. *J Biol Chem*. 2010; 285:33652–33661. [PubMed: 20732877]
13. Horie T, Ono K, Horiguchi M, et al. 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; 107:17321–17326. [PubMed: 20855588]
14. Allen RM, Marquart TJ, Albert CJ, et al. miR-33 controls the expression of biliary transporters, and mediates statin- and diet-induced hepatotoxicity. *EMBO molecular medicine*. 2012; 4:882–895. [PubMed: 22767443]
15. Rayner KJ, Sheedy FJ, Esau CC, et al. Antagonism of miR-33 in mice promotes reverse cholesterol transport and regression of atherosclerosis. *J Clin Invest*. 2011; 121:2921–2931. [PubMed: 21646721]
16. Rayner KJ, Esau CC, Hussain FN, et al. Inhibition of miR-33a/b in non-human primates raises plasma HDL and lowers VLDL triglycerides. *Nature*. 2011; 478:404–407. [PubMed: 22012398]
17. Rottiers V, Obad S, Petri A, et al. Pharmacological inhibition of a microRNA family in nonhuman primates by a seed-targeting 8-mer antimiR. *Sci Transl Med*. 2013; 5:212ra162.
18. Davalos A, Goedeke L, Smibert P, et al. miR-33a/b contribute to the regulation of fatty acid metabolism and insulin signaling. *Proceedings of the National Academy of Sciences of the United States of America*. 2011; 108:9232–9237. [PubMed: 21576456]
19. Ramirez CM, Goedeke L, Rotllan N, et al. MicroRNA 33 regulates glucose metabolism. *Mol Cell Biol*. 2013; 33:2891–2902. [PubMed: 23716591]
20. Karunakaran D, Thrush AB, Nguyen MA, et al. Macrophage Mitochondrial Energy Status Regulates Cholesterol Efflux and Is Enhanced by Anti-miR33 in Atherosclerosis. *Circ Res*. 2015; 117:266–278. [PubMed: 26002865]
21. Mandolini C, Santovito D, Marcantonio P, et al. Identification of microRNAs 758 and 33b as potential modulators of ABCA1 expression in human atherosclerotic plaques. *Nutr Metab Cardiovasc Dis*. 2015; 25:202–209. [PubMed: 25445880]

22. Martino F, Carlomosti F, Avitabile D, et al. Circulating miR-33a and miR-33b are up-regulated in familial hypercholesterolaemia in paediatric age. *Clin Sci (Lond)*. 2015; 129:963–972. [PubMed: 26229086]
23. Marquart TJ, Wu J, Lusis AJ, Baldan A. AntimiR-33 Therapy Does Not Alter the Progression of Atherosclerosis in Low-Density Lipoprotein Receptor-Deficient Mice. *Arteriosclerosis, thrombosis, and vascular biology*. 2013; 33:455–458.
24. Rotllan N, Ramirez CM, Aryal B, et al. Therapeutic silencing of microRNA-33 inhibits the progression of atherosclerosis in *Ldlr*^{-/-} mice—brief report. *Arterioscler Thromb Vasc Biol*. 2013; 33:1973–1977. [PubMed: 23702658]
25. Ouimet M, Ediriweera HN, Gundra UM, et al. MicroRNA-33-dependent regulation of macrophage metabolism directs immune cell polarization in atherosclerosis. *J Clin Invest*. 2015; 2015
26. Horie T, Baba O, Kuwabara Y, et al. MicroRNA-33 Deficiency Reduces the Progression of Atherosclerotic Plaque in *ApoE*^{-/-} Mice. *J Am Heart Assoc*. 2012; 1:e003376. [PubMed: 23316322]
27. 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; 33:455–458. [PubMed: 23288159]
28. 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. [PubMed: 24753547]
29. Goedeke L, Salerno A, Ramirez CM, et al. Long-term therapeutic silencing of miR-33 increases circulating triglyceride levels and hepatic lipid accumulation in mice. *EMBO Mol Med*. 2014; 6:1133–1141. [PubMed: 25038053]
30. Horie T, Nishino T, Baba O, et al. MicroRNA-33 regulates sterol regulatory element-binding protein 1 expression in mice. *Nat Commun*. 2013; 4:2883. [PubMed: 24300912]
31. Karunakaran D, Richards L, Geoffrion M, et al. Therapeutic Inhibition of miR-33 Promotes Fatty Acid Oxidation but Does Not Ameliorate Metabolic Dysfunction in Diet-Induced Obesity. *Arterioscler Thromb Vasc Biol*. 2015
32. Horie T, Nishino T, Baba O, et al. 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. [PubMed: 24931346]

KEY POINTS

1. miRNAs that control the expression of key players of lipoprotein metabolism in liver and/or plaque might be exploited as therapeutic tools in patients at risk of cardiovascular disease.
2. Some studies, especially those targeting miR-33, suggest that anti-miRNA therapies might be useful to modulate the flow of cholesterol through the reverse cholesterol pathway, and attenuate atherogenesis.
3. The data on short- vs. long-term effects of anti-miR-33 therapy on cholesterol and triglyceride lipoproteins are inconsistent among different laboratories.
4. Further studies are necessary to establish definitive proof of the long-term efficacy (i.e., improved lipoprotein profiles and reduced atheromata) and safety of miRNA-based therapies.

TABLE 1

The effects of loss of miR-33 on plasma lipoproteins and atherosclerosis

Animal model	T _X	HDL-c	VLDL-tag	Atherosclerosis	Ref.
C57BL/6	ASO	↑	n.d.	n.d.	[9]
C57BL/6	ASO	↑	n.d.	n.d.	[10]
C57BL/6	ASO	↑	n.d.	n.d.	[11]
miR33 ^{-/-}	–	↑	n.d.	n.d.	[13]
<i>Ldlr</i> ^{-/-}	ASO	↑	n.d.	↓	[15]
A. green monkey	ASO	↑	↓	n.d.	[16]
A. green monkey	ASO	↑	=	n.d.	[17]
<i>ApoE</i> ^{-/-}	ASO	=	n.d.	↓	[20]
<i>Ldlr</i> ^{-/-}	ASO	=	↑	=	[23]
<i>Ldlr</i> ^{-/-}	ASO	=	=	↓	[24]
<i>Ldlr</i> ^{-/-}	ASO	=	=	↓	[25]
<i>ApoE</i> ^{-/-} ×miR33 ^{-/-}	–	↑	=	↓	[26]
<i>ApoE</i> ^{-/-} ×miR33 ^{-/-}	BMT	=	=	=	[26]
C57BL/6	ASO	=	↑	n.d.	[28]
C57BL/6	ASO	↑	↑	n.d.	[29]
miR33 ^{-/-}	–	=	↑	n.d.	[30]