SREBF2-Embedded mir33 Links the Nuclear Bile Acid Receptor FXR to Cholesterol and Lipoprotein Metabolism

Philippe Lefebvre, Bart Staels

In their article, published in the present issue, Tarling et al1 identify a novel regulatory loop of hepatic cholesterol biosynthesis and export in mice involving the nuclear bile acid receptor FXR/NR1H4. Through transcriptional regulation of the Sreb2 gene and its intronic microRNA mmu-miR33, FXR is now shown to participate in cholesterol homeostasis by post-transcriptional silencing of mir33 targets, including Abca1, a major determinant of hepatic high-density lipoprotein (HDL) production. This mechanism reveals another level of integration of lipid and cholesterol metabolism by FXR, as this bile acid–activated transcription factor is already known to control the expression of genes involved in triglyceride metabolism and the enterohepatic cycling of bile acids, which are cholesterol catabolites.

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Cholesterol is an essential component of cell membranes and a precursor of numerous signaling molecules, from steroid hormones to bile acids. Its synthesis, dietary absorption, and distribution throughout the body are tightly regulated processes as elevated cholesterol concentrations, notably in the low-density lipoprotein (LDL) fraction, increases the risk for cardiovascular disease. The basic helix-loop-helix leucine zipper transcription factors, sterol-regulatory element binding proteins (SREBPs), are key regulators of fatty acid and cholesterol homeostasis. Activated at low cellular cholesterol concentrations through a mechanism involving a sequential docking/release from the Golgi to the endoplasmic reticulum, they undergo proteolytic cleavage and subsequent nuclear translocation. Whereas the nuclear SREBP1 (nSREBP1) isoforms mainly regulate genes of the lipogenic pathway, nSREBP2 primarily regulates genes controlling cholesterol synthesis, such as HMG-CoA (3-hydroxy-3-methylglutaryl-coenzyme A) reductase, and uptake, such as the LDL receptor. Interestingly, the human SREBP–encoding loci SREBF1 and SREBF2 also contain cotranscriptionally regulated miRNAs, respectively, miR-33b and miR-33a, the latter being evolutionary conserved. MiR33a plays a modulatory role in cholesterol homeostasis in rodents and primates, as its target transcripts include notably Abca1 and Abcg1 (Figure).

The authors identified, by analyzing previously published mouse liver FXR ChIP-Seq (chromatin immunoprecipitation followed by sequencing) data, an intronic FXR binding site within the Sreb2 locus, which confers FXR responsiveness to Sreb2 and mmu-miR33a. However, the FXR-mediated accumulation of the Sreb2 transcript was accompanied by neither increased nSREBP2 protein nor cholesterol biosynthesis target genes. This uncoupling between induction of Sreb2 transcription and nSREBP2 production is likely the result of FXR-induced transcription of Insig2a, a known FXR target gene whose product traps SREBPs into the endoplasmic reticulum. Whether mmu-miR33a transcription stems from a transcription unit independent of Sreb2 was investigated indirectly using liver-deficient Scap mice. SREBP cleavage activating protein (SCAP) deficiency phenotypically translates into impaired SREBP processing and low levels of nSREBP. In this background, mmu-miR33a was not responsive to FXR activation, suggesting a cotranscriptional regulation of Sreb2 and of mmu-miR33a. In vivo, FXR activation decreased plasma cholesterol, mainly through a decrease in HDL-cholesterol in the mouse (this work and Ref. 8), an effect also reported in humans. Previous studies by the same authors identified the evolutionary conserved mmu-miR-144 as a FXR-regulated gene targeting Abca1 and hence participating also in the decrease of plasma HDL-cholesterol on FXR activation. In liver-depleted SCAP mice, FXR activation still induced a significant decrease of HDL-cholesterol, suggesting that the mmu-miR33a pathway is modulatory rather than dominant in the control of HDL-cholesterol production and synergizes with mmu-miR-144.

This study thus adds another layer to the regulation of hepatic and plasma cholesterol homeostasis by miRNAs, to which miR-122a, miR-223, and miR370 also contribute. Interestingly, miR-370 regulates the expression of miR-122a and miR122a is in addition controlled by the nuclear receptor REV-ERB-α/NR1D1, which participates in the circadian regulation of bile acid synthesis. It is worth noting that the miR122a locus contains FXR binding sites in the liver, but not in the intestine (PL and BS, unpublished data). How these regulatory pathways operate in pathological conditions has not yet been studied, but is worth investigating as the FXR cistrome seems to be altered in obesity, hence potentially altering FXR-mediated regulation of miR33a. Another pending question is whether this network operates in the intestine, which also contributes to lipoprotein and HDL production, in which FXR is abundantly expressed and binds in the vicinity and within the Sreb2 locus (PL and BS, unpublished data). Similarly, cholesterol efflux from macrophages is an ABCA1 (ATP-binding cassette sub-family A member

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findings in rodent models are thus eagerly awaited. Statins. Translational studies examining the relevance of the activation may interfere with the LDL-lowering activity of HMG-CoA reductase inhibition, the uncoupling of subsequent LDL-receptor activation is a major response to Because induction of SREBP2 transcription activity and obeticholic acid significantly increases LDL-cholesterol. Finally, in addition to a slight decrease in HDL-cholesterol, treatment of humans with the semisynthetic FXR agonist unlike mice, also express the SREBF-1–coregulated miR33b. In human macrophages, it remains to be established whether the observed modulation of HDL-cholesterol on FXR activation in mice faithfully predicts the response in humans, which, unlike mice, also express the SREBF-1–coregulated miR33b. Finally, in addition to a slight decrease in HDL-cholesterol, treatment of humans with the semisynthetic FXR agonist obeticholic acid significantly increases LDL-cholesterol. Because induction of SREBP2 transcription activity and subsequent LDL-receptor activation is a major response to HMG-CoA reductase inhibition, the uncoupling of Sreb2 transcription and nSREBP2 accumulation suggests that FXR activation may interfere with the LDL-lowering activity of statins. Translational studies examining the relevance of the findings in rodent models are thus eagerly awaited.

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Disclosures
Dr Staels is a member of the Institut Universitaire de France. The other author reports no conflicts.

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