Proprotein convertase subtilisin kexin type 9 and high-density lipoprotein metabolism: experimental animal models and clinical evidence

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Proprotein convertase subtilisin kexin type 9 (PCSK9) belongs to the proprotein convertase family. Several studies have demonstrated its involvement in the regulation of low-density lipoprotein (LDL) cholesterol levels by inducing the degradation of the LDL receptor (LDLR). However, experimental, epidemiologic, and pharmacologic data provide important evidence on the role of PCSK9 also on high-density lipoproteins (HDLs). In mice, PCSK9 regulates the HDL cholesterol (HDL-C) levels by the degradation of hepatic LDLR, thus inhibiting the uptake of apolipoprotein (Apo)E–containing HDLs. Several epidemiologic and genetic studies reported positive relationship between PCSK9 and HDL-C levels, likely by reducing the uptake of the ApoE-containing HDL particles. PCSK9 enhances also the degradation of LDLR's closest family members, ApoE receptor 2, very low–density lipoprotein receptor, and LDLR-related protein 1. This feature provides a molecular mechanism by which PCSK9 may affect HDL metabolism. Experimental studies demonstrated that PCSK9 directly interacts with HDL by modulating PCSK9 self-assembly and its binding to the LDLR. Finally, the inhibition of PCSK9 by means of monoclonal antibodies directed to PCSK9 (ie, evolocumab and alirocumab) determines an increase of HDL-C fraction by 7% and 4.2%, respectively. Thus, the understanding of the role of PCSK9 on HDL metabolism needs to be elucidated with a particular focus on the effect of PCSK9 on HDL-mediated reverse cholesterol transport. (Translational Research 2016;173:19–29)

Abbreviations: Apo = Apolipoprotein; CHRD = cysteine– and histidine–rich domain; CE = cholesteryl esters; CETP = Cholesterol ester transfer protein; ER = Endoplasmic reticulum; EL = Endothelial lipase; EGF-A = Epidermal growth factor–like repeat homology domain A; HNF-1 = Hepatocyte nuclear factor-1; HDL = High-density lipoprotein; HDL-C = HDL cholesterol; mAb = Monoclonal antibody; Lp(a) = Lipoprotein (a); LDL = Low-density lipoprotein; LDL-C = LDL cholesterol; LDLR = LDL receptor; PACE4 = paired basic amino acid-cleaving enzyme 4; PC = Proprotein convertase; PCSK9 = Proprotein convertase subtilisin kexin type 9; PRO = prosegment; SP = signal peptide; SRE = Sterol regulatory element; SREBPs = SRE-binding proteins; TG = Triglyceride; VLDL = Very low-density lipoprotein; VLDL-C = VLDL cholesterol

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INTRODUCTION

Proprotein convertase subtilisin kexin type 9 (PCSK9) is the ninth member belonging to a special family of proteases denoted as the proprotein convertases (PCs), including 8 other members who share identities to bacterial subtilisin and yeast kexin (PC1/3, PC2, Furin, PC4, PC5/6, paired basic amino acid-cleaving enzyme 4 [Pace4], PC7, and SKI-1/S1P).[1,2] Substrates of these proteases, converting inactive secretory precursor into active products, comprise neuropeptides, prohormones, cytokines, growth hormones, cell-surface proteins, and serum proteins.[3] Although in other PC members, a second catalytic cleavage is required to release the prodomain and to activate the protease, the only known substrate of PCSK9 for proteolytic cleavage is itself,[4] with no identified secondary cleavage sites. Hence, it can be seen as functioning only as a binding protein.[5] PCSK9 is secreted mainly by the liver where it regulates the number of cell-surface low-density lipoprotein (LDL) receptor (LDLR). By this virtue, PCSK9 has emerged as a hot new drug target to treat hypercholesterolemia and coronary artery disease (CAD). However, the physiological role of PCSK9 on the metabolism of different lipoproteins, in particular on high-density lipoprotein (HDL), still needs to be elucidated. In the present review, we will provide experimental, epidemiologic, genetic, and pharmacologic evidence on the involvement of PCSK9 on HDL levels and metabolism.

STRUCTURE AND CELL BIOLOGY OF PCSK9

The human *PCSK9* gene encodes a 692-amino acids (aa) full-length PCSK9 (preproPCSK9, about 72 kDa) which is composed of a signal peptide (aa 1–30), a N-terminal prodomain (aa 31–152), a catalytic serine protease domain (aa 153–404), a hinge region (aa 405–454), and a cysteine– and histidine–rich domain in the C-terminal domain (aa 455–692; Fig 1). The autocatalytic processing, which is necessary for the release from the endoplasmic reticulum of the mature PCSK9 (about 62 kDa), involves Gln152 and Ser153 (VFAQ152:SIP).[7] The step forward the autocatalytic process is the travel to the Golgi where PCSK9 sugar residues at the glycosylation site N533CS are further maturated and the propeptide is sulfated at Tyr38 and therefore secreted.[8] Interestingly, PCSK9 levels are finely regulated by 2 PCs, namely, Furin and or PC5/6.[9] Indeed, the mature form is enzymatically cleaved at the RFHR218:QA (R218 and Q219 residues) site resulting in the detachment of the NH2-terminal inhibitory prosegment and the formation of a 55-kDa–truncated PCSK9 form.[10] In humans, this latter constitutes the 15%–40% of the total circulating PCSK9, and it is believed to be inactive.[10-12] Conversely, Lipari et al[13] found that circulating furin-cleaved PCSK9 was able to regulate LDLR and serum cholesterol levels although somewhat less efficient than the intact PCSK9. To sum up, the naturally occurring PCSK9 R218S missense mutation has been reported to promote autosomal-dominant hypercholesterolemia (ADH) among heterozygous carriers.[14]

EFFECT OF PCSK9 ON LDLR CLOSEST FAMILY MEMBERS

The best and mainly characterized activity of secreted PCSK9 is to post-translationally regulate the number of cell-surface LDLR (see Benjannet et al[11] and Dewpura et al[15] for more comprehensive reviews). Notably, by binding to the epidermal growth factor–like repeat homology domain A of human LDLR, PCSK9 has a dual effect (1) acting as a courier, facilitating the exit of LDLR from the endoplasmic reticulum, and (2) fostering the degradation of the LDLR at the cell surface.[16]

Noteworthy, in addition to LDLR, although with less efficiency, PCSK9 has been shown to enhance the degradation of LDLR's closest family members apolipoprotein (Apo)E receptor 2 (46% identity) and very low-density lipoprotein (VLDL) receptor (59% identity),[17] whereas that of LDLR-related protein 1 depends on the cell line considered.[18] Finally, recent studies reported that the use of PCSK9 monoclonal antibodies (mAbs) reduced lipoprotein (a) (Lp[a]) plasma levels up to 30%[19] as a consequence of greater LDLR-mediated catabolism of Lp(a) itself.[20] Thus, the extended action of PCSK9 on different lipoprotein receptors may have an impact on several lipoproteins particles.

PHARMACOLOGIC MODULATION OF PCSK9 LEVELS BY DRUG AFFECTING LIPID METABOLISM

Statins. Because statins determine low-sterol conditions, turning on the activity of the sterol regulatory element (SRE)–binding protein transcription factor, they simultaneously increase the synthesis of LDLR and PCSK9.[21] This paradoxical mechanism might counteract the hypolipidemic action of statin, although this observation is not supported by all of the studies.[22] Serum PCSK9 was significantly increased in response to 10 mg/d, 40 mg/d, and 80 mg/d atorvastatin by +14%,[23] +34%,[24] and +47%,[25] respectively. Data from 500 men and 500 women participating in the Justification for the Use of Statins in Prevention: An Intervention Trial Evaluating Rosuvastatin trial showed that participants randomized to rosuvastatin 20 mg/d had approximately 30% increment (35% in women and 28% in men) in median concentrations of PCSK9.[22] A similar trend