Editorial

The Mystery of PCSK9

Alan D. Attie

Many of the most important breakthroughs in science do not come from hypothesis-driven experiments. Apart from serendipity, genetics is perhaps our best route to discoveries that elude our intuition. Genetics establishes a relationship between a gene and a phenotype, but does not necessarily provide mechanistic information. The emerging story of PCSK9 (originally called Narc-1) provides a case in point.

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Last year, Abifadel and coworkers mapped a region on human chromosome 1 that segregated with autosomal dominant hypercholesterolemia in French families.[1] In a region containing 41 genes, they identified PCSK9 as a candidate gene. They found two missense mutations, S172R and F216L. Subsequently, a D374Ymutation was also detected in an unrelated Norwegian kindred[2] and in Utah pedigrees.[3]

PCSK9 is a member of the proprotein convertase family of proteases, most closely related to proteinase K.[4] Apart from its expression in liver and neuronal tissue, it is also expressed in kidney mesenchymal cells and intestinal epithelia.[4] It is induced during liver regeneration and neuronal differentiation.[4] Its substrate specificity is different from many other proprotein convertases because it can cleave at nonbasic amino acids, a feature it shares with subtilisin-kexin isozyme-1/site 1 protease, although the latter enzyme requires a basic residue at position -4. Presently, the only known substrate of PCSK9 is itself; it autocatalytically cleaves its own propeptide between Gln-151 and Ser-152 (rat sequence).[5]

The PCSK9 gene is regulated by sterols. Indeed, dietary cholesterol potently suppresses its expression.[6] Transgenic mice overexpressing the transactivation domain of SREBP-1a or SREBP-2 also showed substantial upregulation of PCSK9.[6] The SREBP-1c isoform is regulated by both insulin[7] and the liver X receptor (LXR) transcription factor.[8] PCSK9 was upregulated by a synthetic LXR agonist, TO901317, suggesting that it is regulated by the SREBP-1c isoform.[6] Horton et al identified genes upregulated in the aforementioned SREBP transgenic mice and downregulated in mice lacking the sterol responsive element cleavage activating protein (SCAP), an escort protein required for SREBP activation.[9] Among these genes was PCSK9.

The function of PCSK9 remains elusive. Maxwell and Breslow recently reported that adenoviral expression of the wild-type form of PCSK9 decreased the abundance of the low-density lipoprotein (LDL) receptor protein, but not its mRNA.[10] They did not study the effect of a mutant form of PCSK9, leaving unanswered the question of whether overexpression of mutant PCSK9 results in the same phenotype as overexpression of the wild-type allele. A further mystery is how PCSK9 affects the abundance of a protein that is apparently not a substrate for the protease.

Hobbs and coworkers identified a protein responsible for another syndrome affecting LDL receptor function, autosomal recessive hypercholesterolemia, ARH.[11] The protein is apparently essential for the internalization of the LDL receptor in lymphocytes and hepatocytes, but not fibroblasts.[12,13] Interestingly, a recent study in lymphocytes from ARH patients shows that it regulates the ability of the receptor to bind to LDL, which binds to the receptor through apolipoprotein (apo)B, but not β-VLDL, which binds through apoE.[14] Thus, we have a precedent for a protein regulating the function of the LDL receptor in at least two steps of its itinerary. In addition, the ARH precedent establishes that the activity of the LDL receptor can be modulated in trans.

In this issue of *Arteriosclerosis, Thrombosis, and Vascular Biology*, Dubuc et al show that statins, inhibitors of cholesterol synthesis, upregulate the expression PCSK9 in the human hepatoma cell line HepG2.[15] Statins are known to upregulate the expression of the LDL receptor,[16] thus these results imply that statins might simultaneously reduce the abundance of the LDL receptor protein if their induction of PCSK9 is sufficient. These contrary effects resemble the dual effects of cholesterol on SREBP-1c; increased transcriptional activation through LXR and decreased production of the active mature fragment of the protein. Because statins depend on the presence of the LDL receptor for their LDL-lowering ability[17] and the extent of cholesterol lowering correlates with the abundance of its mRNA,[16] the exact "status" of the LDL receptor under these conditions bears reexamination, especially in light of the recent results from the Hobbs laboratory.[14]

The lipoprotein profile of patients with PCSK9 mutations closely resembles that of individuals with other forms of hypercholesterolemia; those with mutations in the LDL receptor, apolipoprotein B, or ARH. These individuals do not have hypertriglyceridemia, and most of the cholesterol elevation is caused by an increase in LDL particles. Oouguerrram et al recently studied lipoprotein kinetics in two subjects with the S127R mutation in PCSK9. They found a 30% reduction in the fractional clearance rate (FCR) of LDL relative to eleven control subjects. The most striking result was a 3-fold increase in the production rate of VLDL apoB, along with a $\approx$2-fold increase in the production rate of LDL apoB.

From the Department of Biochemistry, University of Wisconsin-Madison, Wisc.

Correspondence to Alan D. Attie, Department of Biochemistry, University of Wisconsin-Madison, 433 Babcock Dr, Madison, WI 53706-1569. Email attie@biochem.wisc.edu

Can all of the lipoprotein phenotypes seen in the patients with mutant PCSK9 be explained as a consequence of impaired LDL receptor abundance and/or activity? The in vivo lipoprotein turnover data fall well within the range of measurements that have been made in familial hypercholesterolemia patients with LDL receptor mutations. Most studies have found a reduced clearance rate of LDL of ≈30% in heterozygotes and ≈65% in homozygotes, as seen in the early study of Bilheimer et al.[18] Many studies have also reported an increase in apparently "direct" LDL apoB production; ie, without a traceable VLDL apoB precursor pool.[19–23]

Whether or not there is an increase in VLDL apoB production in FH patients, as seen by Cummings et al,[24] has been more controversial. The early lipoprotein turnover studies did not characterize the nature of the LDL receptor mutations. A recent study by Tremblay et al in subjects homozygous for LDL receptor-null alleles showed a 2-fold increase in VLDL apoB production.[25] Several studies found increased VLDL apoB secretion in LDL receptor-null mice.[26–29] Twisk et al predicted that this would occur in patients with null or binding-defective LDL receptors, but not necessarily in those with receptors that stall in the secretory pathway.[27] A study by Millar et al in LDL receptor-null mice concluded that VLDL production is not increased.[30] This study used injection of Triton-WR-1339 to block VLDL clearance under the premise that it has no effects on hepatic lipoprotein production. However, Triton WR-1339 stimulates apoB secretion in wild-type hepatocytes (Horton and Attie, unpublished observations), thus would tend to make the wild-type and mutant mice have similar apparent apoB production rates and thus could explain the negative results of Millar et al.

The sterol responsiveness of the PCSK9 promoter makes it plausible that the wild-type protein plays a role in lipid metabolism. Unlike ARH, mutations in PCSK9 act in a dominant fashion to produce hypercholesterolemia, suggesting that the mutant allele has potentially gained a new function. Several of the mutant alleles that have been studied accumulate the proform of the enzyme, thus the novel function might be caused by that form or by a deficiency of the enzyme itself. A novel function could involve an adventitious interaction with another protein, a phenomenon that has been reported for one of the superoxide dismutase mutations that causes amyotrophic lateral sclerosis.[31] Together with ARH, PCSK9 expands the tableaux for a much more complex picture of the LDL receptor and its role in lipoprotein metabolism.

Acknowledgments

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