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REVIEW

The proprotein convertases are potential targets in the treatment of dyslipidemia

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Abstract The family of the secretory proprotein convertases (PCs) comprises seven basic amino acid (aa)-specific subtilisin-like serine proteinases known as PC1/3, PC2, furin, PC4, PC5/6, PACE4 and PC7, and two other PCs, SKI-1 (subtilisin-kexin isozyme-1)/S1P (site-1 protease) and PCSK9 (proprotein convertase subtilisin kexin 9) that cleave at nonbasic residues. Except for the testicular PC4, all the other convertases are expressed in brain and peripheral organs and play a critical role in various functions including the production of diverse neuropeptides as well as growth factors and receptors, the regulation of cellular adhesion/migration, cholesterol and fatty acid homeostasis, and growth/differentiation of progenitor cells. Some of these convertases process proteins that are implicated in pathologies, including cancer malignancies, tissue regeneration, and viral infections. The implication of some of these convertases in sterol/lipid metabolism has only recently been appreciated. SKI-1/S1P activates the synthesis of cholesterol and fatty acids as well as the LDL receptor (LDLR), whereas PCSK9 inactivates the LDLR. Moreover, furin, PC5 and/or, PACE4 inactivates endothelial and lipoprotein lipases. Humans and mice exhibiting either a gain or loss of function of PCSK9 through specific point mutations or knockouts develop hypercholesterolemia and hypocholesterolemia phenotypes, respectively. A PCSK9 inhibitor in combination with statins offers a most promising therapeutic target to treat cardiovascular disorders including dyslipidemias. Specific inhibitors/modulators of the other PCs should find novel therapeutic applications in the control of PC-regulated pathologies.

Keywords Cholesterol metabolism · Human mutations · Mouse knockouts · Precursor inactivation · Proprotein convertase · Subcellular localization · Zymogen activation

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Introduction

Atherosclerosis and its major sequelae, coronary artery disease (CAD), are the leading causes of mortality and morbidity in developed countries. Incidence of fatal and non-fatal acute myocardial infarctions is expected to increase dramatically in the next two decades. Cardiovascular risk factors include dyslipidemia, hypertension, diabetes, smoking, obesity, age, psychological stress, and male gender. The most potent factor, dyslipidemia, often reflects a high ratio of low-density lipoprotein cholesterol (LDL-C) to high-density lipoprotein cholesterol (HDL-C). The data from the Cholesterol Treatment Trialists (CTT) Consortium [6] reveal that for each 1 mmol/l reduction in LDL-C induced by statins yields ~20% more protection against vascular disease, implying that the lower we drive the ratio of plasma LDL-C/HDL-C, the greater is the benefit to patients at risk to develop cardiovascular complications. Current guidelines recommend a target level of LDL-C <1.8 mmol/l (<70 mg/dl) in high-risk individuals and in the secondary prevention of CAD [67]. Among the effective LDL-C-lowering drugs are statins [14], inhibitors of the rate-limiting hydroxylmethylglutaryl coenzyme A reductase (HMG-CoA) needed for cholesterol synthesis. Although well tolerated statins are limited in their capacity to lower LDL-C. New approaches include NPC1L1 intestinal sterol transporter blocker ezetimibe that reduces LDL-C by an additional 20% [111]. Clearly, novel strategies to further decrease levels of circulating LDL-C in combination therapy are needed [17, 107].

Cardiovascular regulation is dependent on a myriad of factors, including protease activities [107]. The mammalian genome database predicts the presence of 550–700 protease genes (~2% of genes), covering all potential enzymatic cleavages of a given species at all developmental stages [7, 73]. The most abundant serine proteases represent about one third of all five protease classes [73]. However, proteases do not operate alone but form cascades, regulatory circuits, and networks that all dynamically interconnect to form the protease web [69]. All known vasoactive proteins and peptides result from proteolytic processing and activation events. The proprotein convertases (PCs) are implicated in the limited proteolysis of secretory precursor proteins resulting in a diversity of bioactive proteins and peptides, and in some cases, in inactivation of key proteins [88]. Mammalian PCs are the central focus of this manuscript. They are encoded by genes numbered from *PCSK1* to *PCSK9* (PC subtilisin/kexin). The nine known PCs (Fig. 1) are as follows: PC1/3, PC2, furin, PC4, PC5/6, PACE4, PC7, SKI-1/S1P, and PCSK9 [85, 86, 90]. The first seven are basic amino acid (aa)-specific PCs cleaving precursor proteins at single or paired basic residues. These PCs are phylogenetically more closely related to each other and to yeast kexin than to SKI-1/S1P or PCSK9, which belong to the pyrolysin [89] and proteinase K [85] subfamilies, respectively. All PCs contain a signal peptide, a prosegment, and a catalytic domain. Just following the catalytic domain, the basic aa-specific convertases exhibit a β-barrel P-domain that apparently stabilizes the catalytic pocket. The C-terminal domain of each convertase contains

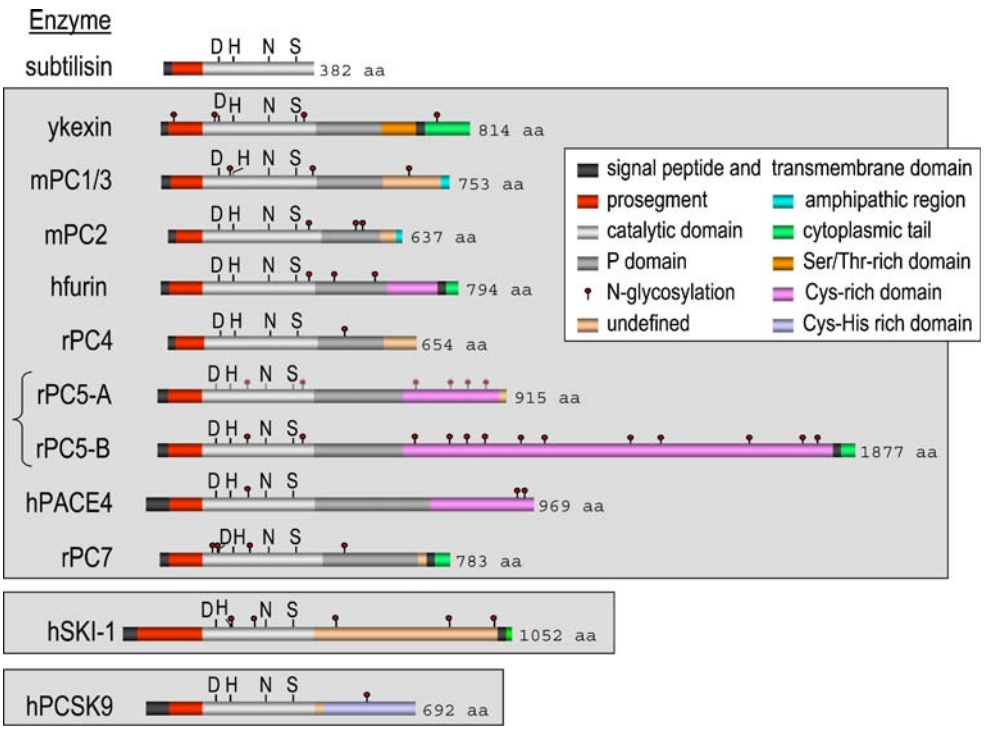

Fig. 1 Schematic primary structures of the nine PCs. The basic aa-specific PCs together with ykexin, SKI-1, and PCSK9 are individually *boxed* to emphasize their distinct subclasses. PC5 exists as two alternatively spliced isoforms, soluble PC5A and membrane-bound PC5B. The catalytic triad residues Asp, His, and Ser and the oxyanion hole Asn are indicated. *h*, human; *r*, rat; *m*, mouse; and *y*, yeast

unique sequences regulating their cellular localization and trafficking. PC5 and PACE4 contain a specific Cys-rich domain (CRD), which, in combination with TIMPs, binds to HSPG at the cell surface. In contrast, PCSK9 exhibits a Cys-His-rich domain (CHRD) that is required for cell surface binding in an LDLR-dependent fashion [85, 89]. Some of these PCs play critical roles in regulating lipids and/or sterols [88]. PCSK9 enhances the degradation of the LDL receptor (LDLR) [10, 55, 56, 70], SKI-1/S1P activates specific membrane-bound transcription factors, e.g., SREBP-1 and -2 [20], PC5A, PACE4, and/or furin cleave/inactivate EL and LPL, which are critical in HDL, VLDL, and chylomicron metabolism (Fig. 2) [37]. Familial autosomal dominant hypercholesterolemia (ADH) is characterized by high levels of plasma cholesterol, xanthomas, and premature CAD. In vivo functions of PCSK9 have been shown in humans exhibiting gain- or loss-of-function mutations associated with hypercholesterolemia [1, 2, 4, 11, 50, 63, 110] or hypocholesterolemia [12, 21, 44, 117] and in mouse knockout (KO) models [76]. This led to classifying *PCSK9* as the third gene associated with familial ADH (incidence ~2.3%) with *LDLR* (incidence ~67%) and *APOB* (apolipoprotein B; incidence ~14%) as the other two (Fig. 3) [1]. Although *LDLR* and *PCSK9* genes are coregulated, PCSK9 triggers the degradation of LDLR [5, 27]. PCSK9 inhibition is thus a promising complement to statin therapy [5, 17, 117]. In vivo functions of PCSK9 have been reported in mouse KO models [76] and in natural gain- or loss-of-function human variants [1, 10, 21, 50, 110].

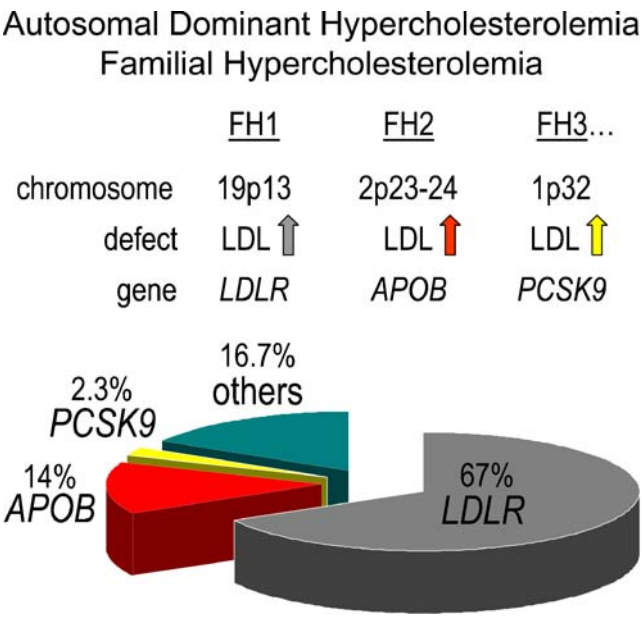

Fig. 3 Incidence of mutations in *LDLR*, *APOB*, *PCSK9*, and other genes in familial hypercholesterolemia. The incidence of mutations are represented, emphasizing that the genetic origin of ~16.7% of ADH cases remains to be elucidated

The role of PCs in cardiovascular functions and disease

Background on lipid homeostasis

Lipoproteins shuttle hydrophobic molecules (cholesteryl esters and triglycerides) between organs in the aqueous environment of plasma. They are macromolecules with a

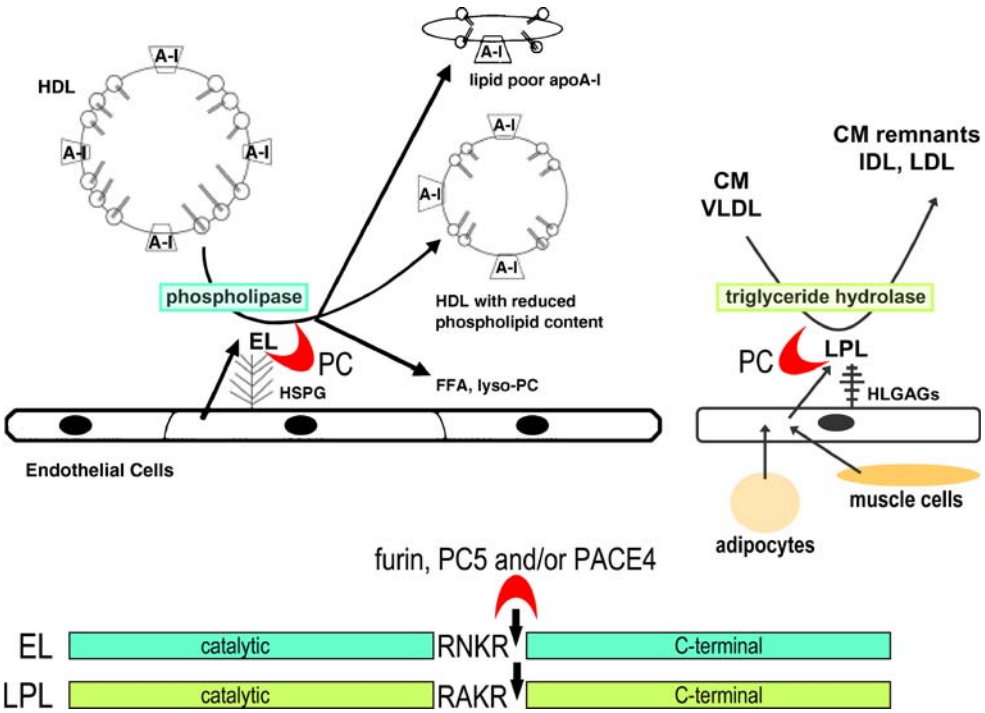

Fig. 2 Inactivation of endothelial lipase (*EL*) and lipoprotein lipase (*LPL*) by PC5, PACE4, and furin. EL and LPL bind heparan-sulfate proteolycans (*HSPGs*) and heparin-like glycosaminoglycans (*HLGAGs*), respectively, and are cleaved by PCs internally at the C-terminus of Arg in the motif RxKR↓. This cleavage hampers the phospholipase role of HSPG-bound EL on HDL and the triglyceride hydrolase function of HLGAG-bound LPL on chylomycrons (*CM*) and VLDL (adapted from Broedl et al. [15])

single envelope of phospholipids and free (unesterified) cholesterol and a core of triglycerides and cholesteryl esters [33]. The major lipoprotein classes are the triglyceride-rich chylomicrons and VLDL, LDL, and HDL lipoproteins. The protein component of LDL is apoB, whereas VLDL also contains apoE and apoCs. The denser HDL contains apoAs, Cs, D, and E [39]. The LDL particles are atherogenic, that is, they cause atherosclerosis [19]. LDLs are cleared from blood by LDLR-mediated endocytosis. SK1-1/S1P and PCSK9 are involved in transcriptional regulation and cellular processing of the LDLR, respectively. HDL-C levels are inversely related to the risk of CAD. HDL particles are formed predominantly in the liver and intestine and are extensively modified in the plasma by EL and hepatic lipase (HL) and lipid transfer proteins. Presently, few therapeutic options raise HDL-C to prevent heart disease [51].

This paper explores the possible role of some of the convertases implicated in HDL metabolism and exclusively concentrates on the lipidemic effects of the PCs and will not detail the other numerous implications of the PCs in cancer, metastasis, atherosclerosis, restenosis, and viral infections and other pathologies. For these, the reader is referred to excellent reviews [9, 40, 83, 90, 97, 108].

– *PCSK9* (originally named NARC-1): PCSK9 was first characterized by our group in 2003; it is highly expressed in the liver, gut, and kidney [85]. PCSK9 mRNA levels are upregulated by SREBP-2 and downregulated by cholesterol [27, 35, 57]. We established the first association between single-point mutations in *PCSK9* and ADH in two French families [1] (Fig. 4). Later on, Cohen et al. [21] showed that two nonsense *PCSK9* mutations resulting in a loss of function are associated with hypocholesterolemia in 1.8% of black subjects [44] (Fig. 4). Only one of these two mutations was also found in European–Americans at a lower frequency (0.12%). PCSK9 mutations associated with hypercholesterolemia result in a gain of function via an enhanced PCSK9 activity that triggers the degradation of LDLR [55] in acidic compartments, likely endosomes/lysosomes [5, 10, 56]. By a yet unknown mechanism, high levels of PCSK9 lead to a faster rate of degradation of the cell surface LDLR, resulting in increased circulating LDL-C, as the LDL uptake in hepatocytes by the Ldlr is diminished. In agreement, $Pcsk9^{-/-}$ mice exhibit an increased LDLR protein, but not mRNA, and a twofold drop in circulating cholesterol [76]; whereas mice overexpressing PCSK9 after recombinant adenoviral infections exhibit high levels of circulating cholesterol [10, 46, 55, 70]. Two healthy and fertile females, 32 [117] and 21 [34] years old, with either homozygote C679X or heterozygote ΔR97 and Y142X variations, respectively, were reported (Fig. 4). Although the Y142X truncation leads to the complete loss of PCSK9 expression, the deletion of ΔR97 and the C679X variant results in either an unprocessed zymogen or an autocatalytically processed PCSK9 that remains in the endoplasmic reticulum (ER) [117]. Their LDL-C is remarkably low, ~15 mg/dl. This, and the observation that loss-of-function nonsense mutations could lead to 88% reduction in CAD over a 15-year-period, indicate that the inhibition of PCSK9 or decreasing its levels may represent a safe and effective strategy for the control of hyperlipidemia [44].

– *Interplay between PCSK9, LDLR, and apoB*—In vivo studies in hypercholesterolemic patients [68] and in a stable transfectant in rat liver cells [104] showed that hypercholesterolemic mutants of PCSK9 resulted in the increased release of apoB-containing lipoproteins. In vivo evidence that PCSK9 enhances apoB release, even in the absence of LDLR, came from its adenovirus-mediated overexpression in $Ldlr^{-/-}$ mice that led to the increased VLDL- and LDL-associated cholesterol [10]. However, adenovirus infection at lower titers revealed no significant effects on apoB secretion [70]. Twenty-four hour-fasted mice overexpressing adenovirus PCSK9 showed massive hyperlipidemia as a consequence of increased secretion of apoB100-containing VLDLs [22, 47]. Primary hepatocytes from $Pcsk9^{-/-}$ mice showed some reduction in secretion of apoB as compared to $Pcsk9^{+/+}$ hepatocytes [76]. That LDLR may attenuate hepatic apoB secretion was first observed by Twisk et al. [113] who showed that, by an unknown mechanism, $Ldlr^{-/-}$ hepatocytes secreted apoB100 at a 3.5-fold higher rate than did $Ldlr^{+/+}$ hepatocytes. The mechanisms of PCSK9-mediated degradation of LDLR or apoB and their interplay along the secretory pathway need clarification. As hepatic overproduction of apoB100 is one of the causes for a subset of familial-combined hyperlipidemia, to better understand the impact of PCSK9 on the synthesis and secretion of apoB-containing lipoproteins is essential.

– *PCSK9 degradation versus gain-/loss-of-function mutations*—To date, almost 35 aa variations have been reported (see Fig. 4; [1, 2, 12, 21, 32, 44, 50, 72, 91, 110, 117]). Until the crystal structure of PCSK9 is solved, the biochemical analysis of the corresponding variants is crucial to better understand its biology. As gain-of-function mutations are rather rare in proteases, we suspected that the level of active enzyme was modulatory and thereby able to modulate in return LDLR levels (Fig. 5). We discovered that two French mutations associated with hypercholesterolemia, F216L and R218S [1, 2], modify a typical $RXXR_{218}$ site for basic aa-specific PCs (Fig. 6), best cleaved by furin and

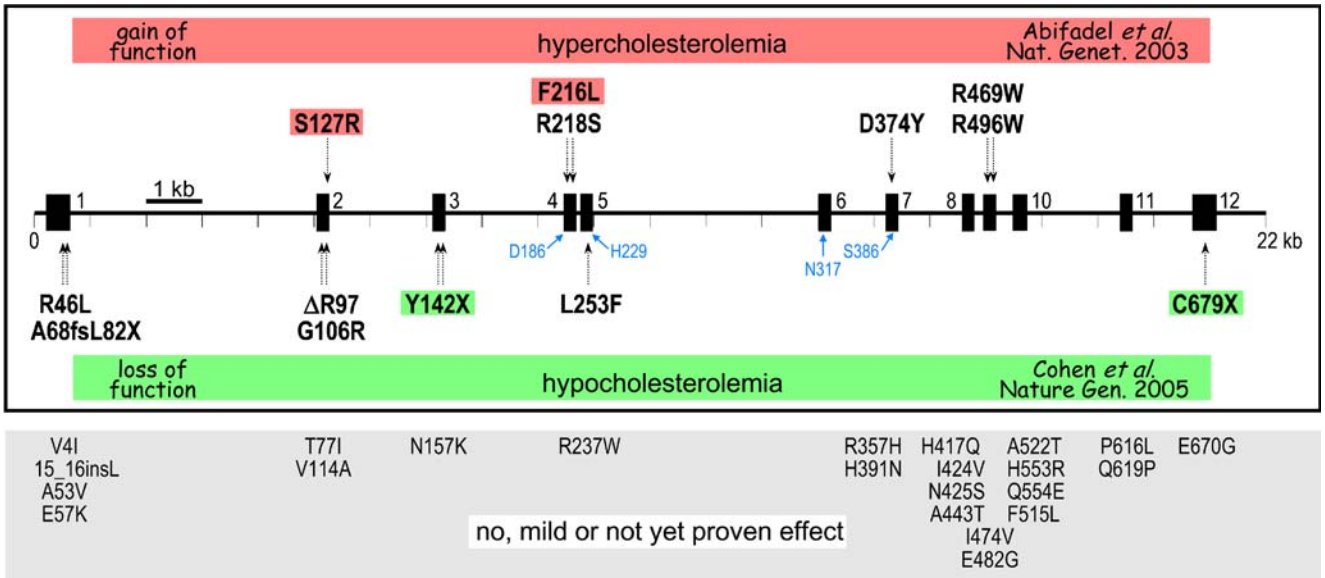

Fig. 4 Known human PCSK9 mutations or single nucleotide polymorphisms (SNPs) with or without effect on the development of hyper-or hypocholesterolemia. A schematic of the 22-kb *PCSK9* gene is shown and the location of the active site *D186*, *H229*, *N317*, and *S386* residues is emphasized. Also shown are the known PCSK9 aa modifications because of exonic nucleotide changes. Some lead to hypercholesterolemia (*top*), a discovery made by Abifadel et al. [1] for the S127R and F216L mutations, whereas others result in the loss-of-function of PCSK9 and hence hypocholesterolemia (*bottom*) as first reported by Cohen et al. [21]. In the *bottom panel* (*grey background*), aa modifications that have no, mild, or not yet proven effect on plasma cholesterol levels are displayed according to their exon location

PC5 [11, 86]. Although not in the vicinity of this PC site, the Anglo-Saxon D374Y mutation also confers resistance to cleavage. The extent of this cleavage was highly enhanced when we optimized the site for PC cleavage into an $RRRR_{218}EL$, resulting in a loss of function of PCSK9, i.e., the inability to trigger LDLR degradation (Fig. 6) [11]. In contrast, the A443T substitution [44] associated with hypocholesterolemia leads to an increase sensitivity to PC-mediated cleavage [11]. Thus, the half-life of PCSK9 seems to directly modulate the circulating LDL-C. Whether cellular and/or circulating PCSK9 is subjected to degradation by other enzymes, such as cell surface metalloproteinases or plasma proteases, is yet to be defined.

– *Cellular trafficking of PCSK9 and colocalization with its dominant partner LDLR*—Like most PCs, PCSK9 is autocatalytically processed in the ER, before its exit from this compartment. Although most PCs are activated after a secondary cleavage of their prosegment before their secretion (Fig. 7), PCSK9 is rapidly secreted as a tight complex with its N-terminal prosegment [10, 85]. As the prosegments of PCs are usually potent inhibitors, this suggests that the secreted PCSK9 is inactive. The secreted form of PCSK9 was shown to be internalized into endosomes via cell-surface binding in an LDLR-dependent manner [18]. In agreement, media swaps [18] or addition of purified PCSK9 to naive cells [45] resulted in the degradation of the LDLR. Very recently, it was demonstrated that PCSK9 and LDLR could interact directly at the cell surface [45], but whether this is cell-type dependant and requires another accessory protein is yet to be determined. We also recently completed an extensive immunocytochemical study of the cellular localization of PCSK9 and its mutants in primary hepatocytes and cell lines [64]. Our data show that wild type and hypercholesterolemic variants of PCSK9 co-localize with LDLR in early and late endosomes, whereas variants associated with hypocholesterolemia do not [64].

SKI-1: In contrast to basic aa-specific PCs, SKI-1 (also known as S1P) cleaves substrates in the general motif RX

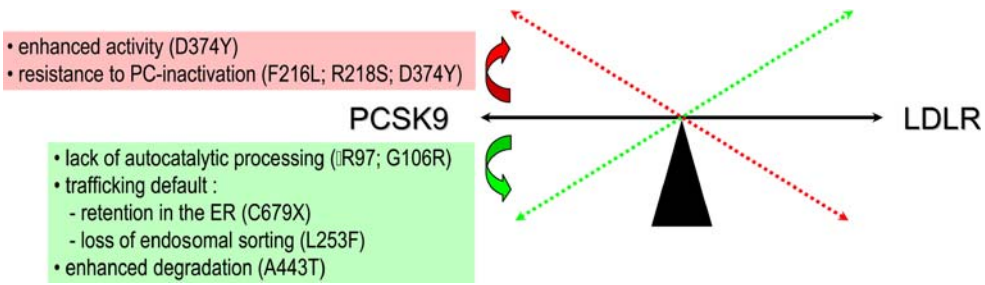

Fig. 5 The PCSK9 and LDLR protein balance. Possible pathways leading to higher (*red arrow*) or lower (*green arrow*) levels of PCSK9 protein or activity are proposed. Their opposite impact on LDLR protein levels, as PCSK9 enhances the degradation of the LDLR, is illustrated

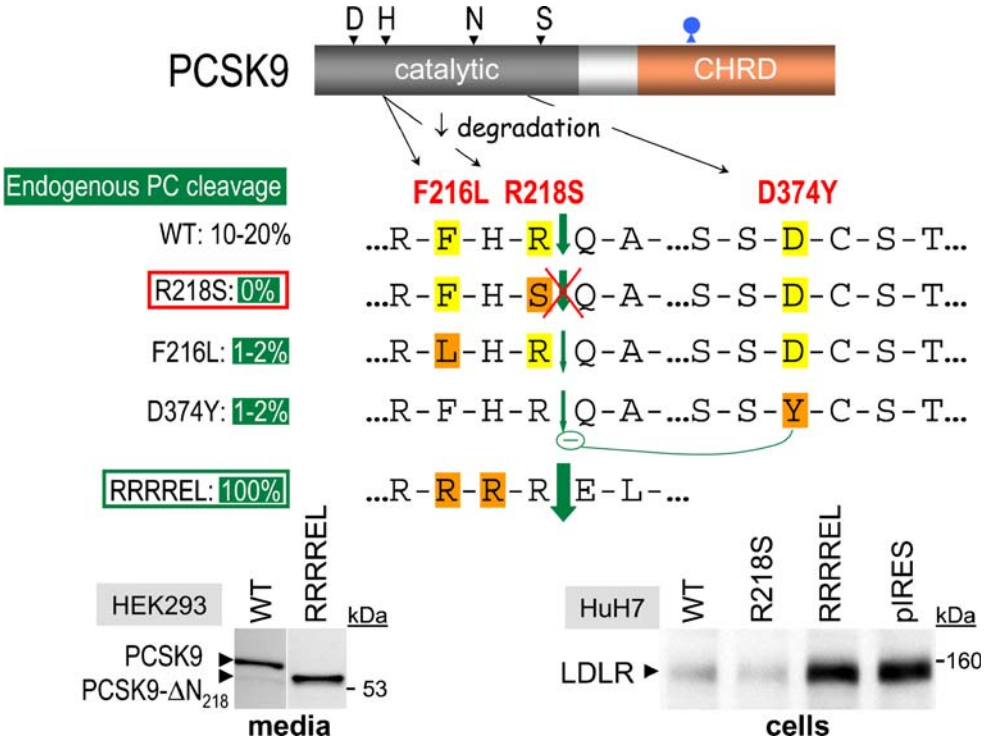

Fig. 6 Functional representation of some of the gain-of-function mutations. The sequences surrounding the *F216L*, *R218S*, and *D374Y* mutations are shown, as well as the percent in cleavage by furin/PC5 of PCSK9 secreted by transfected HEK293 cells. Expression of the mutated ^{215}RRRREL^{220} PCSK9 resulted in 100% cleavage producing ~55 kDa inactive PCSK9-ΔN_{218}. *WT*, wild type; *pIRES*, empty vector

(V,L)(K,F,L)↓ (the downward arrow emphasizes that cleavage occurs C-terminal to K, F, or L) [71, 89]. ProSKI-1 is autocatalytically cleaved into a mature ~106 kDa membrane-bound form [89] and a secreted ~98 kDa shed form [29]. Its *PCSK8* gene, ubiquitously expressed [89], is located on human chromosome 16 and mouse chromosome 8 [105]. In the absence of sterols, SKI-1 cleaves the membrane-bound transcription factors sterol regulatory element-binding proteins (SREBPs) in their luminal loop [81], leading to the release of a cytosolic basic helix-loop-helix transcription factor. In the nucleus, this activates the transcription of *LDLR* and all the genes involved in cholesterol and fatty acid synthesis [81]. In the presence of sterols, SREBP cleavage is inhibited, and hence, transcription of its target genes is reduced, although the reverse is true in the absence of sterols [81]. Other transmembrane transcription factors cleaved by SKI-1 include the ER-stress response factor ATF6 and CREB-like transcription factors Luman and CREB4 [16, 49, 53, 61, 71, 81, 103, 114, 115]. Recently, we developed in vitro fluorogenic assays of SKI-1 activity as well as cellular inhibitors of this convertase that block viral infectivity through the inhibition of viral surface glycoprotein processing [8, 71, 74, 112].

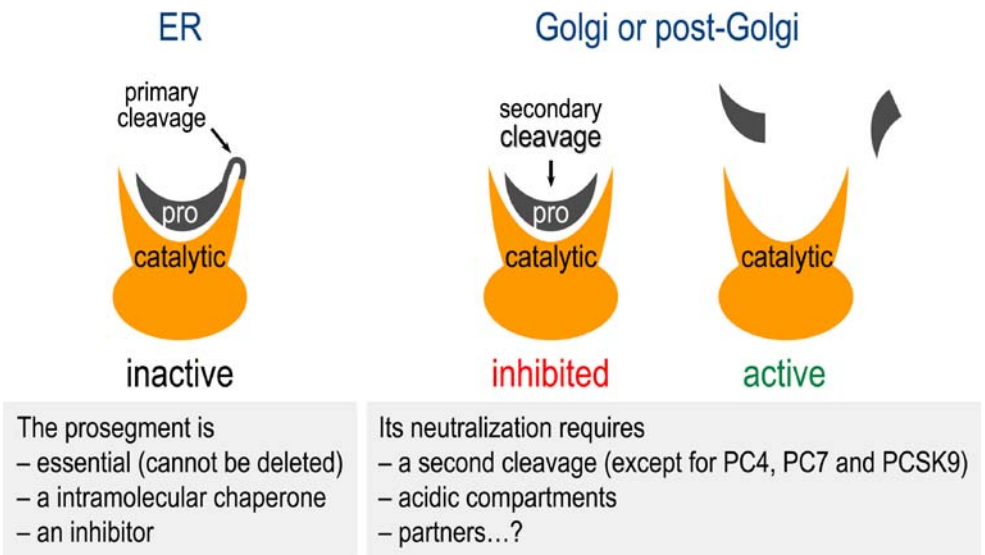

Fig. 7 Zymogen activation of the proprotein convertases. Except for PC2, all other PCs undergo an autocatalytically cleavage of their chaperone/inhibitor prosegment in the ER. The complex prosegment-catalytic enzyme then exits the ER towards the Golgi or post-Golgi compartments, where usually a secondary cleavage of the inhibitory prosegment results in zymogen activation. The conditions favoring such activation vary from one PC to another and include pH, calcium concentration, and in some cases, the presence of specific partners

SKI-1 KO—Lethality occurs at the blastocyst stage in *Pcsk8*$^{-/-}$ mice with the absence of inner cell mass formation [59]. A conditional KO of *Pcsk8* in the liver, using the Mx1-cre transgene, led to a partial disruption to the gene (85%) and caused a 50% reduction in rates of cholesterol and fatty acid synthesis, plus very low levels of nuclear SREBPs and reduced mRNA of their target genes [114]. SKI-1 may be involved in cartilage formation as disorganization of chondrocytes was observed in zebrafish deficient in SKI-1 [84].

PC5: PC5 (also called PC6 [62]) was first identified and cloned by our group [54, 58]. Human *PCSK5* encodes two alternatively spliced isoforms PC5A (915 aa) and PC5B (1870 aa) [54]. Both zymogens undergo a first autocatalytic cleavage in the ER and a second in the *trans*-Golgi network (TGN) [24, 65] or possibly at the cell surface (Mayer and Seidah, in preparation). Although devoid of a transmembrane domain, PC5A can exert its proteolytic action at the cell surface, as it is retained at the plasma membrane as a complex with tissue inhibitors of metalloproteases (TIMPs) and heparan sulfate proteoglycans [66].

Using in situ hybridization and/or quantitative reverse transcription polymerase chain reaction, we documented tissue distribution patterns of PC5 during development, adulthood, and in various cell lines [30]. By embryonic day E15.5, the PC5 pattern of expression becomes similar to the adult: strong labeling in adrenal cortex, small intestine, kidney, and vasculature [30]. PC5A is the predominant isoform in adult mouse tissues except in the intestine and kidney where PC5B predominates [30].

Many substrates are reported to be efficiently processed ex vivo by PC5: matrix metalloproteases and ADAM family enzymes [95, 102]; growth factors such PDGF-A [94], PDGF-B [93], and VEGF-C [92]; and receptors such as IGF-1R [41], integrins [13, 52], renin [58, 77], and lipases [37]. We showed that in atherosclerotic plaques and during arterial restenosis, expression of PC5 is highly upregulated [96, 98–102]. High expression of PC5 in enterocytes suggests a possible role in processing protein substrates that could regulate food and/or sterol/lipid absorption [54, 87]. It was recently reported that a locus on chromosome 9, close to *PCSK5*, is implicated in lipid regulation in humans [31]. PC5 is thus a good candidate proteinase in the control of both arterial restenosis [97] and the levels of circulating HDL.

PC5 KO—To investigate physiological roles of PC5, we generated a *Pcsk5*-deficient allele missing exon 4 that encodes the catalytic Asp_{173}. Although heterozygote Δ4/+ mice are healthy and fertile, homozygote Δ4/Δ4 embryos die at E4.5-E7.5 [30].

Furin: Furin, an ubiquitous membrane protein [78], is initially produced as a ~104 kDa precursor rapidly converted into an active ~98 kDa form [23, 48, 87]. This autocatalytic cleavage, occurring in the ER (Fig. 6), is a prerequisite for the exit of mature furin molecules out of the ER to the TGN and cell surface [60, 106]. Deduced from many studies is that furin and PC5 exhibit partial redundancy of their in vitro selectivity and sensitivity to certain modified serpin inhibitors [36, 86, 109]. Candidate substrates described for furin in vitro include many vasoactive peptides and proteins involved in cardiovascular tissue remodeling [3, 108]. Most of these cleavages occur in the TGN, at cell surface, or in endosomes but rarely in the ER [82]. It is noted that furin, PACE4, and PC5 can inactivate endothelial and lipoprotein lipases [37]. Moreover, furin plays a key role in blood pressure regulation though the activation of transforming growth factor β (TGFβ) [26], a process that was recently shown to be inhibited by the binding of Emilin-1 to proTGFβ [75, 116]. Very recently, it was shown that angiopoietin-like proteins Angptl-3 and Angptl-4 were processed at an internal RRKR site to release these lipoprotein lipase inhibitors in circulation [42, 43]. This suggests that a furin-like enzyme is the responsible convertase of LPL, a hypothesis that will need further confirmation. With tissue-specific conditional KOs, we may soon be able to establish if such ex vivo observations also pan out in vivo.

Furin KO—Furin is detected at E7.5 in the endoderm and mesoderm. During the late somite stages, it is seen in the cardiovascular system [80, 118]. Inactivation of the *fur* gene (*Pcsk3*) causes embryonic death ≈E11 because of hemodynamic insufficiency and cardiac ventral closure defects [80]. Mutant embryos failed to develop large vessels despite the presence of endothelial cell precursors. TGFβ1 was recently shown to be efficiently processed by furin [26], and the inactivation of its gene produces a phenotype similar to that of furin-null embryos [25, 28].

A conditional KO in liver, with the deletion of exon 2 dependent on the Cre expression from the Mx1-cre transgene, resulted in viable *Pcsk3*$^{flox/flox}$ *Tg(Mx1-cre)* mice with almost no phenotype. This demonstrated the existence of some

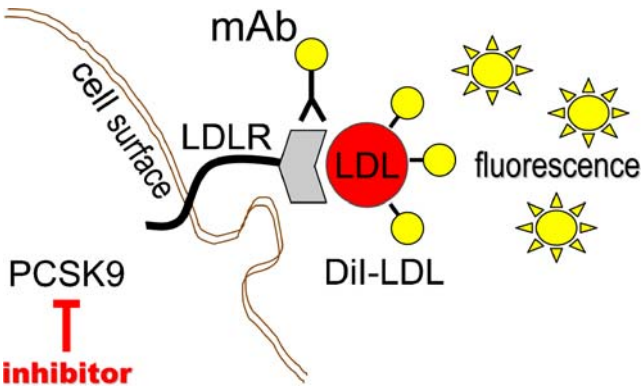

Fig. 8 High throughput functional cell-based screens for PCSK9 inhibitors. Schematic representation of such screens using either fluorescent mAb to LDLR or its ligand DiI-LDL

redundancy with other convertases, as some typical furin substrates were still cleaved, although to a lesser extent [79].

The need for better treatments of dyslipidemias

It is becoming very clear that lowering the levels of circulating LDL-C and/increasing that of HDL-C over a lifetime has profound effects on the incidence of cardiovascular disorders and dyslipidemias. This includes myocardial infarcts, hypertension, and endothelial dysfunction leading to atherosclerosis, as well as stroke. Indeed, recent clinical trials indicated that treatment of these disorders should be based on global cardiovascular risks rather than only on the levels of circulating cholesterol. It is now recognized that the major risk factors in cardiovascular disorders are as follows: abnormal lipid contents, smoking, diabetes, high blood pressure, abdominal obesity, unhealthy diet, and lack of physical activity. Although improved diet and increased physical activity can be achieved by modifications of lifestyle, the other parameters are usually regulated with specific medication, e.g., the use of statins, ezetimibe, thiazide, β-blockers, angiotensin converting enzyme inhibitors, or angiotensin receptor blockers.

The available data suggest that some of the basic aa-specific PCs may be implicated in lipid and sterol regulation. Examples include the role of furin, PACE4, and PC5 in the inactivation of endothelial and lipoprotein lipases and in the processing of receptors such the lipoprotein-related receptor protein LRP1. On the other hand, it is very clear that SKI-1/S1P is directly implicated in the regulation of SREBPs activation and hence the synthesis of cholesterol and fatty acids. Finally, PCSK9, through degradation of the LDLR, indirectly contributes to the regulation of the levels of circulating LDL-C.

How can we envisage a drug design against the convertases implicated in regulating the levels of cholesterol and/or fatty acids? Concerning the role of furin, PACE4, and PC5 in the inactivation of lipases (Fig. 2), it would not be recommended to use an inhibitor of these enzymes, as this would likely result in a decreased level of HDL [37, 38]. This assumption should be verified by measuring lipase activity and HDL-C levels in knockout mice of either furin, PACE4, or PC5. As the knockouts furin [80] and PC5 [30] are embryonic lethal, it will be necessary to use conditional knockouts in either liver or endothelial cells to answer this question. In the case of SKI-1/S1P that activates SREBPs and/or PCSK9 that enhances the degradation of LDLR, a direct cell permeable functional inhibitor obtained through high throughput screens (HTS) of combinatorial libraries of nonpeptidic compounds may be successful. This could take the shape of in vitro assays screens with fluorogenic substrates or cell-based assays. The identification of "hits" would then be followed by medicinal chemistry methods to enhance the potency, efficacy, and kinetic bioavailability of the "hits" and drastically reduce their possible toxicity. As in the case of PCSK9, the enzyme is secreted as a tight binding complex with its inhibitory prosegment [10, 11, 85], it may be difficult to achieve an in vitro enzymatic activity. However, as addition of the PCSK9-prosegment complex outside cells allows its internalization into endosomes and results in a functional protein [18, 64], a cell-based functional assay using the level of LDLR as end point would allow HTS for an inhibitor of the function of PCSK9 or its more active D374Y mutant [11, 45] on the degradation of endogenous LDLR in either HuH7, HepG2, or HEK293 cells (and hence higher levels of cell-surface LDLR). For example we could use either automated FACS with a monoclonal antibody (mAb) to LDLR, or fluorescent LDLR-mAb or even DiI-LDL ligand (Fig. 8). As heterozygotes lacking 50% of the functional PCSK9 already show marked reduction in circulating LDL-C [21, 44], it may not be necessary to reduce the levels or activity of PCSK9 by more than 70–80% to achieve the desired effect.

Although PC-based hypolipidemic treatments are still in their infancy, the future will tell whether modulating the levels or activity of some of these convertases may represent viable therapeutic approaches that when combined with the other drugs, may well prove to be very beneficial in treating cardiovascular disorders.

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