Small RNA Overcomes the Challenges of Therapeutic Targeting of Microsomal Triglyceride Transfer Protein

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MicroRNA-30c Reduces Hyperlipidemia and Atherosclerosis in Mice by Decreasing Lipid Synthesis and Lipoprotein Secretion

Soh et al


The plasma level of apolipoprotein B (apoB) is among the strongest risk factors for coronary artery disease. Microsomal triglyceride transfer protein (MTP) plays a key role in the lipidation of nascent apoB and the secretion of apoB-containing lipoproteins enriched with triglycerides and is thus a promising target for the treatment of hyperlipidemia. Yet, the development of MTP inhibitors to lower plasma lipid concentrations has been hindered by adverse effects on hepatic steatosis. A study recently published in *Nature Medicine* identifies microRNA-30c (miR-30c) as a potent repressor of MTP that controls plasma apoB-containing lipoprotein levels, in addition to decreasing hepatic lipid synthesis through direct targeting of lysophosphatidylglycerol acyltransferase 1 (*LPGAT1*). These findings identify miR-30c as a novel therapeutic target that coordinately reduces lipid biosynthesis and lipoprotein secretion to suppress circulating apoB lipoproteins, while sparing the liver from steatosis.

Hyperlipidemia resulting from elevated levels of cholesterol or triglycerides is a primary risk factor for atherosclerotic cardiovascular disease, a major cause of morbidity and mortality in the developed world. The prevailing approach to decrease coronary artery disease risk has largely focused on lowering plasma low-density lipoprotein cholesterol levels, particularly through the use of statins. Although effective, a substantial disease burden remains in patients receiving statin therapy, and statin intolerance limits its use for others, high-density lipoprotein (HDL) metabolism and reverse cholesterol transport have come into focus.

Many miRNAs have been demonstrated to control cholesterol efflux (miR-33, miR-758, miR-26, miR-106, and miR-144) and scavenger receptor BI–mediated HDL cholesterol uptake (miR-223, miR-455-5p, miR-96, miR-185, and miR-125a). Furthermore, studies of miR-33–targeting oligonucleotides in mice and nonhuman primates have shown efficacy in modulating plasma levels of HDL cholesterol, illustrating the potential of such approaches for the treatment of dyslipidemias. Yet, the functional impact of miRNA regulation of apoB-containing particles—low-density lipoproteins, very low-density lipoproteins (VLDL), and chylomicrons—has been unknown to date.

In the July 1, 2013, issue of *Nature Medicine*, Soh et al. deftly define a miRNA (miR-30c) circuitry that independently controls both hepatic lipid synthesis and the secretion of apoB-containing lipoproteins to determine plasma lipid levels. The authors showed that miR-30c represses MTP, which mediates the packaging of VLDL particles with triglycerides in the liver and small intestine, an essential step in the secretion of apoB-containing lipoproteins. miR-30c was found to target *MTTP* mRNA directly through a conserved site within the 3’ untranslated region. Overexpression of miR-30c reduced MTP expression, activity, and apoB secretion in vitro. Despite relatively low levels of miR-30c expression in hepatocytes, Soh et al. found that inhibiting endogenous miR-30c in Huh7 hepatoma cells significantly increased MTP expression (protein and miRNA), activity, and apoB secretion in the media without altering apoB synthesis or general protein synthesis and secretion (albumin), thereby confirming its important physiological role. Notably, lentiviral-mediated manipulation of miR-30c in C57BL/6 wild-type mice fed a Western diet for 3 weeks impacted plasma lipid levels. *MTTP* mRNA and protein expression, as well as activity, were increased or decreased by hepatic delivery of anti-miR-30c or miR-30c, respectively. Moreover, corresponding changes were observed in total plasma cholesterol and non-HDL cholesterol; however, the authors found no change in HDL-C levels. Whereas miR-30c manipulation did not alter plasma triglycerides after 3 weeks, in a separate study the authors demonstrated that miR-30c inhibition or overexpression altered newly synthesized triglycerides in the plasma up and down, respectively. Collectively, these data provide strong support for miR-30c inhibition of VLDL secretion through the direct targeting of *MTTP* mRNA.

During the course of their in vivo studies, the authors observed an MTP-independent effect of miR-30c on lipid synthesis. Whereas miR-30c had no effect on hepatic fatty acid (FA)
oxidation, cholesterol, and triglyceride levels after 3 weeks, FA, phospholipid, and triglyceride synthesis were each found to be significantly altered by miR-30c manipulation [anti-miR-30c]; decreased [miR-30c]). The decrease in FA and triglyceride synthesis associated with miR-30c overexpression is likely responsible for keeping hepatic triglyceride levels steady in the face of reduced VLDL secretion. Through a series of clever experiments, Soh et al. identified in the face of reduced VLDL secretion. Through a series of clever experiments, Soh et al. identified LPGAT1 as a direct target of miR-30c and the biochemical link between miR-30c and FA synthesis control. Although miR-30c was found to repress several genes associated with lipid synthesis—LPGAT1, FA elongase 5 (ELOVL5), StAR-related lipid transfer domain protein 3, and membrane bound O-acyltransferase domain containing 1—using siRNAs, the authors showed that only ELOVL5 and LPGAT1 knockdown decreased de novo lipid synthesis. Transfection of miR-30c with ELOVL5 or LPGAT1 siRNAs was used to establish that LPGAT1, but not ELOVL5, was required for miR-30c repression of lipid synthesis. Notably, miR-30c control of VLDL secretion and FA synthesis is mutually exclusive as siRNA knockdown of MTP had no effect on LPGAT1 expression or lipid synthesis, and conversely, LPGAT1 knockdown had no effect on MTP activity and VLDL secretion. Moreover, miR-30c overexpression in liver-specific miR-30c control of VLDL secretion and FA synthesis is mutually exclusive as siRNA knockdown of MTP had no effect on LPGAT1 expression or lipid synthesis, and conversely, LPGAT1 knockdown had no effect on MTP activity and VLDL secretion. Moreover, miR-30c overexpression in liver-specific MTP−/− mice was found to cause a significant reduction in hepatic triglyceride content likely because of reductions in FA, triglyceride, and phospholipid hepatic synthesis.

Interestingly, the regulation of Mtp and Lpgat1 by miR-30c is selective for this member of the highly conserved miR-30 family. Although all 5 members of this family—miR-30a, miR-30b, miR-30c, miR-30d, and miR-30e—share a common seed region and thus are predicted to target the same set of genes, miR-30c differs from miR-30e and miR-30b by 2 bases in the 3′ end of miRNA (Figure). In addition to complementary pairing of miR-30c to the Mttp 3′ untranslated region at bases 2 through 7 at the 5′ end (seed region) and bases 11 and 12 in the middle, there is another run of 7 complementary bases (16–22) on the 3′ end created by the 2 bases that distinguish miR-30c from miR-30e and miR-30b. These 7 bases at the 3′ end of miRNA likely significantly enhance miR-30c targeting and repression of Mtp, as neither overexpression nor inhibition of miR-30e and miR-30b affected MTP activity and apoB secretion. However, miR-30c repression of the Mtp 3′ untranslated region was abolished when the putative seed binding region was mutated, suggesting that both the seed region and the 3′ pairing of bases 16 to 22 (which is highly conserved in vertebrates) are required for miR-30c repression of MTP. A similar scenario exists for Lpgat1, which harbors 2 target sites for miR-30 family members within its 3′ untranslated region: 1 site is predicted to only pair through the seed region, whereas the second predicted site also contains pairing in the middle (bases 12–15) and at the 3′ end, specifically bases 19 to 22. These bases that distinguish miR-30c from other miR-30 family members likely account for the specificity of miR-30c targeting of Mtp and Lpgat1.

**Figure.** Alignment of microRNA (miR)-30 family members with the 3′ untranslated region (3′ UTR) of Mttp and Lpgat1. A, miR-30c differs from miR-30e and miR-30b by 2 bases in the 3′ end of the miRNA that generates a run of 7 complementary bases (16–22), which enhance miR-30c targeting and repression of Mttp. B, miR-30c targeting of Lpgat1 is enhanced by additional pairing of the miRNA in the middle (bases 12–15) and at the 3′ end, specifically bases 19 to 22. These bases that distinguish miR-30c from other miR-30 family members likely account for the specificity of miR-30c targeting of Mtp and Lpgat1.

mice showed that overexpression and inhibition of miR-30c decreased and increased plasma lipid levels and atherosclerosis. Lentiviral overexpression of miR-30c reduced plasma cholesterol and triglyceride levels, whereas anti-miR-30c increased plasma lipid levels in ApoE−/− mice fed a Western diet. As might be predicted, triglyceride secretion rates and apoB levels (apoB100 and apoB48) were also significantly altered up and down in mice treated with anti-miR-30c or miR-30c, respectively. Most importantly, overexpression of miR-30c significantly reduced atherosclerotic plaque burden in both the aorta and the aortic root of ApoE−/− mice compared with treatment with a scrambled control. One interesting finding observed in Western diet–fed ApoE−/−, but not wild-type mice, was the ability of miR-30c treatment to reduce plasma triglyceride levels. Strikingly, miR-30c repressed triglyceride synthesis in both wild-type and ApoE−/− mice. The authors suggest that this difference could be due in part to loss of lipoprotein lipase activity in the ApoE−/− mice, suggesting that the reduced triglyceride levels are buoyed by loss of triglyceride catabolism. Multiple studies have sought to explain the observed reduced lipoprotein lipase activity associated with apoE deficiency; however, the mechanism remains to be described definitively. Nonetheless, these data strongly suggest that miR-30c reduction of plasma lipid levels prevents atherosclerosis progression in mice.

Nucleic acid–based therapies represent a new frontier in lipid management. Recently, Mipomersen, an antisense oligonucleotide drug targeting apoB, was awarded Food and Drug Administration approval to treat familial hypercholesterolemia. This will likely pave the way for other oligonucleotide–based drugs, including many miRNA-targeting or –enhancing drugs that are currently in clinical trials. Based on miR-30c’s robust control of lipid synthesis and secretion, which overcomes the steatosis associated with conventional MTP inhibitors, it is a prime candidate for drug therapy. However, unlike single-stranded antisense oligonucleotides, the development of
double-stranded oligonucleotide therapies, such as miRNA mimetics and siRNAs, face many challenges, including delivery to the appropriate organ or tissue, injection site reactions, and interferon responses. Several delivery vehicles for miRNA mimetics are under development, including liposomes, polymeric micelles, and lipoprotein-based drug carriers. We recently showed that endogenous HDL particles carry miRNAs in the circulation and thus hold potential for the delivery of miRNA mimetics to target tissues such as the liver. However, an effective miR-30c therapy would likely require increasing miR-30c activity in both the liver and small intestine for maximal control of lipid metabolism. Although the strategies currently under development are likely to target the liver effectively, delivery of oligonucleotides to the small intestine may prove more difficult. A recent study reported that dietary miRNAs are transported to the small intestine where they can have functional impact, opening the possibility that oral delivery of miR-30c or other miRNAs could one day be used to target this tissue.  

In summary, this seminal work by Soh et al. uncovers critical functions of miR-30c in regulating cellular and systemic lipid homeostasis and provides a sound blueprint for experimentation in future miRNA studies. This study illustrates how small differences between miRNA family members outside the seed sequence can produce a major impact on miRNA function. Moreover, it uncovers a clever miRNA circuit that can control the secretion of triglyceride-rich apoplipoproteins, in addition to protecting the liver from steatosis. The findings hold great potential for drug targeting to treat dyslipidemias and coronary artery disease. This work exemplifies a vast potential for discovery as the fields of noncoding RNAs and lipid metabolism converge.

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Disclosures
None.

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