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The Multifaceted Proprotein Convertases: Their Unique, Redundant, Complementary, and Opposite Functions*

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Nabil G. Seidah[‡1], Mohamad S. Sadr[‡], Michel Chrétien[§¶], and Majambu Mbikay[§¶]

From the Laboratories of [‡]Biochemical Neuroendocrinology and [§]Functional Endoproteolysis, Clinical Research Institute of Montreal (IRCM, affiliated with the University of Montreal), Montreal, Quebec H2W 1R7 and the [¶]Chronic Disease Program, Ottawa Hospital Research Institute (OHRI), Ottawa, Ontario K1Y 4E9, Canada

The secretory proprotein convertase (PC) family comprises nine members: PC1/3, PC2, furin, PC4, PC5/6, PACE4, PC7, SKI-1/S1P, and PCSK9. The first seven PCs cleave their substrates at single or paired basic residues, and SKI-1/S1P cleaves its substrates at non-basic residues in the Golgi. PCSK9 cleaves itself once, and the secreted inactive protease escorts specific receptors for lysosomal degradation. It regulates the levels of circulating LDL cholesterol and is considered a major therapeutic target in phase III clinical trials. *In vivo*, PCs exhibit unique and often essential functions during development and/or in adulthood, but certain convertases also exhibit complementary, redundant, or opposite functions.

A large number of secretory proteins are produced as precursors that are cleaved at specific sites to generate mature bioactive products (1, 2) or, in some cases, inactive ones (Fig. 1). Most of these cleavages occur after basic amino acids (aa)[2] at the general motif (R/K)-2nX-R↓ (where $n = 0-3$ aa) (3). They are achieved by one or more of the seven basic aa-specific members of the proprotein convertase (PC) family, which share identities with bacterial subtilases and yeast kexin (genes *PCSK1–PCSK7*) (3). PC1/3 and PC2 are the prototypical PCs of the regulated secretory pathway of endocrine and neuronal cells and are stored in dense core secretory granules (SGs). Often coexpressed in the same tissues and in the same cells (4), they are dedicated to the proteolytic activation of polypeptide hormones and neuropeptides precursors (3, 5). Four of the basic aa-specific PCs (furin, PC5/6, PACE4, and PC7) are widely or ubiquitously expressed and are responsible for most of the processing events occurring in the constitutive secretory pathway: the *trans*-Golgi network (TGN), cell surface, or endosomes. This leads to the activation/inactivation of receptors, ligands, enzymes, viral glycoproteins, or growth factors (6). Although these PCs exhibit a certain degree of functional redundancy when overexpressed in cell lines, their inactivation in mice or humans results in specific phenotypes revealing that, *in vivo*, each PC primarily fulfills unique processing events and/or functions.

Silencing of the genes coding for PC1/3 (7) and PC2 (8) in mice revealed dominant unique neuroendocrine functions for each protease. Furin knock-out (KO) in mice results in numerous embryonic malformations, including the absence of axial rotation and heart looping, leading to death around embryonic day 11 (9). PC5/6 KO leads to death at birth with an altered anteroposterior pattern, including extra vertebrae, lack of a tail, kidney agenesis, hemorrhages, collapsed alveoli, and retarded ossification (10). PACE4 KO results in an altered left-right patterning, including cyclopism and craniofacial and cardiac malformations in some embryos (11). Finally, silencing the expression of PC7 (12) in mice results in anxiolytic and novelty-seeking phenotypes (13), and in human genome-wide association studies as well as cellular studies, PC7 was shown to regulate iron metabolism via shedding of transferrin receptor-1 (14).

SKI-1/S1P (15, 16) is implicated in cholesterol and fatty acid synthesis via activation of the precursors of membrane-bound transcription factors such as sterol regulatory element-binding proteins SREBP1 and SREBP2 (16) and endoplasmic reticulum (ER) stress via cleavage of ATF6 (17). Processing by SKI-1/S1P is often followed by a second luminal cleavage by the protease S2P, resulting in the release of an N-terminal cytosolic domain that acts as a transcription factor upon its translocation into the nucleus (16).

PCSK9, the last member of the PC family (18), uniquely acts as a binding protein to some of the LDL receptor (LDLR) family members (3). The absence of its expression in mice (19, 20) or humans (21) leads to severe hypocholesterolemia, whereas human gain-of-function (GOF) mutations (22, 23) or overexpression in mice (20) results in hypercholesterolemia.

Studies aimed at defining the physiological functions of the PCs *in vivo* heavily relied on the simple or complex phenotypes of mice in which one or more PC gene had been knocked out throughout the body or in a tissue-specific manner and on those derived from transgenic mice overexpressing these PCs (Fig. 2). Because a number of excellent reviews summarized the discovery, general properties, and physiological roles of the PCs (1, 3, 6, 24–26), in this minireview, we will focus on their novel functions and the clinical relevance of their targeting. We will also present our thoughts on the observation that, although often acting in concert, the PCs process specific substrates in ways that result in unique, complementary, redundant, or opposing physiological consequences (Fig. 1*B*).

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[1] To whom correspondence should be addressed. E-mail: seidahn@ircm.qc.ca.
[2] The abbreviations used are: aa, amino acid(s); PC, proprotein convertase; SG, secretory granule; TGN, *trans*-Golgi network; KO, knock-out; ER, endoplasmic reticulum; LDLR, LDL receptor; GOF, gain-of-function; iCT, inhibitory C-terminal peptide; AL, anterior lobe; NIL, neuro-intermediate lobe; α-MSH, α-melanocyte-stimulating hormone; GLP, glucagon-like peptide; LOF, loss-of-function; NCAD, N-cadherin; LDLc, LDL-cholesterol; CHRD, Cys- and His-rich domain; VLDLR, VLDL receptor.

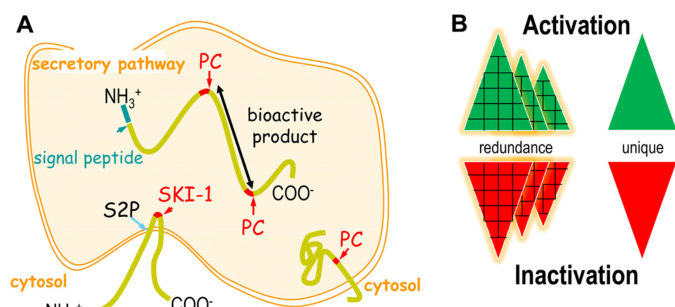


FIGURE 1. **Substrates of the PCs.** *A*, these include precursors of hormones, growth factors, receptors, transcription factors, and surface glycoproteins. Cleavage at one or more PC sites usually results in the release of one or more bioactive moieties for soluble or membrane-bound precursors. Some of them are first cleaved by SKI-1/S1P and then processed a second time by S2P to release a cytosolic transcription factor that is then translocated into the nucleus. *B*, processing of polypeptide precursors by PCs results in the activation or inactivation of their substrates predominantly in a redundant or complementary fashion between PCs. However, unique activation or inactivation PC cleavages have also been reported, some of which are essential during embryonic development.

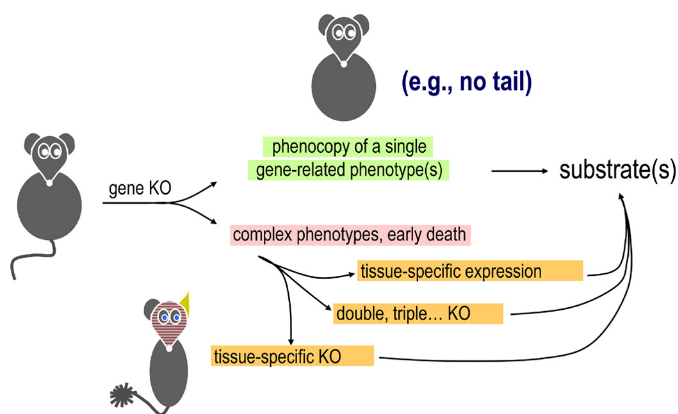
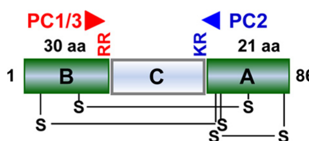
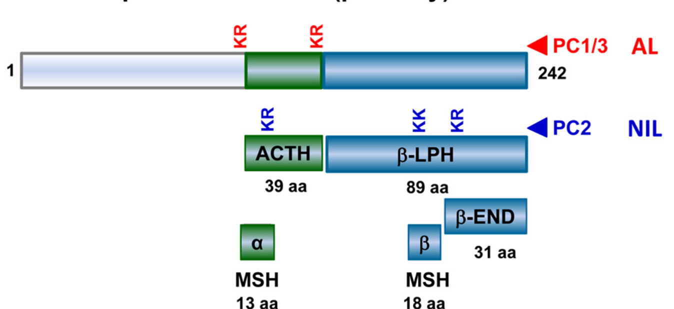


FIGURE 2. **Identification of PC substrates from the simple or complex phenotypes observed following modulation of their expression.** The KO of a PC gene may result in an obvious phenotype (*e.g.* lack of a tail, as for PC5/6 KO) that would phenocopy the absence of a cognate substrate, as for PC5/6 and GDF11 (10). However, of the nine PCs, the loss of expression of four of them (furin, PACE4, PC5/6, and SKI-1/S1P) reveals phenotypes that are more complex and that lead to early death. The identification of *in vivo* substrates may require tissue-specific KO, double or triple KOs, and even transgenesis in some tissues.

Functional Overlap and Complementary and Opposing Roles of PC1/3 and PC2

In the ER, the newly biosynthesized proPC1/3 rapidly converts itself to PC1/3 by cleavage after its prosegment, forming an inactive PC1/3-prosegment complex. In the TGN, the prosegment undergoes a second cleavage and comes off the complex, thereby activating the enzyme. Later, PC1/3 is activated even further in SGs by C-terminal truncations. Even though proPC2 does not need the assistance of the helper protein 7B2 to navigate out of the ER, the presence of this chaperone-like protein ensures its proper folding and activation into PC2 in SGs. This protein and its inhibitory C-terminal peptide (iCT) form a double lock on PC2, keeping it inactive until it reaches the SGs. Thus, PC1/3 can efficiently process substrates in the TGN and SGs, thereby acting before PC2, which is active only in SGs (27). The activities of these enzymes are temporarily inhibited by SG-resident polypeptides: the PC1/3 helper pro-





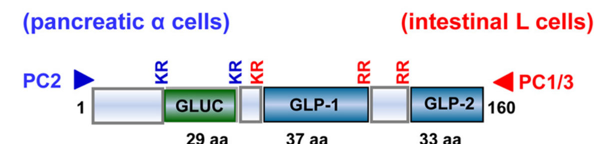


FIGURE 3. **Cooperation and distinctiveness of PC1/3 and PC2 in the processing of three representative substrates.** *A*, proinsulin processing in pancreatic β-cells implicates the two enzymes within the same cell, cleaving successively at two distinct preferred sites. *B*, pro-opiomelanocortin in two different lobes of the pituitary is cleaved to distinct end products according to the relative abundance of the enzymes in each lobe: ACTH and β-lipotropic hormone (β-*LPH*) in the AL and α-MSH, β-MSH, and β-endorphin (β-*END*) in the NIL. *C*, proglucagon is converted to glucagon (*GLUC*) in PC2-rich pancreatic α-cells and to GLP-1 and GLP-2 in PC1/3-rich intestinal L-cells. Note that after cleavage by PC1/3 and PC2, the C-terminally exposed basic residues must be trimmed off by carboxypeptidase E to generate fully active peptides.

tein proSAAS (28) and the PC2 chaperone-like protein 7B2 iCT (29).

PC1/3 and PC2 generally cleave their substrates after paired basic residues and occasionally after single basic residues. They often act on the same substrates, sometimes partially substituting for one another, but more frequently complementing each other. For example, in human pancreatic β-cells, PC1/3 most efficiently cleaves proinsulin after Arg^{31}-Arg^{32} at the B-C peptide junction, and PC2 cleaves after Lys^{64}-Arg^{65} at the C-A peptide junction (30, 31). Thus, it takes both enzymes to efficiently convert proinsulin to insulin and the C peptide (Fig. 3*A*). Although the absence of either PC1/3 or PC2 does not abrogate insulin production, it reduces it and causes accumulation of processing intermediates (32, 33).

The subcellular co-localization and relative abundance of PC1/3 and PC2 also shape the processing end products of a particular substrate. Thus, although both enzymes are present in the two lobes of the rodent pituitary, PC1/3 is more abundant in the anterior lobe (AL), and PC2 is more abundant in the neuro-intermediate lobe (NIL) (4). Pro-opiomelanocortin is expressed in both pituitary lobes: in the AL, it is converted by PC1/3 mostly to ACTH and β-lipotropic hormone, and in the NIL, it is converted by PC2 mostly to α-melanocyte-stimulating

hormone (α-MSH) and β-endorphin (Fig. 3*B*) (34). Similarly, in intestinal L-cells rich in PC1/3, proglucagon is converted to glucagon-like peptides (GLPs) (35), whereas in PC2-rich pancreatic α-cells, it is converted to glucagon (Fig. 3*C*) (36).

Physiological phenotyping of PC1/3- and PC2-null mice revealed the implications of both enzymes in growth and metabolism. PC1/3-null mice exhibit partial prenatal and perinatal lethality, microsomia at birth, and gastrointestinal disturbances (7). Heterozygotes display accelerated weight gain, glucose intolerance, and susceptibility to diet-induced obesity (7, 37). PC2-null mice exhibit microsomia at birth, catch-up growth after birth, lipodysgenesis, glucose tolerance, and diet-induced obesity resistance as adults (8, 38). It therefore appears that the two enzymes are the ying and yang of body mass and energy homeostasis, with PC1/3 playing a restraining role and PC2 playing a promoting one. More insight into their opposing roles in various tissues and cells could be gained by qualitative, quantitative, and functional characterization and validation of the peptides generated in the absence of either one, as recently reported in the brains of mice lacking expression of PC1/3 or PC2 (39, 40).

It is likely that various "modifier" gene products influence the observed phenotypes of PC1/3 or PC2 deficiency in mice. The lack of penetrance of the perinatal lethality of PC1/3-null mice of mixed genetic background (7) probably reflects variable inheritance of modifier loci. Similarly, pathological hypercorticosterolemia and lethality in PC2-deficient mice depend on the genetic background (41).

SAAS and 7B2 iCT could be counted among such modifier gene products. Overproduction of SAAS in transgenic mice by inhibiting PC1/3 also causes obesity and diabetes (42). 7B2 gene (*Sgne1*) inactivation in mice is associated not only with complete PC2 deficiency but also with a mouse strain-dependent (C57BL/6 *versus* 129/Sv background) lethal Cushing-like syndrome (41, 43). However, in FVB/N congenic mice, 7B2 deficiency causes a unidirectional circling behavior (see supplemental video), reminiscent of the *hyt*/*hyt* thyroid-stimulating hormone-deficient mouse (44). A PC2-independent neuronal role of 7B2 has been suggested by its more extended distribution in the central nervous system (45) and by its recently reported anti-protein aggregation properties, implying that it could protect neurons against aggregation-associated neurodegenerative pathologies such as Alzheimer disease (46).

In humans, severe genetic lesions at the *PCSK1* locus have implicated PC1/3 activity in body mass regulation and gastrointestinal dysfunction. Early in life, carriers often exhibit a remarkable obesity and malabsorption. These symptoms are sometimes accompanied by diabetes insipidus, hypothyroidism, and hypogonadism (47). No similar lesions that significantly reduce PC2 expression or activity have been reported.

Interestingly, in invertebrate species such as *Caenorhabditis elegans* and *Drosophila melanogaster*, only PC2 and 7B2 are expressed, but not PC1/3. A review of exome databases for known SNPs suggests that *PCSK1* is more variable than *PCSK2*. One can speculate that *PCSK2* is more critical for survival, as it contributes to the basic buildup of body mass and adipose tissue. On the other hand, *PCSK1* might be an adaptive gene capable of modulating this buildup through phenotypic loss-of-function (LOF) variations positively selected for by environmental pressures.

Unique and Complementary Functions of Furin, PACE4, PC5/6, and/or SKI-1/S1P

Although the completion of the zymogen activation of furin occurs in the TGN, where it begins to cleave some of its cognate substrates, it can also do so at the cell surface or in recycling endosomes (48). In contrast, PC5/6 and PACE4 are activated predominantly at the cell surface, where they bind heparin sulfate proteoglycans and process their substrates at this location and/or within the extracellular matrix (6). Therefore, the substrates of furin are wider and much more varied than those of PC5/6 and PACE4. Some of the unique furin substrates include TGFβ-like precursors such as BMP10 during embryonic development (49) and the iron-regulating protein hepcidin in adults (14). However, redundant functions of furin with those of other PCs have also been observed, as exemplified in the liver (50), and with PACE4 in the early embryonic processing of the TGFβ-related Nodal precursor (51).

GDF11 (growth differentiating factor 11) was shown to be a unique PC5/6 substrate that is activated during development (10). The only validated unique substrates physiologically activated by PACE4 are the matrix metalloproteases ADAMTS-4 and ADAMTS-5; cleavage occurs in the extracellular milieu of cartilage and results in aggrecan degradation (52). The binding of both PC5/6 and PACE4 to heparin sulfate proteoglycans (6) likely accounts for their spatial segregation from furin, which cycles between the TGN and the cell surface (48), and favors their cleavage of specific substrates during embryogenesis (51) and in the adult. In fact, such spatial segregation of PCs and/or other cellular factors may be in part responsible for the differences observed *in vitro* and *in vivo*, whereby some substrates are cleaved by more than one PC *in vitro*, whereas a single PC is responsible for such processing *in vivo*. However, this may be tissue-specific, as in the case of prohepcidin in the liver (14), or could vary during development in specific cellular environments.

Recently, it was shown that a single substrate can be cleaved by some of the constitutive PCs and/or SKI-1/S1P at similar or different sites, resulting in redundant or complementary functions. For example, the human lipoprotein lipase inhibitor ANGPTL4 (angiopoietin-like 4) is redundantly processed and activated by furin, PC5/6, PACE4, and PC7 at the same site: $RRKR^{164} \downarrow LPE$ (53).

Complementarities between PC activities of different specificities are exemplified by the combined processing of specific substrates by furin and SKI-1/S1P under physiological or pathological conditions. In the developing embryo, the combined action of furin and SKI-1/S1P reduces axonal growth by the generation of multiple forms of the repulsive guidance molecule RGMa, which are essential for inhibition of neogenin-mediated neuronal outgrowth (54). RGMa is a tethered membrane-bound molecule, and proteolytic processing amplifies RGMa diversity by creating soluble versions with long-range effects as well (54). A pathological situation involves the enhancement of viral infection by the combined SKI-1/S1P-

and furin-mediated activation of the surface glycoprotein of the Crimean-Congo hemorrhagic fever virus (55, 56).

Opposite Activation/Inactivation of Substrates through Processing by Furin, PC5/6, PACE4, and/or PC7

Cleavage of precursor substrates by PCs commonly generates one or more bioactive moieties. Such an activation process is not generalized because, in some cases, it may result in inactivation of the substrate (Fig. 1). One of the earliest examples is the inactivation of ACTH in the intermediate lobe of the pituitary, resulting in the generation of an acetylated-amidated N-terminal peptide known as α-MSH. However, this is strictly not an inactivation *per se* because a new active melanotropic peptide is generated. Examples of *bona fide* inactivation of proteins include the processing inactivation of endothelial and lipoprotein lipases by furin (57); activation of the surface glycoprotein gp160 of HIV by furin (58) but inactivation of the HIV accessory protein Vpr by PACE4 and PC5/6 (59); inactivation of PCSK9 by furin (60); and finally, activation of the adhesion molecule N-cadherin by furin (61) but its inactivation by PC5/6 (62).

N-cadherin (NCAD), a member of the cell adhesion superfamily, is expressed as a non-adhesive proprotein (proNCAD). Before reaching the plasma membrane, proNCAD undergoes proteolytic processing of its prosegment at **R**Q**KR** ↓ DW^{161} by furin (61). Homophilic adhesion of NCAD requires the evolutionarily conserved Trp^{161} (63). However, downstream of Trp^{161} resides another PC site, **R**I**R**SD**R** ↓ DK^{189}, which is best cleaved by PC5/6 (62). The presence of two adjacent sites on NCAD implies that its adhesive function is regulated by opposing furin and PC5/6 activities. Indeed, in malignant gliomas, in which NCAD is the major cell-cell adhesion component, the equilibrium of cellular adhesion *versus* cellular dissemination is dependent on the expression of furin and PC5/6 (62). More specifically, invasive glioma cells abolish homophilic NCAD interactions either by down-regulating furin, which results in non-adhesive proNCAD expression at the cell surface (61), or by overexpressing PC5/6, which cleaves and inactivates NCAD (62). Thus, it would be interesting to test this model *in vivo* by inhibiting PC5/6 function in invasive cancer cells.

The convertase PC7 is unique in its ability to shed transferrin receptor-1 and hence regulate iron metabolism (14). However, some redundancy with furin has been observed for the processing of the HIV gp160 into gp41 and gp120, as well as in the generation of bioactive proBDNF.[3] This convertase seems to play an important role in mood modifications in part via the regulation of central dopaminergic circuits (13). So far, no opposite function to the other convertases has been reported.

The Convertase SKI-1/S1P: A Unique Protease Acting in the *cis/medial*-Golgi

The convertase SKI-1/S1P is a type 1 membrane-bound protease with a cytosolic tail rich in acidic residues (15). Subcellular localization studies revealed that SKI-1/S1P can be found in the active state in the *cis*/*medial*-Golgi and in endosomes/lysosomes, but not at the cell surface (64). *In situ* hybridization data revealed a widespread if not ubiquitous distribution of SKI-1/S1P mRNA (15). In the central nervous system, SKI-1/S1P mRNA labeling is confined mostly to neurons.

SKI-1/S1P uniquely processes precursors exhibiting the consensus motif (R/K)X(V/L/I)Z ↓, where Z is any amino acid except Val, Pro, Cys, Glu, or Asp, and the spacer X is often a basic residue (65). SKI-1/S1P cleaves transmembrane transcription factors, including the cholesterol and fatty acid regulators SREBP1 and SREBP2, the ER stress response factor ATF6, and the cAMP response element-binding protein-like transcription factors Luman and CREB4 (65). It also processes soluble precursors such as those of proBDNF (15) and somatostatin (65), activates envelope glycoproteins of highly infectious hemorrhagic fever viruses (55, 66), and regulates lysosomal targeting of proteins by activating the addition of phosphate moieties onto mannose residues (67).

Targeted disruption of the SKI-1/S1P-encoding gene (*Mbtsp1*) prevents normal epiblast formation and subsequent implantation of the embryo into the uterus (68). Interestingly, both hypopigmentation and maternal-zygotic embryonic lethality before embryonic day 8 were reported in mice harboring a homozygous hypomorphic Y496C mutation (69). This mutation affects SREBP2 processing (RSVL ↓ SF) more than SREBP1 processing (RNVL ↓ GT), suggesting that the latter may be a better substrate of SKI-1/S1P. The reason behind this is unknown, although it may be due to the possible Ser *O*-glycosylation of the SREBP2 cleavage site at the P3 and/or P1′ position, which would impair processing (70).

Tissue-specific KO of the SKI-1/S1P gene in the liver or cartilage in mice confirmed its regulation of cholesterol and fatty acid synthesis (68) and its essential role in endochondral bone formation (71). Finally, the non-peptidyl PF-429242 inhibitor of SKI-1/S1P was shown to be very effective in inhibiting Lassa virus infection (72) and hepatitis C replication (73).

Mucolipidosis II is a severe lysosomal storage disorder caused by defects in the α- and β-subunits of the hexameric GlcNAc-1-phosphotransferase complex essential for the formation of the mannose 6-phosphate targeting signal on lysosomal enzymes. The observation that SKI-1/S1P activates the α/β-subunit precursor of the GlcNAc-1-phosphotransferase by cleavage at the **R**Q**L**K ↓ site (67) is physiologically pertinent in the biogenesis of lysosomes. In fact, loss of SKI-1/S1P expression (or by extension, its inhibition) may result in lysosomal dysfunction because instead of sorting proteins to lysosomes via the mannose 6-phosphate receptor, those lacking mannose 6-phosphate will then be secreted into the extracellular matrix (67).

PCSK9: A Key Player in Cholesterol Homeostasis and a Major Target in the Treatment of Hypercholesterolemia

In 2003, the discovery of the ninth member of the PC family, PCSK9 (18), an enzyme encoded by a third gene involved in autosomal dominant hypercholesterolemia (22), revealed an unsuspected regulation of the LDLR, with major implications for cardiovascular disease. PCSK9 binds to the LDLR and directs it to lysosomes for degradation (74, 75). This was one of the most exciting developments in cardiovascular research in the last decade (3). This fundamental knowledge went from

[3] N. G. Seidah, unpublished data.

bedside to bench and back in <9 years. Indeed, a number of pharmaceutical companies are now racing to develop PCSK9 inhibitors that may in the future substitute or complement the use of statins (3, 76).

Because of the high expression of PCSK9 in the liver and intestine and its chromosomal localization (1p32) (18), coinciding with a locus linked to hypercholesterolemia, a relationship between mutations in *PCSK9* and hypercholesterolemia was established in French families by Abifadel *et al.* (22). GOF mutations in *PCSK9* were shown to deplete the LDLR (22, 74), with ensuing increased LDL-cholesterol (LDLc) levels in plasma. Conversely, *PCSK9* LOF mutations result in hypocholesterolemia (77). Low concentrations of active PCSK9 are also associated with lower incidence of atherosclerosis (78).

Cell Biology of PCSK9—Similar to other PCs, PCSK9 is synthesized as an ~74-kDa precursor (proPCSK9) that undergoes an autocatalytic cleavage at the VFAQ$^{152}\downarrow$ site, allowing it to exit the ER. (i) However, the inhibitory prosegment remains tightly bound to PCSK9 (18, 79). (ii) The ER exit of the enzymatically inactive PCSK9-prosegment complex requires its binding to an undefined membrane-bound hinge protein. The cytosolic tail of this putative hinge protein is proposed to interact with the Sec23A/Sec24A complex, leading to its packaging into COPII (coat protein complex II) vesicles, which are then routed to the *cis*-Golgi and extracellular secretion (80). (iii) The catalytic domain of PCSK9 binds to the EGF-A domain of the LDLR, and the resulting PCSK9-LDLR complex is then directed to lysosomes for degradation by still poorly defined intracellular and extracellular pathways (3, 81). (iv) PCSK9 exhibits a C-terminal Cys- and His-rich domain (CHRD) essential for PCSK9-triggered LDLR degradation (3). Internalization of the PCSK9-LDLR complex in early endosomes (82) does not require the C-terminal CHRD of PCSK9 (83) or its acidic N-terminal 31–59 segment (84), which was shown to bind apoB in LDL (85), and the cytosolic tail of the LDLR is not essential (86). (v) However, targeting of the PCSK9-LDLR complex to lysosomes for degradation does require the integrity of the CHRD (83, 87). This led to the hunt for the protein responsible for targeting of the complex to lysosomes. A recent report suggested that such a protein in HepG2 cells could be APLP2 (amyloid precursor-like protein 2) (88). However, a careful analysis of APLP2 KO mice would be required to validate this intriguing observation.

In human and mouse plasma, both full-length PCSK9 (aa 152–692) and a truncated form, PCSK9-ΔN_{218} (aa 219–692), can be detected. The latter, which has little activity on the LDLR, is likely generated by hepatocyte-derived furin by cleavage at Arg$^{218}\downarrow$ (60, 89). That cleavage of PCSK9 by purified furin *in vitro* does not completely inactivate its function (90) may be due to the artificially remaining C-terminal Arg^{218} in the segment comprising aa 153–218, which is usually removed by cellular carboxypeptidases *in vivo*, resulting in the separation of this fragment as well as the prosegment from the rest of the molecule and inactivation of the remaining PCSK9 (89).

Animal Models for Studying PCSK9 Functions—PCSK9 KO (*Pcsk9*$^{-/-}$) mice exhibit higher levels of LDLR protein in the liver and 42% less circulating total cholesterol, with an ~80% drop in LDLc (19, 20). In contrast, transgenic mice overexpressing PCSK9 exhibit 5–15-fold higher levels of LDLc (20). Total KO and liver-specific KO mice exhibit 42 and 27% less circulating total cholesterol, respectively, indicating that hepatic PCSK9 is responsible for approximately two-thirds of the phenotype. Analysis of liver KO livers demonstrated that PCSK9 expression is restricted to hepatocytes, from where most circulating PCSK9 originates (20, 91). The plasma lipid profile of double KO mice (*Pcsk9*$^{-/-}$ *Ldlr*$^{-/-}$) lacking both PCSK9 and the LDLR is identical to that of *Ldlr*$^{-/-}$ mice, confirming that PCSK9 activity on the LDLR mediates most of its role in cholesterol homeostasis (20, 91). Thus, although SKI-1/S1P increases the levels of the LDLR via activation of SREBP2, PCSK9 enhances the degradation of the LDLR as well as the closely related VLDL receptor (VLDLR), ApoER2 (92), and LRP-1 (93). Indeed, VLDLR proteins accumulate in the adipose tissue of PCSK9 KO mice, resulting in marked adipocyte hyperplasia (91). PCSK9 is thus pivotal in fat metabolism: it maintains high circulating LDLc levels via hepatic LDLR degradation, but it also limits visceral adipogenesis likely via adipose VLDLR regulation.

Here, we present some information regarding the possible consequences of the loss of PCSK9 expression. 1) Partial hepatectomy of *Pcsk9*$^{-/-}$ mice revealed the development of lesions and a delay in hepatocyte proliferation (20). Interestingly, when fed a high cholesterol diet 1 week prior to partial hepatectomy, KO mice no longer exhibited necrotic lesions. 2) Compared with control mice, PCSK9-null male mice are hyperglycemic and glucose-intolerant, suggesting that, following stimulation by high glucose levels, PCSK9 may be necessary for the normal function of pancreatic islets (94). 3) The absence of PCSK9 can be protective against melanoma invasion in mouse liver (95), opening the door to novel applications of PCSK9 inhibitors/silencers in cancer/metastasis. 4) Intriguingly, lack of PCSK9 is not associated with lipid accumulation in the liver or increased hepatobiliary cholesterol excretion (96), but it significantly reduces liver and plasma sphingolipids in both humans and mice (97). In that context, it was recently demonstrated that ~30% of the circulating LDLc is rather excreted from blood through the gut via a *trans*-intestinal cholesterol excretion pathway. *Pcsk9*$^{-/-}$ mice exhibit increased *trans*-intestinal cholesterol excretion in part due to the PCSK9-regulated LDLR and another receptor (98).

Human Mutations and Clinical Trials Using Monoclonal Antibodies to PCSK9—Human *PCSK9* is a highly polymorphic gene, with >150 coding mutations compiled (LOVD Database), many of which result in either LOF or GOF. In 2011, Chrétien and co-workers (99) identified, in a French Canadian family, the unique LOF Q152H point mutation situated at the autocatalytic zymogen-processing site of PCSK9. This mutation is associated with marked hypocholesterolemia and an ~70–80% reduction in the level of circulating PCSK9 (99). The resulting proPCSK9 acts as a dominant-negative in the ER, preventing the zymogen processing of the non-mutated protein derived from the wild-type allele (99, 100). Mimicking this genetic defect with a specific small molecule inhibitor may lead to an orally bioavailable drug.

Two women lacking functional PCSK9 have extremely low levels of LDLc (~0.4 mM), indicating that PCSK9 inhibition

may be safe (3). Thus, anti-PCSK9 monoclonal antibodies that interfere with PCSK9-LDLR complex formation are efficient in reducing LDLc levels. So far, the clinical data are very encouraging, with phase II trials showing a drop of >60% in LDLc for at least 2 weeks after a single subcutaneous injection of a humanized anti-PCSK9 monoclonal antibody in the presence or absence of the cholesterol-lowering "statin" therapy. Phase III clinical trials have now started with >20,000 individuals being tested, and anticipated results should be forthcoming in 2016/2018 (reviewed in Ref. 76). Other approaches including the use of recombinant adnectins, antisense RNAi, or small molecule inhibitors are also undergoing early preclinical testing or are already in phase I clinical trials (3, 76).

Conclusions

The original hypothesis of the existence of secretory precursors that, upon specific cleavage(s), release bioactive peptides or proteins has been amply tested and confirmed in many precursor proteins from various species (1, 2). The long process of hunting for the cognate proteases responsible for such processing led to the identification of nine PCs with multiple physiological functions that, in some cases, are associated with pathology (3). Some PCs are essential for normal development, tissue homeostasis, and metabolic cascades, whereas are others have more limited physiological functions. In humans, *PCSK1* LOF and *PCSK9* GOF mutations can lead to pathology. In contrast, LOF variants of PCSK9 are protective against hypercholesterolemia. The accumulated knowledge over the last 23 years leads us to think that inhibiting some of these convertases may be beneficial, in some cases, to the patient. The most advanced target is by far PCSK9 because data from human genetics and mouse models encouraged the development of efficacious methods to reduce its activity on the LDLR through the use of injectable monoclonal antibodies. This brings about a powerful new strategy to reduce hypercholesterolemia, especially in patients that do not tolerate statins, are resistant to their action, or do not achieve low levels of LDLc by the available treatments. In the future, modulation of some of the other PCs for specific conditions may also find its way into the clinic.

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