MicroRNA Modulation of Cholesterol Homeostasis

Carlos Fernández-Hernando, Kathryn J. Moore

Abstract—Although the roles of the sterol response element binding protein-1 (SREBP1) and SREBP2 transcription factors in regulating fatty acid and cholesterol synthesis and uptake have been known for some time, it was recently discovered that 2 related microRNAs (miRs), miR-33a and miR-33b, are embedded in these genes. Studies indicate that miR-33a and miR-33b act with their host genes, Srebp2 and Srebp1, respectively, to reciprocally regulate cholesterol homeostasis and fatty acid metabolism in a negative feedback loop. miR-33 has been shown to posttranscriptionally repress key genes involved in cellular cholesterol export and high-density lipoprotein metabolism (Abca1, Abcg1, Npc1), fatty acid oxidation (Crot, Cpt1a, Hadhb, Ampk), and glucose metabolism (Sirt6, Irs2). Delivery of inhibitors of miR-33 in vitro and in vivo relieves repression of these genes, resulting in upregulation of the associated metabolic pathways. In mouse models, miR-33 antagonism has proven to be an effective strategy for increasing plasma high-density lipoprotein cholesterol and fatty acid oxidation and protecting from atherosclerosis. These exciting findings have opened up promising new avenues for the development of therapeutics to treat dyslipidemia and other metabolic disorders. (Arterioscler Thromb Vase Biol. 2011;31:2378-2382.)

Key Words: ABC transporter ■ lipids ■ lipoproteins ■ metabolism

Unraveling the role of microRNAs (miRNAs) in the regulation of gene pathways is an exciting new frontier in many different areas of biological research. This class of short (22-nucleotide), noncoding RNAs posttranscriptionally represses gene expression through binding to complementary target sites in the 3′-untranslated regions (3′UTRs) of messenger RNA (mRNA).1–4 Since their discovery in Caenorhabditis elegans,5,6 our understanding of miRNA processing and action has increased tremendously through the work of many investigators in this field. miRNAs are encoded in intergenic or intronic regions of the genome of metazoan animals, plants, and viruses and are processed from primary transcripts through the sequential actions of Drosha and Dicer enzymes.1,3,4 Mature miRNAs are then incorporated into the RNA-induced silencing complex in the cytoplasm and bind to partially complementary target sites in mRNAs, thereby inhibiting their expression through mRNA destabilization, repression of translation, or a combination of both processes.1–4 Bioinformatic predictions and experimental approaches indicate that a single miRNA may simultaneously target more than 100 mRNAs that function in the same or related pathways, thus providing a mechanism of “fine-tuning” entire gene networks involved in a physiological process or biological pathway.

Sterol Response Element Binding Protein–miRNA-33a/b

The elegance of this mechanism of posttranscriptional gene control is exemplified by the recent identification of microRNA-33a and b (miR-33a/b) as intronic miRNAs located within the sterol response element binding protein (SREBP) genes Srebp2 and Srebp1.7–9 These loci code for the membrane-bound transcription factors SREBP1 and SREBP2, which activate the synthesis of fatty acid and the synthesis and uptake of cholesterol.10–12 Coincident with the transcription of Srebp1 and Srebp2, the embedded miR-33a and miR-33b are transcribed, and these negative regulators act to repress a number genes involved in fatty acid oxidation and cholesterol export.7–9,13–15 This cleverly designed negative feedback loop helps to boost intracellular cholesterol and fatty acid levels by simultaneously balancing transcriptional induction and posttranscriptional repression of lipid metabolism genes.

miR-33 Regulates Cholesterol Metabolism

The presence of miR-33a in the intron of Srebp2 is remarkably conserved in many species, including large and small mammals, chickens, and frogs, suggesting a critical function.7–9 By contrast, there is a gap in the evolutionary conservation of miR-33b, which is present in the Srebp1 gene of mammals, with the exception of rodents. Interestingly, miR-33 and the Srebp gene are conserved in some cholesterol auxotroph animals, where the regulation of SREBP protein and its transcriptional targets are related to fatty acid and phospholipid metabolism. This observation suggests that the function of ancestral miR-33 may have been more related to miR-33b than to miR-33a. The 2 isoforms of miR-33 differ by

Received on: July 11, 2011; final version accepted on: August 23, 2011.
From the Departments of Medicine and Cell Biology, Leon H. Charney Division of Cardiology and the Marc and Ruti Bell Vascular Biology and Disease Program, New York University School of Medicine, New York, NY.
Correspondence to Carlos Fernández-Hernando, PhD, 522 First Ave, Smilow 703, New York, NY 10016. E-mail carlos.fernandez-hernando@nyumc.org
© 2011 American Heart Association, Inc.
Arterioscler Thromb Vase Biol is available at http://atvb.ahajournals.org

DOI: 10.1161/ATVBAHA.111.226688
miR-33 regulates fatty acid metabolism

miR-33a is found within the same intron of Srebp2 from many animal species, including large and small mammals. Interestingly, the fruit fly Drosophila melanogaster also has a highly conserved mature form of miR-33a, but this organism does not synthesize sterols. This observation points to broader roles for miR-33 and leads to the identification of additional targets of miR-33a/b. Of note, several genes involved in fatty acid metabolism, including Crot, Cpt1a, Hadhb, and Ampk, contain predicted binding sites for miR-33/a/b. Overexpression of miR-33a/b reduces fatty acid oxidation and leads to the accumulation of triglycerides in human heptic cells and in the fat body of miR-33 transgenic flies. Of particular interest is the inhibitory effect of miR-33 on Srebp1, which acts in concert with the activation of downstream targets of miR-33a/b. The role of AMPK in regulating cellular energy places this enzyme at a central point in maintaining energy homeostasis. AMPK promotes hepatic fatty acid β-oxidation and inhibits cholesterol and triacylglyceride synthesis. In this way, miR-33 inhibition of AMPK also increases cellular cholesterol and triacylglyceride content. Together, these data implicate miR-33a and miR-33b as central regulators of multiple aspects of lipid metabolism by limiting cholesterol efflux and fatty acid degradation as SREBP2 boosts their production.

miR-33 regulates glucose metabolism and insulin signaling

miR-33 has also been implicated in regulating insulin signaling via targeting of insulin receptor substrate-2, an essential signaling molecule that mediates the effects of insulin. miR-33a/b overexpression reduces insulin receptor substrate-2 levels and inhibits the activation of downstream messenger cascades, including AKT. Moreover, miR-33a/b also target fibroblast growth factor receptor substrate 2,
which has been suggested to participate in insulin signaling by recruiting Src-homology-phosphatase-2 and to function as a docking molecule similar to insulin receptor substrate-2. In addition to insulin receptor substrate-2 and fibroblast growth factor receptor substrate 2, miR-33 also regulates the expression of other genes involved in glucose metabolism, such as sirtuin-6. Interestingly, hepatic-specific disruption of sirtuin-6 in mice results in fatty liver formation because of enhanced glucolysis and triglyceride synthesis, which correlates with the increased triglyceride content observed in human hepatic cell lines transfected with miR-33.

Other Functions of miR-33

A recent report has suggested a role for miR-33 in regulating stem cell self-renewal via downregulation of p53. p53 has 2 putative miR-33 binding sites in the 3’UTR, and miR-33 transfection represses p53 expression and p53-mediated apoptosis. This study suggests that miR-33 may promote the repopulation capacity of hematopoietic stem cells. Interestingly, SREBP1 and cellular cholesterol content have also been shown to regulate cell cycle progression. Thus, miR-33a/b might cooperate with their host genes in regulating cell proliferation and cell cycle progression. Indeed, it has been recently reported that miR-33 overexpression reduces cell proliferation by direct targeting the serine/threonine-protein kinase Pim-1.

Preclinical Studies With miR-33 Inhibitors

The physiological relevance of miR-33 targeting of cellular cholesterol efflux has been demonstrated by short-term overexpression or silencing of miR-33 in mice using strategies such as viral delivery of miR-33 mimics or hairpin inhibitors or parenteral administration of modified antisense oligonucleotides. In vivo overexpression of miR-33 reduced expression of ABCA1 in the liver and decreased plasma HDL levels by 25%. Conversely, various methods of miR-33 inhibition increased hepatic ABCA1 expression, resulting in up to 40% increases of plasma HDL cholesterol. The results of these miR-33 antagonism studies were recently confirmed by the generation of a miR-33 knockout mouse. Targeted deletion of miR-33a from the intron of SREBP2 generated mice that were viable and fertile and showed no disruption of SREBP2 function. These miR-33 deficient mice had circulating HDL cholesterol levels that were 25% to 40% higher than wild-type C57BL/6 mice. Notably, whereas no differences in male and female mice were observed in studies using pharmacological inhibitors of miR-33, female miR-33 knockout mice showed larger increases in plasma HDL than their male counterparts. The molecular mechanisms of this difference are currently being investigated.

Plasma HDL cholesterol levels bear a strong inverse correlation with cardiovascular disease risk, and thus the finding that HDL levels can be modulated by manipulating miR-33 has generated considerable interest in its therapeutic potential. In mouse models of atherosclerosis, overexpression of apoAI to increase HDL has been shown to hinder plaque progression and to promote regression. Furthermore, direct infusion of HDL in apolipoprotein E–deficient mice, cholesterol-fed rabbits, or human subjects with established atherosclerosis reduces plaque size. To test whether miR-33 inhibition might have a similar impact, low-density lipoprotein receptor–/– mice were treated with an oligonucleotide inhibitor of miR-33 for 4 weeks following establishment of atherosclerotic plaques by Western diet feeding for 14 weeks. Notably, in this mouse model of atherosclerosis, miR-33 inhibition increased HDL by 35%, as previously seen in wild-type mice, and this was associated with a 35% reduction in both plaque size and lipid content. Using an in vivo assay to measure the efficiency of reverse cholesterol transport, it was shown that the HDL generated by miR-33 inhibition was functional and increased the transport of cellular radiolabeled cholesterol to the plasma, liver, and feces. The atheroprotective effects of HDL have been largely attributed to its function in reverse cholesterol transport, and in line with this, atherosclerotic lesions in anti-miR-33–treated mice showed increased markers of plaque stability, including reduced macrophage accumulation and inflammatory gene expression, as well as an increase in collagen content. Notably, in addition to raising ABCA1 in the liver, anti-miR-33 oligonucleotides were also detected within macrophages of the atherosclerotic plaque. Isolation of these macrophages by laser capture microdissection showed an increase in ABCA1 expression of anti-miR-33–treated mice, as well as a decrease in inflammatory gene expression. Thus, oligonucleotide inhibitors of miR-33 may promote the reverse cholesterol transport pathway in 2 ways: by directly increasing HDL biogenesis in the liver and by increasing cellular cholesterol efflux from plaque macrophages.

Although the preclinical studies of miR-33 inhibition in mice are encouraging, extrapolation of these findings to humans is complicated by the fact that mice lack miR-33b. This difference between mice and humans may be particularly relevant under conditions in which the transcription of Srebp1 is highly upregulated, such as hyperinsulinemia, which would lead to profound increases in miR-33b expression. Not only would such a condition lead to greater downregulation of cellular cholesterol efflux and plasma HDL levels, but the increased miR-33b/Srebp1 transcription in insulin-resistant states would be predicted to promote hypertriglyceridemia by inhibiting fatty acid oxidation and promoting fatty acid synthesis. Thus, inhibitors of miR-33a/b may relieve repression of both of these metabolic pathways, resulting in reduced plasma triglycerides, as well as increased plasma HDL. However, a comprehensive understanding of the effects of inhibiting both miR-33a and miR-33b awaits translational studies in animal models containing both isoforms of miR-33, such as a nonhuman primate model.

Future Aspects

The therapeutic manipulation of miRNA-regulated pathways is emerging as a promising avenue for the treatment of dyslipidemia and other metabolic disorders. Given the role of miR-33a/b in repressing cholesterol efflux, fatty acid oxidation, and insulin signaling (Figure 2), pharmacological targeting of miR-33a/b may be a promising strategy to treat metabolic syndrome. Cardinal features of this syndrome include dyslipidemia, characterized by an increase in plasma
triglycerides and a decrease in plasma HDL, as well as obesity and insulin resistance. The metabolic syndrome is a growing public health concern worldwide, with complex and interrelated risk factors for both cardiovascular disease and diabetes. Despite widespread use of statins to lower levels of low-density lipoproteins and apolipoprotein B-containing lipoproteins, considerable residual cardiovascular disease risk persists in this patient population. Major goals in the pursuit of novel therapies to target this residual risk have focused on raising levels of HDL to exploit its atheroprotective functions, lowering triglycerides, and improving insulin signaling. Whether miR-33 could be such a panacea awaits future studies.

**Sources of Funding**

Dr Fernández-Hernando is supported by grants from the National Institute of Health (R01-HL106063 and R01-HL107953) and the American Heart Association (SDG-0835585D). Dr Moore is supported by grants from the National Institute of Health (R01-A0020255 and R01-HL108182).

**Disclosures**

None.

**References**


MicroRNA Modulation of Cholesterol Homeostasis
Carlos Fernández-Hernando and Kathryn J. Moore

Arterioscler Thromb Vasc Biol. 2011;31:2378-2382
doi: 10.1161/ATVBAHA.111.226688

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://atvb.ahajournals.org/content/31/11/2378

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Arteriosclerosis, Thrombosis, and Vascular Biology can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Arteriosclerosis, Thrombosis, and Vascular Biology is online at:
http://atvb.ahajournals.org//subscriptions/