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Micromanaging cholesterol

By Lev Osherovich, Senior Writer

Two reports from independent teams in New York and Boston have unveiled the function of microRNA-33 and describe how the miRNA helps fine-tune high-density lipoprotein cholesterol levels both in the liver and in macrophages found in atherosclerotic plaques.[1,2] The findings open a new opportunity to increase levels of good cholesterol using RNA-disrupting therapeutics; attempts to do this with small molecules have not yet resulted in a marketed drug.

MicroRNA-33 (miR-33) is an miRNA tucked within introns of the gene encoding the cholesterol-regulating transcription factor sterol regulatory element binding protein (SREBP). The new research shows that miR-33 helps to raise intracellular cholesterol by blocking the production of ATP-binding cassette sub-family A member 1 (ABCA1), a protein that exports high-density lipoprotein (HDL) cholesterol.

"We've identified a new way cholesterol homeostasis is regulated," said Kathryn Moore, associate professor of medicine and cell biology at the **New York University School of Medicine** and a coleader of one of the studies.

"The discovery of this unexpected player in regulating HDL levels could open a new therapeutic area of RNA-targeting therapies," said Anders Näär, associate professor of cell biology at **Harvard Medical School** and **Massachusetts General Hospital**, who led the other team.

Peter Linsley, CTO of miRNA company **Regulus Therapeutics Inc.**, said the studies provide one of the first examples of coordinated activity of a protein and an miRNA encoded by the same gene, hinting at an essential supporting role for miR-33 in regulating cholesterol levels.

"It's quite common to find miRNAs in introns, but it's not often that there's such a clear link between the miRNA and the protein-coding region," said Linsley.

Regulus is collaborating with the NYU team to study the feasibility of blocking miR-33. Other companies focused on miRNA targets also are following the miR-33 story to see whether the target can be validated in disease models.

Gaze into the miR

The two teams converged on miR-33 from different directions. The group co-led by Moore and Carlos Fernández-Hernando, an assistant professor of medicine and cell biology at NYU, came upon miR-33 in a screen for miRNAs that influenced cholesterol levels. Näär's group noticed miR-33 while studying *SREBP* gene sequences from flies, mice and humans.

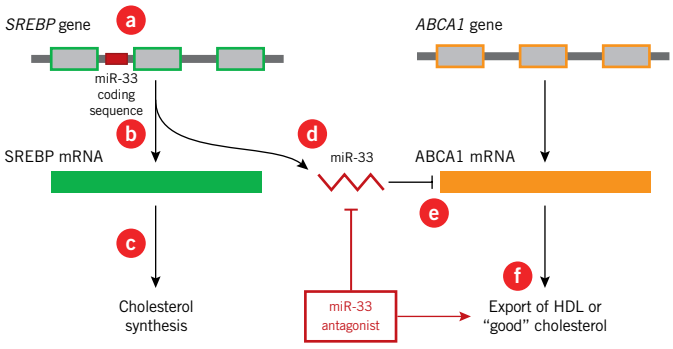

Figure 1. Fine-tuning cholesterol metabolism with microRNA. Studies by Najafi-Shoushtari *et al.* and Rayner *et al.* worked out how microRNA-33 (miR-33) regulates cholesterol metabolism.

The *sterol regulatory element binding protein* (*SREBP*) gene [**a**] consists of exons (light grey rectangles) separated by introns (dark grey lines), which harbor the miR-33 coding sequence (red rectangle) within the intronic sequence. When *SREBP* is transcribed [**b**], the exons are spliced together to produce the mRNA that codes for SREBPs, which go on to upregulate the biosynthesis of cholesterol [**c**]. Meanwhile, the introns are processed to produce miR-33 [**d**]. The miRNA binds to the mRNA for ATP-binding cassette sub-family A member 1 (ABCA1) and inhibits its translation [**e**]. As a result, ABCA1 protein levels decrease, reducing the export of high-density lipoprotein (HDL) cholesterol [**f**].

Rayner *et al.* used antisense oligonucleotides to block miR-33 and thus restore ABCA1 activity and raise HDL cholesterol levels in mice. Najafi-Shoushtari *et al.* obtained similar results using locked nucleic acid (LNA) oligonucleotides.

Both teams confirmed that miR-33 is expressed in the liver, pancreas and macrophages, which are innate immune cell components of atherosclerotic plaques.

miRNAs typically downregulate protein production by binding to cognate sequences in their mRNA targets, triggering mRNA degradation. Thus, the teams scoured the genome for miR-33 target sites and converged on *ABCA1*.

Prior work had shown that when levels of all types of cholesterol are low, SREBP turns on cholesterol synthesis.[3] As a check, ABCA1 acts counter to SREBP by pumping cholesterol out of cells in the form of HDL particles.

The researchers therefore anticipated that because miR-33 and SREBP are made from a common transcript, they would work toward the same goal of raising cholesterol levels, and so miR-33 would antagonize ABCA1.

Indeed, in murine and human cell cultures, overexpression of miR-33 reduced levels of both ABCA1 protein and extracellular HDL. Conversely, knockdown of miR-33 increased ABCA1 protein and stimulated HDL secretion compared with normal miR-33 expression. Both teams obtained comparable *in vivo* results with miR-33 overexpression and knockdown in mice.

In addition, Näär's team reported that miR-33 knockdown did not affect low-density lipoprotein (LDL) cholesterol or triglyceride levels.

The findings suggest that SREBP and miR-33 work together to raise intracellular cholesterol by increasing its synthesis and reducing its export, respectively (*see* **Figure 1, "Fine-tuning cholesterol metabolism with microRNA"**).

"The discovery of this unexpected player in regulating HDL levels could open a new therapeutic area of RNA-targeting therapies."

—Anders Näär, Harvard Medical School

miR mortals

Näär, Moore and Fernández-Hernando suspect that because naturally high HDL levels protect against atherosclerosis and other cardiovascular diseases, blocking miR-33 could help raise HDL and improve cardiovascular health. However, efforts to raise HDL levels by targeting various components of the cholesterol biosynthesis and transport machinery with small molecules haven't yet borne fruit in the clinic.

One advantage of targeting miR-33 over other cholesterol-related targets is that the miRNA "appears to be a fundamental player in homeostasis," said William Marshall, president and CEO of **miRagen Therapeutics Inc.** "Here, you have an opportunity to go after a whole pathway."

miRagen is developing antagonists of miRNAs involved in cardiac disease. The company's lead compounds, which are in preclinical development, target miRNAs associated with myocardial infarction (MI) and chronic heart failure.

In contrast, small molecules in development to increase HDL inhibit specific enzymes involved in cholesterol synthesis and transport.

"Blocking miR-33 would raise HDL by a different mechanism than previously attempted, such as cholesteryl ester transfer protein (CETP) inhibitors," said Moore.

CETP moves cholesterol and triglycerides from HDL to LDL particles, so blocking the enzyme raises HDL levels and lowers LDL levels, which is thought to protect against atherosclerosis and coronary artery disease (CAD).

Late-stage CETP antagonists include anacetrapib (MK-0859) from **Merck & Co. Inc.** and dalcetrapib (JTT-705; R1658; RG1658) from **Japan Tobacco Inc.** and **Roche**. Both are in Phase III trials for dyslipidemia and atherosclerosis. In 2006, **Pfizer Inc.** discontinued development of its torcetrapib CETP inhibitor following a failed Phase III trial in dyslipidemia.

Moore and Fernández-Hernando told *SciBX* that Regulus is collaborating with the NYU team to test more drug-like antisense inhibitors of miR-33.

Arthur Levin, chief development officer and president of U.S. operations for **Santaris Pharma A/S**, thinks miR-33 could fit nicely into the company's portfolio of cardiovascular miRNA compounds.

Santaris has preclinical programs targeting the mRNAs encoding apolipoprotein B (APOB) and proprotein convertase subtilisin kexin type 9 (PCSK9), two proteins that influence LDL levels. Santaris' locked nucleic acid (LNA) technology uses synthetic, degradation-resistant RNA analogs that have high binding affinity to complementary RNA and better tissue uptake than conventional small interfering RNA.

"The Näär paper is especially interesting because they use a locked nucleic acid" to inhibit miR-33, Levin noted.

Although Santaris was not involved in the two studies, the proof of principle achieved by Näär's team using off-the-shelf, research-grade LNAs "begs the question of how to get a drug like this into our portfolio," said Levin. "This is a nice new mechanism of action that is perfectly suited to our technology."

Levin noted that the presence of miR-33 in macrophages as well as in the liver makes the miRNA an especially enticing target, as macrophages are thought to be critical players in inflammation and the structural stability of atherosclerotic plaques. He noted that LNAs are readily taken up by macrophages and liver cells.

Linsley agreed that the tissues in which miR-33 is active are among the most readily targetable with nucleic acid therapeutics. "The liver is the sweet spot for oligonucleotides" such as those used by Regulus, he noted.

Linsley, Levin and Marshall all said the next step is to show that miR-33 antagonists have good pharmacodynamics and that knocking the miRNA down has a disease-modifying effect in mouse models of cholesterol-associated diseases like atherosclerosis.

Näär, Moore and Fernández-Hernando said such experiments are under way, and both groups hope to test the effect of miR-33 knockdown in nonhuman primates.

Both teams have filed patents on their discoveries, which are available for licensing.

Osherovich, L. *SciBX* 3(21); doi:10.1038/scibx.2010.635
Published online May 27, 2010

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