Abstract The identification and characterization of proprotein convertase subtilisin-like/kexin type 9 (PCSK9) have provided new insights into LDL metabolism and the causal role of LDL in coronary heart disease (CHD). PCSK9 is a secreted protease that mediates degradation of the LDL receptor by interacting with the extracellular domain and targeting the receptor for degradation. Individuals with loss-of-function mutations in PCSK9 have reduced plasma levels of LDL cholesterol and are protected from CHD; these observations have validated PCSK9 as a therapeutic target and suggested new approaches for the treatment and prevention of CHD.—Horton, J. D., J. C. Cohen, and H. H. Hobbs. PCSK9: a convertase that coordinates LDL catabolism. J. Lipid Res. 2009. 50: S172–S177.

Supplementary key words low density lipoprotein receptor • proprotein convertase subtilisin-like/kexin type 9 • low density lipoprotein • hypercholesterolemia

For the past four decades, characterization of patients with genetic disorders of lipid metabolism has revealed key components of the molecular machinery involved in biosynthetic and regulatory pathways that produce, transport, and eliminate cholesterol. These findings have led to the development of potent cholesterol-lowering drugs that reduce coronary heart disease (CHD). The identification of new forms of genetic hyperlipidemias continues to reveal novel participants, as exemplified by the discovery that mutations in proprotein convertase subtilisin/kexin type 9 (PCSK9) have profound effects on plasma levels of LDL cholesterol (LDL-C).

Here, we focus on advances in PCSK9 biology and structure during the last 2 years.

MISSENSE MUTATIONS IN PCSK9 REVEAL A KEY REGULATOR OF LDL METABOLISM

In 2003, Abifadel et al. (1) identified three families with autosomal dominant hypercholesterolemia and premature CHD caused by missense mutations in the ninth member of the proprotein convertase family, PCSK9. PCSK9 encodes a 692 amino acid protein that is expressed predominantly in liver, intestine, and kidney (2). The protein contains a signal sequence, a prodomain (amino acids 31–152), a catalytic domain (amino acids 153–451), and a C-terminal domain (amino acids 452–692) that are rich in cysteines and histidines (Fig. 1A). Overexpression of PCSK9 in livers of mice markedly reduces hepatic LDL receptor (LDLR) protein (but not mRNA) levels, causing hypercholesterolemia (3). This finding suggested that the missense mutations identified by Abifadel et al. (1) conferred a gain-of-function to the mutant protein. Subsequent studies revealed that inactivation of PCSK9 in humans and mice resulted in hypocholesterolemia (3).

PHYSIOLOGY OF PCSK9

Cellular itinerary of PCSK9

PCSK9 is synthesized as a 73 kDa zymogen in the endoplasmic reticulum (ER) and is modified en route to the cell surface (Fig. 1B). The protein undergoes autocatalytic cleavage between residues 152 and 153 (FAQ152↓SIP). The N-terminal prodomain remains tightly associated with the 63 kDa mature protein and acts as a chaperone to transport PCSK9 through the secretory pathway (4). A mutation in PCSK9 (C679x) that prevents folding of the C-terminal domain does not prevent autocatalytic cleavage, suggesting that cleavage is cotranslational (3). After cleavage, the last four amino acids of the prodomain blanket the catalytic triad, thereby restricting access to potential substrates. PCSK9 undergoes a series of posttranslational modifications, including glycosylation (4), phosphorylation (5), and tyrosine sulfation (6) (Fig. 1A). None of these modifications is required for secretion of PCSK9, and their role in PCSK9 function remains obscure.
Circulating levels of PCSK9

Mice in which PCSK9 has been selectively inactivated in liver have no detectable PCSK9 in the blood, suggesting that the liver is the major source of circulating PCSK9 (2). Recently, several laboratories have developed ELISAs to measure plasma PCSK9 levels in humans (3, 7–9). The mean concentration of PCSK9 varies widely between these assays (ranging from 500 ng/ml to 4 μg/ml) likely due to differences in antibody specificities and the standards used in the assays. PCSK9 levels correlate with LDL-C (r = 0.3–0.6) but not HDL-C (8, 9). Plasma levels of LDL-C and PCSK9 may be directly related because expression of PCSK9 promotes the degradation of hepatic LDLRs. The levels of these two proteins are not invariably coupled; treatment with high-dose statins reduces plasma levels of LDL-C but increases levels of circulating PCSK9 (8).

Kinetics of PCSK9 clearance

Recombinant PCSK9 has a half-life of ~5 min in the blood of wild-type mice (10). Inactivating the LDLR increases the half-life of PCSK9 to ~15 min, implicating LDLR as a major conduit for PCSK9 removal (10). The rapid clearance of PCSK9 from plasma, even in Ldlr−/− mice, indicates that the rate of PCSK9 synthesis must be high, given the low plasma concentrations of PCSK9.

Site of action of PCSK9

The effect of PCSK9 on LDL-C levels appears to be mediated solely through LDLRs since inactivation of Pck9 does not reduce plasma cholesterol levels in Ldlr−/− mice (2). Parabiosis experiments in wild-type and PCSK9 transgenic mice demonstrate that circulating PCSK9 can mediate degradation of LDLRs in the liver (3). Infusion of recombinant human PCSK9 into mice to levels comparable with those in human plasma caused a significant reduction of hepatic LDLRs (10). These experiments support PCSK9 acting primarily at the cell surface, although it remains possible that the protein interferes with the movement of the LDLR in the secretory pathway (Fig. 1B) (11).

It is not clear to what extent PCSK9 affects LDLRs in tissues other than the liver. Intravenous infusion of PCSK9 into mice at levels as high as 32 μg/h for 6 h abolished hepatic LDLR expression but failed to reduce LDLRs in adrenals (10). Moreover, LDLRs were not increased in adrenals of Pck9−/− mice (3). However, in another study,
LDLRs were reduced in several tissues, particularly in lung, after the injection of 100 µg of PCSK9 (12).

Why PCSK9 preferentially degrades LDLRs in hepatocytes is unknown. A similar mystery surrounds ARH (LDLRAP), an adaptor protein required for LDLR internalization in hepatocytes but not in most other cell types (3). A physiological explanation might be that PCSK9 selectively suppresses LDLRs in the liver to prevent the immediate reuptake of nascent VLDL particles, thereby promoting their delivery to peripheral tissues.

Regulation of PCSK9

PCSK9 is regulated primarily at the level of transcription by sterol regulatory element binding proteins (SREBPs) (3), which regulate genes involved in fatty acid synthesis (SREBP-1c) and cholesterol metabolism (SREBP-2) (13). An SREBP binding site in the promoter of PCSK9 has been characterized (14). Although SREBP-1c has been implicated in the regulation of PCSK9 (15), SREBP-2 appears to play a more central role under physiological conditions (3). Manipulations that suppress SREBP-2, such as fasting or cholesterol feeding, are associated with lower PCSK9 mRNA levels in livers of mice (14). Mice that lack hepatic SREBP-2 have reduced PCSK9 mRNA levels, whereas mice that lack SREBP-1c do not (unpublished observations).

Activators of the nuclear receptors farnesoid X receptor or peroxisome proliferator-activated receptor α reduce PCSK9 mRNA in cultured human hepatocytes (16, 17). The physiological significance of these regulators requires further study.

PCSK9:LDLR BINDING AND STRUCTURE

Biochemical and cryo-electron microscopy studies have provided new insights into how PCSK9 binds LDLRs and promotes degradation. The extracellular domain of the LDLR consists of an N-terminal ligand binding domain (R1-R7) that mediates binding to LDL, followed by an epidermal growth factor (EGF)-precursor homology domain that contains a pair of EGF-like repeats (EGF-A and EGF-B) separated from a third EGF-like repeat (EGF-C) by a β-propeller domain (18). After LDL binds to the LDLR, the receptor-ligand complex undergoes endocytosis and is delivered to the endosome (Fig. 1B) (13). The bound lipoproteins are released in the acidic environment of the endosome, and the LDLR recycles to the cell surface.

Structural requirements for PCSK9 binding and function

PCSK9 binds specifically to the EGF-A domain of the LDLR in a calcium-dependent manner (19). Other regions of the LDLR that do not contact PCSK9 are required for PCSK9-mediated degradation, including the β-propeller domain and at least three ligand binding repeats (20). The reason for these structural requirements is not known. The C-terminal domain of PCSK9 does not bind to the LDLR, but this region is required for LDLR degradation (20). Thus, the C terminus may bind another protein that directs LDLRs to lysosomes, or the domain may prevent the binding of a protein required for recycling of the LDLR from endosomes to the cell surface.

In the crystal structure of the LDLR at acidic pH, the ligand binding repeats 4 and 5 closely associate with the β-propeller domain (18). These findings suggested that the LDLR changes from an open to a closed conformation at acid pH, thereby facilitating the release of LDL particles (18). The pH-dependent conformational change in LDLR is not required for LDLR degradation by PCSK9 (20), but the affinity of PCSK9 binding to the LDLR is enhanced at acidic pH, suggesting that PCSK9 binds more avidly to LDLRs in the endosome/lysosomal compartments (19, 21, 22). An unresolved question is whether PCSK9 and LDL compete for binding to the LDLR.

PCSK9 crystal structure

The structure of PCSK9 was reported by three groups in 2007 (21, 23, 24), all of whom obtained similar structures despite using a wide range of pH conditions. The catalytic domain resembles that of other subtilisin-like serine proteases except for the high-affinity interactions with the prodomain that shields the active site from exogenous substrates. The C-terminal domain is a unique structure that contains three six-stranded β-sheet subdomains arranged with quasi-three-fold symmetry.

PCSK9:LDLR-AB cocrystal

The structure of PCSK9 in complex with the EGF-AB fragment from the LDLR revealed that PCSK9 primarily contacts the N-terminal region of the EGF-A repeat (25). The prodomain and the C-terminal domain of PCSK9 do not contact the EGF-A domain (Fig. 1C). The interface between PCSK9 and EGF-A is >20 Å from the catalytic site and is formed primarily by hydrophobic residues surrounded by several specific polar interactions. High levels of PCSK9 and LDLR expression in cells promotes associations of PCSK9 with regions of the LDLR outside of EGF-A, which may explain reports that PCSK9 binds VLDLR and apoER2 under certain conditions (27).

GAIN-OF-FUNCTION MUTATIONS IN PCSK9

Mutations in PCSK9 are responsible for a small fraction (<2%) of genetic hypercholesterolemias (28). In contrast with familial hypercholesterolemia (FH), which is caused by >1,000 different mutations in the LDLR, only a small number of missense mutations in PCSK9 cause hypercholesterolemia. Structural studies of PCSK9 provide insight into the mechanisms by which two gain-of-function mutations in PCSK9, D374Y and F216L, may function to increase LDLR degradation.

PCSK9(D374Y) is ~10-fold more active than wild-type PCSK9 in mediating degradation of LDLRs, owing to an ~5- to 30-fold increased affinity for the receptor (3, 21, 22). In the structure of the PCSK9:EGF-AB interface, PCSK9:D374Y forms a salt bridge with EGF-A-H306. Changing D374 to Y places the hydroxyl group of tyrosine close enough to the carbonyl oxygen of EGF-A-C319 to permit formation of
Loss-of-function mutations in PCSK9 and CHD

Several loss-of-function alleles in PCSK9 have been identified in individuals with hypocholesterolemia (3). Most of these mutations are rare, but three were sufficiently common to examine the relationship between LDL-C levels and CHD. In the Atherosclerosis Risk in Communities Study, a missense mutation in the prodomain (R46L), present in 3% of Caucasians, was associated with a 15% reduction in LDL-C and a 46% reduction in CHD (3). In the same study, two nonsense mutations (Y142X and C679X), present in 2% of African-Americans, caused a 28% reduction in LDL-C and an 88% reduction in CHD (3). The reduction in CHD associated with the PCSK9-R46L mutation has been replicated in two case-control studies (35, 36).

Could the greater than expected reduction in CHD observed in association with PCSK9 variants be due to an effect of PCSK9 on CHD that is independent of the effect on LDL-C levels? Recently, common sequence variations in other genes (e.g., \( \text{APOE} \), \( \text{HMGCR} \), and \( \text{APOB} \)) have been linked to LDL-C levels and CHD risk (37). These findings are consistent with the loss-of-function variants in PCSK9 reducing CHD risk through lowering plasma levels of LDL-C.

To probe the association between hypocholesterolemia and cancer, Folsom, Peacock, and Boerwinkle (38) compared the frequency of PCSK9 loss-of-function alleles in subjects with and without various cancers (colon, lung, breast, and prostate). No differences in allele frequencies between the two groups were found. Although additional studies are required, these data suggest that low plasma levels of cholesterol are a consequence, not a causal contributor, to cancer.

Two young women with total PCSK9 deficiency have been identified (39). Both subjects had very low plasma levels of LDL-C (14 mg/dl and 16 mg/dl), providing perhaps the most compelling evidence of the importance of PCSK9 in LDL metabolism. No adverse clinical sequellae were reported in either individual. Determining the long-term consequences of PCSK9 deficiency will require careful clinical assessment of additional, older PCSK9-deficient individuals.
An alternative strategy to inhibit PCSK9 synthesis is to degrade the PCSK9 mRNA with antisense oligonucleotides or small interfering RNA. Antisense oligonucleotides administered to mice reduced PCSK9 expression by >90% and lowered plasma cholesterol levels by ~53% (40). A single intravenous injection of an small interfering RNA delivered in lipidoid nanoparticles to cynomologous monkeys reduced plasma PCSK9 levels by ~70% and plasma LDL-C levels by ~56% (41). Plasma LDL-C levels remained significantly lower 3 weeks after injection.

A third option to inhibit PCSK9 activity is to prevent binding of PCSK9 to LDLRs at the cell surface with small molecules, peptides, or antibodies directed against PCSK9. Overexpressing soluble LDLR (42) or adding EGF-A fragments to cultured cells (43) inhibits the ability of exogenously added PCSK9 to mediate LDLR degradation. The success of this approach depends on PCSK9 functioning primarily at the cell surface.

New agents to treat hypercholesterolemia should work additively or synergistically with statins. Several lines of evidence suggest that an inhibitor of PCSK9 will augment the hypolipidemic effects of these powerful drugs. LDL clearance is accelerated and plasma cholesterol levels are reduced in statin-treated Pcsk9−/− mice compared with untreated Pcsk9−/− mice (3). Statins increase PCSK9 transcription, resulting in higher plasma PCSK9 levels (7, 8). The elevation in PCSK9 attenuates the increase in hepatic LDLR, which may explain why most LDL-C lowering occurs with the first dose of statin (>25%), and further doublings of the dose reduce plasma LDL levels by only ~6% (44).

ARE THERE OTHER FUNCTIONS OF PCSK9?

Currently, there is little evidence that PCSK9 has functions other than promoting LDLR degradation. No other proteins that contains an EGF-A repeat are predicted to interact with PCSK9, based on the structure of the PCSK9: EGF-AB complex. The only phenotype of PCSK9 deficiency detected in humans is lower plasma cholesterol levels, but it remains possible that aging or exposure to environmental challenges, such as infectious agents or dietary toxins, may reveal other phenotypes associated with absence of PCSK9. For example, mice expressing no PCSK9 only in liver have impaired liver regeneration after a partial hepatectomy (2).

IMPLICATIONS FOR HUMAN HEALTH

The reduced CHD risk in individuals with loss-of-function mutations in PCSK9 indicates that LDL is necessary for the development of CHD and suggests that a modest, lifelong decrease in LDL-C levels would confer substantial protection against CHD, even in the presence of other risk factors (e.g., hypertension, diabetes, and smoking) (3). The reduction in CHD associated with defective PCSK9 alleles is greater than would be predicted from multiple statin trials. This observation highlights the importance of duration of exposure to LDL-C as well as the absolute level of LDL-C. Loss-of-function mutations in PCSK9 are associated with reduced plasma levels of LDL-C, starting in childhood (45). In contrast with a static measurement, such as a plasma level of LDL-C, genotypes provide an integrated measure of cholesterol exposure.

Figure 2 compares the cumulative LDL exposure (expressed as grams of cholesterol per year) over a lifetime in FH patients (46) and normal individuals (47). CHD occurs after a theoretical threshold of LDL exposure is exceeded (indicated by a red line). This threshold is reached in childhood in FH homozygotes and in early middle age in FH heterozygotes and is significantly delayed in individuals who are heterozygous for PCSK9 loss-of-function mutations. These findings raise new questions. Would modest reductions in plasma levels of LDL-C starting at an earlier age enhance the benefit of cholesterol-lowering therapy? Will new methods to estimate the lifetime exposure of blood vessels to LDL provide better indicators of absolute CHD risk than do current guidelines?

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