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HDL *miR*-ed Down by *SREBP* Introns

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Abstract

Animal cells must maintain a membrane cholesterol-to-phospholipid ratio within tight limits for normal function. Elaborate mechanisms control the cellular input of cholesterol from endogenous synthesis or uptake from plasma lipoproteins. Much less is known about the factors that regulate the output of cholesterol from cells. On pages 1566 and 1570 of this issue, Najafi-Shoushtari et al. (1) and Rayner et al. (2) show that cholesterol output is controlled by the same genes that regulate cholesterol input, but in a reciprocal manner and through an unexpected mechanism.

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The regulatory genes in question direct the synthesis of sterol regulatory element-binding proteins (SREBPs), which are membrane-bound transcriptional activators (3). Vertebrates have two *SREBP* genes. *SREBP-2* preferentially activates the synthesis and uptake of cholesterol, whereas *SREBP-1* preferentially activates the synthesis of fatty acids (4). Najafi -Shoushtari *et al.* and Rayner *et al.* reveal that both genes also encode, within their introns, a microRNA (*miR-33*) that has the reciprocal effect. *miR-33* blocks the egress of cholesterol from cells by reducing the mRNA and protein levels for ABCA1, a transporter in the plasma membrane that secretes cholesterol from cells (5). When cells are depleted of cholesterol, both the transcription of *SREBP*s and the intron-encoded *miR-33* rise modestly.

SREBP-1 and *SREBP-2* encode *miR-33b* and *miR-33a*, respectively, which differ in only 2 of 19 nucleotides that constitute the mature microRNA. Both *miR-33* isoforms target for destruction several mRNAs—most prominently the mRNA encoding ABCA1— that contain a highly conserved target sequence in their 3′-untranslated regions. When cultured mammalian cells were transfected with *miR-33*, the amount of mRNA encoding ABCA1 decreased, whereas the opposite occurred when cells were transfected with RNA molecules that specifically reduced *miR-33* levels.

ABCA1 functions most prominently in macrophages and hepatocytes (5). In macrophages, it excretes cholesterol that accumulates as a result of the uptake of oxidized cholesterol-carrying lipoproteins. In liver, ABCA1 is essential for the production of the precursor forms of high-density lipoprotein (HDL). Indeed, Najafi -Shoushtari *et al.* and Rayner *et al.* show that delivery of a *miR-33* antagonist leads to a small but significant increase in plasma HDL. So far, the most remarkable feature of the *miR-33a/b* story is the pattern of evolutionary conservation. The precursor for mature *miR-33a* is found within the same intron of *SREBP-2* from many animal species, including large and small mammals, chickens, and frogs. There is even a perfectly conserved mature form of *miR-33a* in the single *SREBP*-like gene of the fruit fly *Drosophila*

melanogaster. The latter is most remarkable because insects do not synthesize sterols. Their single *SREBP* gene controls fatty acid production (6). Moreover, the fruit fly genome does not contain *ABCA1*, so the target of *miR-33a* in *Drosophila* is unknown.

In contrast to the uniform conservation of *miR-33a* in *SREBP-2*, there is a gap in the evolutionary conservation of *miR-33b* in *SREBP-1* (according to the U.S. National Center for Biotechnology Information database). The *SREBP-1* genes from large mammals encode *miR-33b*, but there is no trace of *miR-33b* in the *SREBP-1* genes of small mammals (rats and mice) or chickens.

Although the amount of mature *miR-33a* rises and falls in concert with *SREBP-2* mRNA, the amplitude of variation is quite small in the systems studied by Najafi-Shoushtari *et al.* and Rayner *et al.* This is likely because variations in cellular cholesterol levels cause relatively minor changes in the transcription of the *SREBP* genes. Cholesterol regulates SREBP activity most profoundly at the level of protein processing (3). SREBPs are synthesized as membrane proteins in the endoplasmic reticulum and transported to the Golgi complex, where they are proteolyzed to release active fragments that enter the nucleus. There, they enhance transcription of cholesterol-synthesizing genes, such as those encoding 3-hydroxy-3-methylglutaryl coenzyme A (HMG CoA) synthase and HMG CoA reductase. When cells are depleted of cholesterol, the level of nuclear SREBP-2 increases by orders of magnitude owing to increased proteolytic processing, and mRNAs encoding HMG CoA synthase and reductase increase correspondingly (4). By contrast, the mRNA encoding SREBP-2 increases by less than a factor of 2, explaining why *miR-33a* also shows relatively small changes. Whether or not such small changes influence plasma HDL in humans is yet to be determined.

One circumstance in which transcription of an *SREBP* gene is profoundly regulated in vivo and where changes in *miR-33* are likely to be important clinically is in the liver (see the figure). Hepatocytes produce two alternatively spliced transcripts of *SREBP-1*, called *SREBP-1a* and *SREBP-1c*. The promoter and first exon of *SREBP-1c* differ from those of *SREBP-1a*, the isoform that predominates in most nonhepatic tissues (7). Transcription of *SREBP-1c* in liver is enhanced by insulin, working in concert with nuclear liver X receptors (8,9). When insulin levels are high, *SREBP-1c* is transcribed at extremely high levels, and the resultant nuclear SREBP-1c activates genes necessary to produce fatty acids, which are incorporated into triglycerides (4). As a result, in states of hyperinsulinemia, the liver becomes engorged with fat, and plasma triglyceride levels rise. The usual cause of hyperinsulinemia is peripheral insulin resistance, which leads to hyperglycemia and enhanced insulin secretion. Inasmuch as *miR-33b* is encoded in human (but not in rodent) *SREBP-1c*, the hepatic level of *miR-33b* would be predicted to be markedly elevated in insulin-resistant states in humans, but not in mice and rats.

In humans, insulin resistance is a hallmark of metabolic syndrome, which is provoked by obesity (10). In addition to hyperinsulinemia, hyperglycemia, and fatty liver, cardinal features of metabolic syndrome include an increase in plasma triglyceride levels, owing to elevated very-low-density lipoproteins (VLDL), and a decrease in plasma HDL. Low HDL is believed to contribute to the increase in coronary heart disease in these subjects. Evidence suggests that the hypertriglyceridemia is caused by the insulin-induced increase in *SREBP-1c* mRNA and protein. Is it possible that the reduction in HDL is caused by a decrease in ABCA1, owing to the increased production of *miR-33b* from the insulin-stimulated *SREBP-1c* gene?

Unfortunately, this question cannot be answered by study of hepatic *miR-33b* in the usual models of insulin resistance in obese rats or mice, because the *SREBP-1c* genes of these model animals lack *miR-33b*. This is consistent with the observation that obese insulin-resistant mice manifest all of the cardinal features of metabolic syndrome except a reduction in HDL (11).

Here is a situation where geneticists may come to the rescue, by searching for mutations in *miR-33b* in patients with metabolic syndrome who manifest inappropriately elevated HDL. Alternatively, the hypothesis can be tested by treating metabolic syndrome subjects with agents that antagonize *miR-33b* and testing for increased HDL.

Acknowledgments

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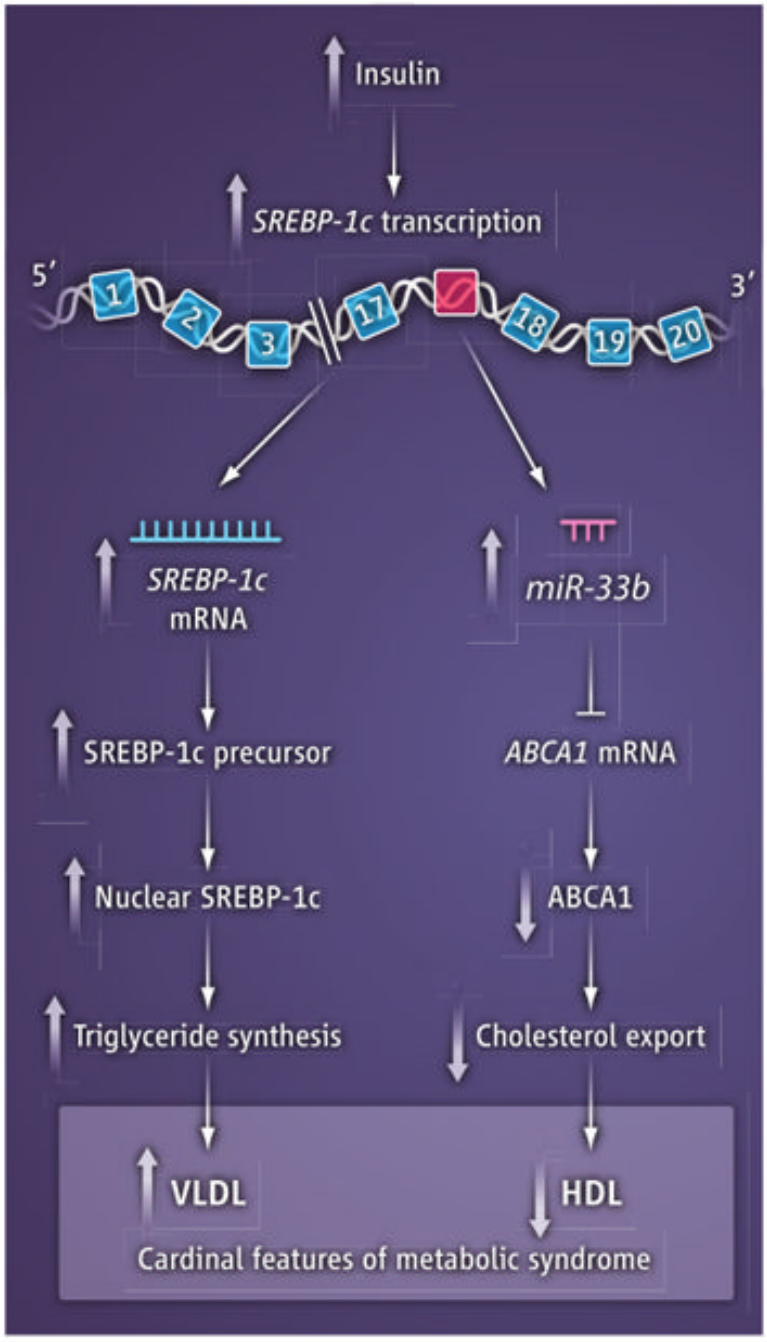

1. . Potential dual role of SREBP in metabolic syndrome

In obese subjects with metabolic syndrome, insulin resistance leads to elevated insulin, which activates transcription of *SREBP-1c* in the liver. This leads to increased VLDL triglycerides and decreased HDL cholesterol in the blood. *miR-33b* is encoded in intron 17 (red); exons are in blue.