Regulation of Carcinogenesis, Angiogenesis and Metastasis by the Proprotein Convertases (PCs)

Regulation of Carcinogenesis, Angiogenesis and Metastasis by the Proprotein Convertases (PCs)

A New Potential Strategy in Cancer Therapy

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Cover Legend: Cascade events implicating the proprotein convertases (PCs) in tumor growth and metastasis. PCs control tumor cell adhesion by activating or/and inducing the expression of adhesion molecules, regulate cell proliferation by activating growth factors, cytokines, and their receptors and induce migration and invasion of tumor cells that leads to metastases formation by activating MMPs.

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PREFACE

To date, cancer therapies are mainly based on the use of cytotoxic drugs and/or radiotherapy that have a relatively low therapeutic index with significant side effects, although they have a potent antitumor activity, since they target both cancer and normal cells. Furthermore, several cancer types are intrinsically not sensitive or become resistant to treatment with cytotoxic drugs or radiotherapy. For this reason, there is a need for the development of novel effective anticancer agents that are more specific for cancer or less toxic for normal cells. The recent discovery regarding the implication of the proprotein convertases in the processes of tumor progression and metastasis, made these enzymes potential new targets in cancer therapy. The first studies demonstrating experimentally the importance of the convertases in cancer was reported in the year 2001. Since this period, the growing body of knowledge and evidence regarding the importance of these molecules in the activation and the regulation of molecules involved in tumor progression and metastasis such as growth factors, adhesion molecules and MMPs is now leading to the increased understanding of the processes of tumorigenesis, angiogenesis and metastasis processes.

However, although the level of the PCs was reported to be higher in various cancers and the idea to inhibit their activity and/or expression may constitute a promising new strategy in cancer therapy, these enzymes are also required for the homeostasis of normal cells. Thereby, the development of PCs inhibitors-derived drugs whose actions can target specific tissues and cell types is required. Similarly, although the use of general PC inhibitors may be advantageous, in some cases it may be necessary to target only one member of the PC family. Therefore, one of the important future developments would be to find and express PC inhibitors specific for each member of the PC family. This is feasible, as was demonstrated by the identification of the specific and natural inhibitor of the convertase PC1 (pro-SAAS) and the convertase PC2 (7B2). In the long term, these inhibitors may provide a rationale for testing this family of compounds as anti-metastatic agents or in conjunction with standard therapy in clinical settings.

In this book, leading experts and pioneers in the area of convertases research dealing with cancer provide an overview that summarizes the current state of knowledge on the role of these enzymes in carcinogenesis and metastasis.

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INTRODUCTION

THE EVER EXPANDING SAGA OF THE PROPROTEIN CONVERTASES: FROM BENCH TO BEDSIDE

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The number of protein/peptide products that result from a given genome depends on multiple factors that generate both diversity and specificity. Prominent among these are processes that regulate post-translational modifications of the primary product of mRNA translation, the precursor protein. The primary events governing the modification of the amino acid chain of secretory proteins include the N-glycosylation and signal peptide cleavage by signal peptide peptidase. This is then followed by trimming of the glycosylation chain and remodeling up until it reaches its final form in the Trans Golgi Network (TGN). Since the early/mid 1960s it was realized that most secretory proteins undergo at least one peptide bond cleavage along their trafficking pathway, e.g., by signal peptide peptidase in the endoplasmic reticulum (ER) and/or by one or more proteinase in the Golgi apparatus to release the final form of the protein and/or its processing products. Proteolysis is essentially an irreversible process, because no known enzyme can repair broken peptide bonds under normal physiological conditions. The primary event of peptide bond cleavage induces conformational changes in the resulting product, thereby generating productive biological activity. The repertoire of the secretory protein precursors that undergo limited proteolysis is large and varied. It includes many proteins that are translocated across membranes such as polypeptide endocrine and neural hormones, growth factors and their receptors, membrane bound transcription factors, adhesion molecules, extracellular matrix proteins, proteases and other types of enzymes, as well as a number of surface glycoproteins of opportunistic pathogenic viruses and bacteria.

While it is predicted that the mammalian genome codes for 460 human and 525 mouse functional proteases [1], only a handful of these are implicated in the intracellular limited proteolysis of precursor proteins.

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Prominent amongst the proprotein processing enzymes are the members of the family of subltilisin/kexin-like proprotein convertases (PCs). It took more than 15 years to identify these serine proteinases that can be subdivided into three subfamilies: [**A**] The basic amino acid specific kexin-like PCs include seven members: PC1/3, PC2, Furin, PC4, PC5/6, PACE4 and PC7 [2]; [**B**] The pyrolysin-like subtilisin-kexin isoform SKI-1/S1P, also known as site 1 protease S1P [3]; and [**C**] The proteinase K-like neural apoptosis regulated convertase NARC-1/PCSK9 [4]. The last two convertases cleave at non-basic residues and process precursors that are distinct form those of the basic amino acid-specific convertases [3–6].

The discovery of these convertases from 1989–2003, elicited a wide interest in the scientific community as it was realized that these enzymes play key roles in various homeostatic as well as pathogenic events [2, 5–10]. The most evident role came from studies of the tumorigenic potential of these convertases, where it was shown that overexpression of one or more of the basic amino acid specific PCs leads to increased cell proliferation and enhanced metastasis, while their inhibition reverses this effect [11–14]. However, this is not universally the case, as a decreased expression of the Cys-rich domain containing PC5 [15, 16] and PACE4 [17] has been observed in various cancers including breast and ovarian cancers, as well as the increased metastatic potential of the human colon carcinoma HT-29 cells overexpressing α1-PDX, a potent inhibitor of the constitutively secreted convertases [18].

On another front, the implication of the PCs in viral infections became apparent from the processing sites of the surface glycoproteins of infectious viruses and of bacterial toxins [19]. In fact, data on various infectious viruses and bacterial toxins showed that cleavage of surface/spike glycoprotein precursors of these pathogens by one or more member of the PC-family, including the basic amino acid- specific Furin, PC7, PACE4 and/or PC5 (2) and the pyrolysin-like SKI-1/S1P (20) is a required step for the acquisition of fusiogenic potential and thus for their infectious and/or cell-cell spreading capacity [19, 21].

Recently, some of the convertases such as PC5/6, SKI-1/S1P and NARC-1/PCSK9, were implicated in cardiovascular complications. Examples include the vital role of SKI-1/S1P in the regulation of the synthesis of cholesterol and fatty acids via the cleavage within the Golgi of the two master switches of sterol, and fatty acid metabolism, the sterol regulatory element binding proteins [SREBP-1 and SREBP-2] [22, 23]. The convertase PC5/6 has also been implicated in vascular remodeling and the development of atherosclerosis [24, 25], as well as in the phenomenon known as restenosis that occurs following balloon angioplasty or stint implantation [26]. In addition, PC5/6, which is highly expressed in endothelial cells [27, 28] has been implicated in the activation of endothelial lipase, and hence could positively regulate the level of high density lipoproteins (HDL) [29].

Finally, the last member of the family NARC-1/PCSK9 has clearly been associated with the development of dyslipidemias, as specific mutations in its coding sequence are directly responsible for the development of a dominant form of either familial hyper-cholesterolemia [5] or hypo-cholesterolemia [30]. This is

the first case of a dominant disease associated with mutations in one of the PCs. It seems that these mutations [6] result in either a gain/enhancement of an existing function, for those causing hyper-cholesterolemia [5], or in a loss of function in hypo-cholesterolemia patients [30]. The mechanism behind these pathologies is essentially related to one of the major roles of NARC-1/PCSK9 which is to enhance the degradation of the low density lipoprotein receptor (LDLR) [31] through a mechanism requiring entry into low pH endocytotic vesicles [32]. This exciting development opens the way to the development of anti-cholesterogenic drugs that could supplement the widely prescribed HMG-CoA reductase inhibitors, known as "statins" that themselves upregulate the expression of NARC-1/PCSK9 [33]. Indeed, supplementation of statins to the diet of mice lacking the expression of *PCSK9*, resulted in a marked additional decrease in the level of circulating total cholesterol [34].

The present monogram deals with multiple aspects of the proprotein convertases, from their discovery, to their analysis and to the projected pharmacological and clinical applications that may result from the inhibition of these enzymes. Thus, this is one example of "bench to bedside" directly applicable to the convertases. It is hoped that the use of modern day multiplexing technologies including various RNA and protein/peptide arrays should result in the development of specific convertase inhibitors that should find applications to control a wide variety of pathologies, including cancer and associated metastasis as well as dyslipidemias such as atherosclerosis and hypercholeste-rolemia. The importance of the PCs in the self renewal and maintenance of cancer stem cells [35] is a future area that begs extensive investigation, as it may opens the door towards stem cell-specific targeting of convertase inhibition. It took more than 30 years to unravel some of the mysteries of the proprotein convertases. It is hoped that the next decade will consolidate and expand the genetic, cellular and molecular knowledge of the PCs, including their 3D structures [36], in order to rationally design potent drugs that regulate their levels and/or activities *in vivo*.

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CHAPTER 1

DISCOVERY OF THE PROPROTEIN CONVERTASES AND THEIR INHIBITORS

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Abstract: The members of the convertase family play a central role in the processing of various protein precursors ranging from hormones and growth factors to viral envelope proteins and bacterial toxins. The proteolysis of these precursors that occurs at basic residues is mediated by the proprotein convertases (PCs), namely: PC1, PC2, Furin, PACE4, PC4, PC5 and PC7. The proteolysis at non-basic residues is performed by subtilisin/kexin-like isozyme-1 (S1P/SKI-1) and the newly identified neural apoptosis-regulated convertase-1 (NARC-1/PCSK9). These proteases have key roles in many physiological processes and various pathologies including cancer, obesity, diabetes, neurodegenerative diseases and autosomal dominant hypercholesterolermia. Here we summarize the discovery of the proprotein convertases and their inhibitors, discuss their properties, roles, resemblance and differences

Keywords: Proprotein convertases, SKI-1/S1P, NARC-1/PCSK9, Prosegments, α1-PDX, 7B2, ProSAAS

1. PROPROTEIN CONVERTASES (PCs)

To date, seven mammalian members of subtilisin-related PCs that process substrates at basic residues have been identified. These include Furin/*PACE*, PC1/*PC3*, PC2, PC4, PACE4, PC5/*PC6*, and PC7/*LPC/PC8/SPC7* (Figure 1).

This somewhat confusing nomenclature arose from the simultaneous discovery of some of these enzymes by different groups. PCs are multi-domain serine proteinases

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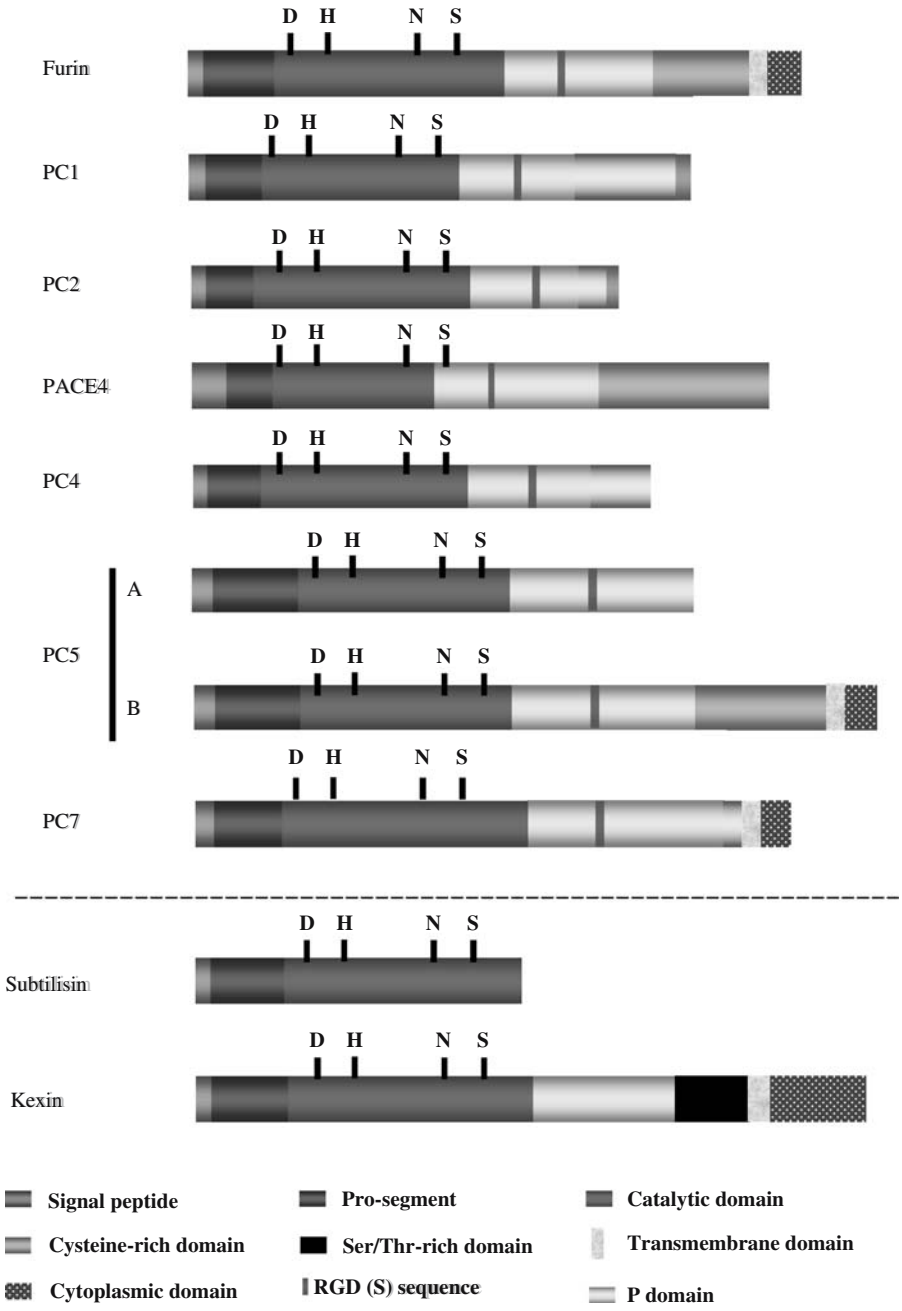

Figure 1. Schematic representation of the prohormone convertases PC1, PC2, Furin, PACE4, PC4, PC5 (A and B isoforms) and PC7. These PCs are multi-domain serine proteinases consisting of a signal peptide followed by prosegment, catalytic, middle, and cytoplasmic domains. Homology is highest in the catalytic domains and lowest in the carboxyl-terminal domains. The schematic representation for Kexin and subtilisin are given for comparison

Convertases	Amino acid number	Autocatalytic site	Accession number
Furin	**794**	**^{101}A-K-R-R-T-K-R-D**	**NP_002560**
PC1	**751**	**^{105}K-E-R-S-K-R-S-V**	**P21662**
PC2	**638**	**^{103}G-F-D-R-K-K-R-G**	**P16519**
PACE4	**969**	**^{141}Q-E-V-K-R-R-V-K**	**P29122**
PC4	**654**	**^{105}R-R-R-V-K-R-S-L**	**A54306**
PC5	**1870**	**^{109}V-K-K-R-T-K-R-D**	**Q04592**
PC7	**785**	**^{134}R-L-L-R-R-A-K-R**	**NP_004707**
SKI-1	**1052**	**^{131}K-V-F-R-S-L-K-Y**	**NP_003782**
NARC-1	**692**	**^{145}E-D-S-S-V-F-A-Q**	**NM_174936**

Figure 2. Amino acid sequences of the autocatalytic sites of the PCs. Like their substrates, the pro-segments of the PCs are removed at sites cleaved by the PCs. Indicated are the number of amino acid and accession number for every PC

consisting of a signal peptide followed by prosegment, catalytic, middle, and cytoplasmic domains (Figure 1). Homology is highest in the catalytic domains and lowest in the carboxyl-terminal domains.

These enzymes cleave precursor proteins at basic residues within the general motif $(\boldsymbol{K/R})$-$(\boldsymbol{X})_{\boldsymbol{n}}$-$(\boldsymbol{K/R})\downarrow$, where n = 0, 2, 4 or 6 and X any amino acid except Cys [1–4]. Usually most of the PCs cleave their substrates at pairs of basic amino acids, but several of them, with monobasic sites are also cleaved [1–4]. Some PCs, such as PC1, PC2 and PC5A, are sorted and activated in the regulated secretory pathway and thus process protein precursors whose secretion is regulated. In contrast, the transmembrane proteins Furin, PACE4, PC5B and PC7 (Figure 1), cycle between the cell surface and the *trans* Golgi Network (TGN) and are involved in the processing of precursor proteins in the constitutive secretory pathway [1–4]. Like their substrates, the pro-segments of the PCs are also removed at a cleavage site containing a basic–amino acid PC motif (Figure 2), befitting their autoactivation [1–4].

1.1 Furin

Furin was the first convertase identified. Its discovery was made just after the availability of the Kex2 cDNA sequence. Kex2 is a cellular processing endoprotease that is required for cleavage at dibasic sites within the killer toxin and the mating pheromone, α–factor precursors [5, 6]. In 1989, in an effort to find other