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REVIEW

Proprotein convertase subtilisin/kexin type 9 (PCSK9): From structure–function relation to therapeutic inhibition

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Abstract *Aims:* This short review aims at summarizing the current information on Proprotein Convertase Subtilisin/Kexin type 9 (PCSK9) structure and function focusing also on the therapeutic possibilities based on the inhibition of this protein.
Data synthesis: PCSK9 has been recently discovered as the third gene involved in autosomal dominant hypercholesterolemia. PCSK9 binds and favors degradation of the low-density lipoprotein receptor (LDLR) and thereby modulates the plasma levels of LDL-cholesterol (LDL-C). Some of the natural occurring PCSK9 mutations increase the protein function (gain of function) and cause hypercholesterolemia, whereas loss of function mutations associate with hypocholesterolemia. Since the loss of a functional PCSK9 in humans is not associated with apparent deleterious effects, this protease is an attractive target for the development of lowering plasma LDL-C agents, either alone or in combination with statins.
Conclusion: Inhibition of PCSK9 is emerging as a novel strategy for the treatment of hypercholesterolemia and data obtained from pre-clinical studies show that use of monoclonal antibodies, antisense oligonucleotides and short interfering RNA are effective in reducing LDL-C, clinical studies, accompanied by a better understanding of PCSK9 biology, are now necessary to address whether these new compounds will have a future in clinical practice.

Abbreviations: LDL, low-density lipoprotein; VLDL, very low-density lipoprotein; apoB-100, apolipoprotein B-100; LDL-C, LDL-cholesterol; CHD, coronary heart disease; LDLR, LDL receptor; FH, familial hypercholesterolemia; PCSK9, Proprotein convertase subtilisin/kexin type 9; HMG CoA, 3-hydroxy-3-methylglutaryl coenzyme A; SREBP, sterol responsive element binding protein; apoER2, apolipoprotein E receptor 2; ADH, autosomal dominant hypercholesterolemia; mAb, monoclonal antibody; siRNA, short interfering RNA.

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Introduction

Low-density lipoproteins (LDL) transport in humans about 60—70% of the total plasma cholesterol. LDL derive from the metabolism of very low-density lipoproteins (VLDL) and contain apolipoprotein B-100 (apoB-100) as major protein constituent. High plasma levels of LDL-cholesterol (LDL-C) are a major determinants of cardiovascular disease risk, with a strong positive correlation between plasma LDL-C levels and the incidence of coronary heart disease (CHD) [1]. Clinical trials with lipid lowering drugs show a progressive greater relative risk reduction as the LDL level drops supporting the concept "the lower the LDL the better" [2]. Under normal circumstances LDL are removed from the circulation mainly by hepatic uptake via the LDL receptor (LDLR), the process is highly specific and involves the endocytosis of LDL/LDLR complexes within clathrin coated vesicles. Plasma levels of LDL-C show a great variability between individuals, which depends on both environmental and genetic factors [3]. Autosomal dominant hypercholesterolemia (ADH) is a heterogeneous genetic disorder characterized by elevated LDL-C and premature CHD, the most frequent form of ADH is caused by mutations in the gene that encodes the LDLR with a frequency of 1:500 for heterozygotes and 1 per million for homozygotes, a second and less frequent form is caused by mutations in the apoB-100 gene. Great progress in this field has come recently from the genetic mapping of a locus on chromosome 1, where a candidate gene has been identified as responsible for a third form of ADH. In 2003 Abifadel et al. identified three patients with a clinical diagnosis of ADH and with no detectable mutations in both LDLR and apoB-100 genes, these subjects carried two rare mutations in the gene encoding PCSK9, which co-segregated with plasma LDL-C [4]. Soon after many mutations in the PCSK9 gene were associated with both hypercholesterolemia (gain of function) and hypocholesterolemia (loss of function) (see Table 1), making this protein an attractive target for the treatment of dyslipidemia.

Structure and biosynthesis of PCSK9

The human PCSK9 gene is located on chromosome 1p32.3, it empasses 12 exons and encodes a 692 aminoacid glycoprotein belonging to the family of protein convertases [5]. PCSK9 is synthesized as a ~75 kDa soluble zymogen (proPCSK9) that undergoes autocatalytic cleavage at position 152 in the endoplasmic reticulum to release the propeptide (14 kDa) from the N-terminus resulting in a mature enzyme of about 60 kDa [5]. The cleavage of the pro-domain is strictly required for PCSK9 maturation and activation. Unlike other convertases, activated by the dissociation of the inhibitory pro-domain, the C-terminus of the PCSK9 pro-domain, Gln152, form hydrogen bonds with His226 preventing further access of substrates to the catalytic site [6]. The noncovalently bound PCSK9/prosegment complex exits the endoplasmic reticulum and moves through the secretory pathway until secretion as a ~75 kDa enzymatically inactive form [7]. Hepatic PCSK9 gene expression is strictly dependent on the intracellular sterols content and coordinated with genes involved in cholesterol metabolism, including 3-hydroxy-3-methylglutaryl coenzyme A (HMG CoA) synthase, HMG CoA reductase, the rate-limiting enzyme that catalyzes the conversion of HMG CoA to mevalonate, and the LDLR [8,9], with a consistent up-regulation following pharmacological blockade of cholesterol synthesis. The promoter region of the PCSK9 gene contains an Sp1 site, a HNF1 site and two sterol responsive elements (SRE-1), the latter are responsible for the sterol-dependent regulation of PCSK9 transcription, mediated by the nuclear translocation of sterol responsive element binding protein 2 (SREBP-2) [10]. In agreement with these observations overexpression of nuclear forms of SREBPs in mice upregulates PCSK9 expression. Furthermore treatment of primary human hepatocytes and HepG2 with statins or lipoproteins deficient medium increases the levels of both PCSK9 and LDLR mRNA, whereas incubation with sterols such as 25-hydroxycholesterol down-regulates PCSK9 expression [10,11]. In addition to sterols also the nutritional status modulates PCSK9 levels which closely follow the diurnal variation of cholesterol synthesis and are strongly reduced by fasting [12,13]. PCSK9 gene expression is also suppressed by glucagon, berberine and bile acids and stimulated by inflammation [14—17]. Several PCSK9 post-translational modifications, including N-linked glycosylation, sulphation and phosphorylation have been described, their physiological significance is, however, unclear. PCSK9 may also be further cleaved at the residue Arg218 to a ~53 kDa protein by furin/PC5A [5,18]. The ~53 kDa cleaved form can be detected in mouse and human plasma and its concentration is significantly reduced in Furin but not in proprotein convertases 5/6 knockout mice [19], suggesting that furin processing may be a key factor in the regulation of PCSK9 plasma levels.

Degradation of LDLR protein by PCSK9

A body of evidence indicates that PCSK9 directly interacts with the LDLR both within the cell and at the surface of the plasma membrane [20,21]. The interaction of PCSK9 with the LDLR occurs with 1:1 stechiometry and a Kd of 170 nM at neutral pH, PCSK9 binds the first epidermal growth factor homology domain (EGF-A) within the extracellular portion of the LDLR [22]. The LDLR EGF-A domain contains a highly conserved leucine residue (Leu318) located between cysteine residues 4 (Cys317) and 5 (Cys319) that contributes to the specificity of the PCSK9—LDLR interaction. Furthermore two calcium-binding sites located at the N-terminus of EGF-A and at the interface with EGF-B are necessary for binding to PCSK9 [22]. The PCSK9—LDLR complex enters the endosomal pathway [20] and, as a consequence of pH lowering, the PCSK9 C-terminus binds the ligand binding domain of the LDLR strengthening the interaction [23]. In contrast to the binding of LDL to the LDLR, PCSK9 affinity for the LDLR is increased in the endosome and failure to release PCSK9 prevents receptor recycling and directs the PCSK9/LDLR complex to the lysosome, where degradation of the LDLR occurs. PCSK9 also targets the LDLR within the cell independently from endocytosis, in fact adenovirally mediated PCSK9 overexpression accelerates the degradation of the LDLR in a post-endoplasmic reticulum compartment [24]. Interestingly overnight incubation of