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Anti-atherosclerosis or No Anti-atherosclerosis That is the miR-33 question

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Cholesterol/lipid abnormalities, such as high low-density lipoprotein (LDL)/high-density lipoprotein-cholesterol (HDL-C) ratio and elevated circulating triglycerides, are associated with cardiometabolic disorders, including metabolic syndrome, type 2 diabetes mellitus, and atherosclerosis. Cholesterol/lipid homeostasis is controlled by complex gene regulatory circuits involving both transcriptional and posttranscriptional mechanisms. For example, the sterol regulatory element-binding protein (SREBP) 1 and 2 family of transcription factors controls the expression of genes involved in both cholesterol biosynthesis and uptake, as well as fatty acid, phospholipid, and triglyceride production.[1] Several groups recently discovered that the human SREBP–encoding genes harbor intronic microRNAs termed miR-33a (present in the *SREBF2* gene) and miR-33b (located in the *SREBF1* gene).[2–6] By contrast with humans and other mammals, rodents lack miR-33b and only have miR-33a in the *Srebf2* gene. The miR-33a/b microRNAs, which differ by 2 nucleotides, act in concert with their host gene products to control cholesterol/lipid homeostasis.[7] For example, miR-33a/b inhibit the expression of ATP-binding cassette transporter A1 (ABCA1), a cholesterol efflux pump with a key role in reverse cholesterol transport from peripheral tissues such as atherogenic macrophages back to the liver.[2–5,8] ABCA1 also promotes the production of nascent lipid-poor HDL-C from the liver and small intestine, and mutation of the ABCA1 gene, as well as aberrant reverse cholesterol transport and low HDL-C levels, has been linked with increased atherosclerosis.[9] Intensive efforts have consequently been focused on finding pharmacological tools for increasing circulating HDL-C and promoting reverse cholesterol transport as a novel therapeutic avenue to treat atherosclerosis and cardiovascular disease.[10]

Experiments in mice and nonhuman primates from several laboratories have shown that miR-33 antagonism by anti-sense inhibition or knockout results in elevation of circulating HDL-C, as well as macrophage cholesterol efflux and reverse cholesterol transport.[2–5,8,11] This has suggested that miR-33–targeting antisense oligonucleotides might serve as a novel therapeutic strategy to decrease atherosclerosis. Indeed, using the LDL receptor ($LDLr^{-/-}$) knockout (KO) mouse atherosclerosis model, Rayner et al[8] recently showed that a 4-week anti-miR-33 treatment not only elevated HDL-C but also caused a striking ≈35% regression

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Anders M. Näär has patents pending on miR-33a and miR-33b anti-sense targeting for the treatment of cardiometabolic disorders.

of established, diet-accelerated, atherosclerotic lesions. No statistically significant effects on other lipid parameters, including circulating triglycerides, were observed in this short-term study.

Although these studies clearly demonstrate a regressive effect of miR-33 antagonism on established atherosclerosis, it was not known whether inhibition of miR-33 might also counter progression of atherosclerosis. Moreover, no longitudinal study of miR-33 antisense-based antagonism had been conducted in an atherosclerosis model, and the duration of potential cardiovascular benefits and whether deleterious effects might emerge after long-term treatment was unknown.

In this issue of *Arteriosclerosis, Thrombosis and Vascular Biology*, Marquart et al[12] have investigated the effects of long-term (12 weeks) anti-miR-33 treatment in LDLr KO mice fed a western-type atherogenic diet. Their results show that miR-33 antisense treatment causes elevated hepatic expression of ABCA1, as expected, as well as of a few additional validated metabolic targets (eg, CPT1α). However, while circulating HDL-C levels were increased during the initial 2-week chow-feeding phase of treatment, the authors surprisingly found that this antimiR-33–dependent increase was not sustained after switching to a high-cholesterol western-type diet for 12 weeks.

It is tempting to speculate that this lack of an effect of anti-miR-33 on HDL-C levels in animals fed a high-fat/cholesterol diet could be owing to homeostatic feedback mechanisms, as metabolic homeostasis is maintained by highly regulated and intricate feedback and feed-forward circuits (eg, cholesterol feedback inhibition of the SREBP2 circuit). Perhaps an intermittent treatment regime should be explored to ascertain whether the potential impact of negative feedback loops that may limit anti-miR-33 efficacy could be diminished? Alternatively, the findings by Marquart et al[12] suggest that future studies should investigate whether anti-miR-33 treatment might be most efficacious when paired with dietary modification (eg, switching to a low-fat/cholesterol diet, as in Rayner et al[8]).

In addition to a lack of sustained effect on HDL-C levels, the Marquart et al[12] study also reported the surprising finding that circulating triglycerides were significantly elevated at the end of the 12-week anti-miR-33 treatment. Moreover, the body weight of animals seemed to increase, although the trend was not statistically significant. Finally, and most importantly, there was also no significant change in the progression of atherosclerosis after the 12-week anti-miR-33 treatment in this model on a high-fat, high-cholesterol atherogenic diet. Although the lack of impact on atherosclerosis by anti-miR-33 treatment is consistent with the absence of an effect on HDL-C levels, the rise in triglycerides is difficult to explain, especially as the fatty acid β-oxidation factor and miR-33 target CPT1α is elevated in the liver of anti-miR-33–treated animals.

Altogether, these findings raise concerns about the efficacy and safety of anti-miR-33 treatment as a therapeutic approach to treat atherosclerosis and cardiovascular disease. Clearly, additional studies are needed to understand at a mechanistic level why anti-miR-33 efficacy is limited under these conditions in the LDLr KO model, and the molecular underpinnings of unwanted consequences, such as elevated triglycerides and body weight.

There are several caveats, however, to these studies. First, a recent study by Horie et al[13] showed that crossing miR-33 KO mice with ApoE null animals not only led to elevated circulating HDL-C but also strongly decreased atherosclerosis normally seen in $ApoE^{-/-}$ mice fed a western-type diet. These results are consistent with a role for miR-33 in contributing to progression of atherosclerosis in this model. By contrast with the Marquart et al[12] study, Horie et al[13] also did not observe a statistically significant increase in

triglycerides in the miR-33/ApoE double KO animals on a western-type diet. Together, this suggests either that different mouse atherosclerosis models could have distinct responses to miR-33 antagonism or that the KO of miR-33 may produce effects different from antisense inhibition.

The second caveat is that most mammals, including humans and nonhuman primates, harbor 2 miR-33 isoforms, whereas mice and other rodents only have miR-33a. This is significant for several reasons. First, miR-33b is coexpressed with the insulin-regulated SREBP 1c host gene[14] and is likely coelevated in insulin-resistant metabolic syndrome patients.[15] Second, miR-33b differs from miR-33a by 2 nucleotides and is likely to have a somewhat different target profile, including stronger effects on targets involved in regulation of fatty acid/triglyceride homeostasis and insulin signaling.[6,11,14,16] Hence, as the authors note, the relevance of miR-33 antagonism data from rodent models to human physiology and metabolic disorders is unclear. Indeed, in marked contrast to the Marquart et al[12] study in this issue, the recent nonhuman primate study of Rayner et al,[11] which is also longitudinal, did show a sustained elevation of HDL-C over 12 weeks in response to miR-33a/b antisense antagonism, as well as a strong decrease in triglycerides. However, it should be noted that the African green monkeys in this study were metabolically normal and were fed either normal chow or a high-carbohydrate diet, and the long-term effects of miR-33 antagonism in nonhuman primates with cholesterol/lipid abnormalities and fed an atherogenic diet on circulating HDL-C and triglyceride levels, as well as atherosclerosis, are unknown.

To conclude, although miR-33–targeting antisense oligonucleotides as a novel atherosclerosis therapeutic strategy has raised considerable excitement in the cardiovascular field, the study by Marquart et al[12] in this issue of *Arteriosclerosis, Thrombosis and Vascular Biology* suggests that caution is warranted and that much additional work is needed to establish whether miR-33 antagonism may indeed represent an efficacious and safe atherosclerosis treatment in humans.

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