Proper cholesterol homeostasis requires a complex network of sterol-sensing proteins, membrane dynamics, and extensive regulation of transcription, translation, posttranscription modifications, and protein turnover. Together, multilayered regulatory modules control 3 key processes to balance cellular cholesterol levels: de novo cholesterol biosynthesis, cholesterol uptake through lipoprotein receptors, and cholesterol efflux or excretion into bile. In addition to maintaining homeostasis, cholesterol efflux to lipid-poor apolipoprotein AI serves to form high-density lipoproteins (HDL) particles, which provide systemic cholesterol homeostasis through the reverse cholesterol transport pathway. The ATP-binding cassette transporter A1 (ABCA1) is a critical transporter of cholesterol and lipids from cells to extracellular apolipoprotein AI, a process that protects against cholesterol overload. ABCA1 expression is regulated by key nuclear receptors, namely the liver X receptor (LXR) family and their heterodimeric partners, retinoic acid receptors. As cellular sterol levels increase, accumulating oxysterols activate LXR/retinoic acid receptors to drive expression of ABCA1 and other transporters, and thus, cholesterol efflux from the cell.

**Articles, see p 1592 and 1602**

In the liver, ABCA1-mediated cholesterol efflux to newly synthesized lipid-poor apolipoprotein AI accounts for the formation of the majority of nascent HDL particles; therefore, hepatic regulation of ABCA1 is an important regulator of plasma HDL cholesterol (HDL-C) levels. Catabolism of HDL-derived cholesterol also occurs in the liver through excretion into the bile as free cholesterol or bile acids. Reduced hepatic ABCA1 expression indirectly increases the excretion of HDL-derived cholesterol into the bile. The farnesoid X receptor (FXR) is a nuclear receptor expressed in the liver and intestine that controls hepatic steroid and bile acid levels through transcriptional regulation of bile acid and lipid-associated genes. Similar to LXR, increased cellular cholesterol levels and bile acid accumulation activate FXR to promote bile acid secretion.

miRNAs are small noncoding regulatory RNAs that bind to complementary sites within mRNA 3′-untranslated (3′-UTR) and coding regions and provide posttranscriptional gene regulation through translation inhibition and mRNA degradation. Often viewed as biological rheostats, the functional relevance of miRNAs in lipid homeostasis has been established. Although multiple miRNAs have been found to regulate lipid metabolism, namely miR-27b and miR-122, miR-33a/b has been extensively studied and represents perhaps the strongest rationale for miRNAs as key mediators of cholesterol homeostasis. During low sterol conditions, miR-33a/b are cotranscriptionally activated to reduce cholesterol efflux through repression of ABCA1 expression. miR-33a/b directly target ABCA1 mRNA, which harbors 4 putative miR-33 target sites in its 3′-UTR. Importantly, inhibition of miR-33 in mice and nonhuman primates was found to increase HDL-C levels and in mice to reduce atherosclerosis and this approach is under development as a novel therapy for atherosclerotic cardiovascular disease.

miRNAs recognize mRNA targets through seed-based complementarity, 5′ bases 2 to 8 of the mature miRNA; therefore, 1 miRNA has the potential to target many miRNAs, and 1 gene (mRNA 3′-UTR) could harbor multiple miRNA targets’ sites for many different miRNAs. As such, genes with extended 3′-UTRs are likely to be repressed by multiple miRNAs. This is likely the case for ABCA1, which has an unusually long (>3.3 kb) 3′-UTR, average is slightly >1 kb, that makes it highly susceptible to miRNA targeting and posttranscriptional regulation. Not surprisingly, other miRNAs have been found to target ABCA1, including miR-758, miR-26, and miR-106b.

In this issue of Circulation Research, 2 independent research groups report that miR-144 directly targets the ABCA1 3′-UTR, thus repressing cholesterol efflux and HDL-C levels. In 1 study, Fernandez-Hernando et al present evidence that LXR activation upregulates miR-144 transcription and that miR-144 directly targets ABCA1 and reduces cholesterol efflux in macrophages and the liver in part of a homeostatic network. In the other study, Edwards et al found that FXR activation drives miR-144 transcription in the liver, which in turn directly targets ABCA1 and represses cholesterol efflux, thus promoting cholesterol excretion in the bile.

Although FXR activation was found to only slightly increase miR-144 levels, previous studies have found that even small changes to ABCA1-targeting miRNAs (miR-33a/b) alter ABCA1 activity and cause large changes to HDL-C levels. Most importantly, the promoter of miR-144/451 was found to harbor 2 functional FXR transcription factor–binding sites. This work was aided by a recent FXR-chromatin immunoprecipitation sequencing study which identified a putative FXR-binding site in the promoter of miR-144/
miR-451,31 and follow-up studies presented here identified a second FXR-binding site in the proximal promoter, both of which were validated using promoter luciferase assays and site-directed mutagenesis. Using gene reporter assays, miR-144, but not miR-451, was found to directly target 2 sites within the mouse Abca1 3′-UTR. Most interestingly, tissue-specific FXR expression was used to demonstrate the requirement of hepatic FXR in miR-144 activation and FXR-mediated reduction in plasma cholesterol and HDL-C levels.26 Interestingly, FXR activation not only suppresses hepatic ABCA1 activity via induction of miR-144 but also was found to upregulate scavenger receptor BI expression. It is notable that pharmacological inhibition of hepatic ABCA1 with probucol reduced plasma HDL-C levels but promoted reverse cholesterol transport by redirecting hepatic cholesterol to biliary excretion15 and that overexpression of scavenger receptor BI was found to increase hepatic cholesterol and bile acid excretion only when ABCA1 was inhibited.33 Thus, FXR activation not only suppresses hepatic ABCA1 activity via induction of miR-144 but also was found to upregulate scavenger receptor BI expression. It is notable that pharmacological inhibition of hepatic ABCA1 with probucol reduced plasma HDL-C levels but promoted reverse cholesterol transport by redirecting hepatic cholesterol to biliary excretion15 and that overexpression of scavenger receptor BI was found to increase hepatic cholesterol and bile acid excretion only when ABCA1 was inhibited.33 Thus, FXR agonists in the liver may work to both promote the clearance of plasma HDL-C (via scavenger receptor BI induction) and channeling of hepatic cholesterol to the bile (via miR-144-mediated repression of ABCA1), thus promoting reverse cholesterol transport. Despite the reduction in HDL-C levels seen with an FXR agonist, the effect on reverse cholesterol transport and atherosclerosis may be favorable as a result of these hepatic effects.

Although miR-144 levels are increased on LXR activation in both macrophages and the liver, LXR is not predicted to directly bind to miR-144 promoter; rather this effect is likely mediated by a currently unknown indirect mechanism. The authors speculate that sterol response element binding protein (SREBP) transcription factors, specifically SREBP1c, may play a role in the regulation. SREBP1c is a direct target of LXR and is predicted to bind to the promoter of miR-144/miR-451 at 2 sites. LXR-mediated SREBP transcription would also be predicted to promote miR-33a/b transcription. As such, miR-144 and miR-33a/b would work in tandem to repress ABCA1, and suggests that miR-33a/b targets ABCA1 in both low- and high-cholesterol conditions in the macrophage and liver. In ABCA1 gene reporter assays, miR-33a/b and miR-144 were each found to reduce luciferase activity individually; however, when combined they reduced luciferase activity by >60%. Further studies are required to resolve LXR-mediated transcriptional activation of miR-144 and the combinatorial effects of miR-33a/b and miR-144 in high sterol states. The LXR-miR-144 network is distinct from the miR-33a/b–ABCA1 network in that miR-33 serves to prevent cellular sterol loss through efflux in low sterol states as the cell adapts to increase cellular cholesterol levels, whereas miR-144 prevents uncontrolled LXR-induced cholesterol efflux through ABCA1 in high sterol conditions.

A common observation between the miR-33a/b literature and these miR-144 studies is the curious lack of mRNA effect on ABCA1 mRNA abundance.31 Overexpression of miR-144 was found to significantly reduce ABCA1 protein and cholesterol efflux in human hepatoma cells (Hep3B) and mouse primary hepatocytes, but failed to alter Abca1 mRNA levels in both cell types.26 In addition, de Aguiar Vallim et al26 only found ABCA1 protein to be affected with miR-144 overexpression in mice; however, Ramirez et al did find miR-144 overexpression to modulate both ABCA1 mRNA and protein levels.19 In addition, although activation of FXR with GSK2324 in mice did significantly reduce hepatic Abca1 mRNA levels, the effect was very modest and disproportionate to the effect on ABCA1 protein.26 Inhibition of miR-144 in basal nonstimulated Huh7 cells resulted in reduced hepatic miR-144 levels and elevated ABCA1 proteins levels but did not increase ABCA1 mRNA levels.27 Interestingly, inhibition of miR-144 in mice was found to affect Abca1 mRNAs levels only in LXR-agonist–treated mice; however, HDL-C levels were found to be significantly elevated in both LXR-agonist–treated and untreated mice.27 The study of de Aguiar Vallim et al26 was consistent with this observation, as inhibition of miR-144 in mice only affected ABCA1 protein levels, which resulted in increased HDL-C levels. Compensatory ABCA1 transcription or other indirect mechanisms may account for the lack of miR-144 or miR-33a/b effect on ABCA1 mRNA abundance, or these miRNAs could target nucleases responsible for mRNA degradation after translational inhibition. Nevertheless, it is clear that miR-144 and miR-33a/b directly target sites harbored within ABCA1’s 3′-UTR.

To demonstrate the functional impact of miR-144 in vivo, both studies successfully used loss-of-function anti-miR approaches in mice. Each study used a slightly different approach to inhibit miR-144 in vivo. de Aguiar Vallim et al used anti–miR-144 2′F/MOs (Regulus Therapeutics) to inhibit endogenous miR-144 levels in wild-type c57Bl/6 mice on a high-fat diet though biweekly treatments (intraperitoneal injections) of 5 mg/kg anti–miR-144 for 4 weeks.26 Ramirez et al27 used mimetics and inhibitors (mirVana, 7 mg/kg) coupled with Invitrogen (Invitrogen) for intravenous injections twice every 3 days. In both studies, inhibition of miR-144 was found to increase ABCA1 expression and function and raise HDL-C levels in mice. In previous reports, anti–miR-33 approaches have been shown to increase ABCA1 protein and raise HDL-C levels in mice and nonhuman primates19–21 and promote reverse cholesterol transport and reduce atherosclerosis in mice.29 On the basis of results from the studies presented here, anti–miR-144 therapy might also be explored as a nucleic acid–based therapy to increase HDL-C levels. However, the relative effects of anti–miR-144 approaches on macrophages compared with the liver may not be the same as for miR-33. It is possible that if the liver effect predominated, the benefits of promoting the biliary excretion of HDL-derived cholesterol could outweigh the downside of reducing plasma HDL-C levels. Studies of HDL metabolism, reverse cholesterol transport, and atherosclerosis will need to be performed in animals with both gain and loss of function of miR-144 before it is known what directionality is preferable and whether this might be an effective approach to atherosclerosis.

Small RNAs, namely miRNAs, are found in all extracellular compartments and biological fluids.34 Extracellular miRNAs are remarkably stable, likely because of their association with lipid and protein complexes, including HDL.35 Using real-time
polymerase chain reaction–based methods, miR-33a/b, miR-144, and miR-758 were not detected on HDL; however, other ABCA1-regulating miRNAs (miR-26 and miR-106b) and miR-451 are consistently found on HDL in humans and mice. Nevertheless, using high-throughput small RNA sequencing to detect and quantify extracellular miRNAs, miR-33, miR-144, and miR-451 were found on HDL in specific samples (unpublished data).

In summary, these studies highlight a common theme with metabolic miRNAs that miRNAs serve as biological rheostats regulating glimpse of the complexity in cellular cholesterol metabolism. Furthermore, these studies highlight the potential for modulating directional cholesterol flux through nucleic acid therapy–based inhibition of miR-144.

Disclosures

None.

References


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Nuclear Receptors and microRNA-144 Coordinately Regulate Cholesterol Efflux
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