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Furin as Proprotein Convertase and Its Role in Normal and Pathological Biological Processes

N. I. Solovyeva*, T. A. Gureeva, O. S. Timoshenko, T. A. Moskvitina, and E. V. Kugaevskaya

Institute of Biomedical Chemistry, ul. Pogodinskaya 10, Moscow, 119121 Russia

**e-mail: Nina.Solovyeva@ibmc.msk.ru*

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Abstract—Furin belongs to intracellular serine Ca^{2+}-dependent endopeptidases of the subtilisin family, also known as proprotein convertases (PC). Human furin is synthesized as a zymogen with a molecular weight of 104 kDa, which is then autocatalytically activated in two stages. This process occurs during zymogen migration from the endoplasmic reticulum to the Golgi apparatus, where a large part of furin is accumulated. The molecular weight of the active furin is 98 kDa. Furin is the enzyme with narrow substrate specificity: it hydrolyzes peptide bonds at the site of paired basic amino acids and is active in a wide range of pH (5.0–8.0). The main biological function of furin as PC consists in activation of functionally important protein precursors. This is accompanied by initiation of cascades of reactions, which lead to appearance of biologically active molecules involved in realization of specific biological functions both in normal and in some pathological processes. The list of furin substrates includes biologically important proteins such as enzymes, hormones, growth/differentiation, receptors, adhesion proteins, plasma proteins. Furin plays an important role in the development of such processes as proliferation, invasion, cell migration, survival, maintenance of homeostasis, embryogenesis, as well as the development of a number of pathologies, including cardiovascular, cancer, and neurodegenerative diseases. Furin and furin-like proprotein convertases are key factors in the realization of the regulatory functions of proteolytic enzymes; the latter is currently considered as the most important function (compared with well recognized protease function in degradation of proteins).

Keywords: furin, domain structure, specificity, MT1-MMP, growth factors, natriuretic peptides

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INTRODUCTION

Proteolytic enzymes are involved not only in degradation of proteins; these enzymes have an important regulatory function as they are responsible for activation, inactivation and modification of the properties of the whole a number of biologically active molecules. Furin (EC 3.4.21.75) belongs to intracellular serine Ca^{2+}-dependent endopeptidases of the subtilisin family, also known as proprotein convertases (PC) [1, 2]. Nine PCs have been recognized; they share homology with subtilizin-like bacterial endopeptidases and yeast kexins (proteases of budding yeast). These include: furin itself also known as PACE (Paired basic Amino acid Cleaving Enzyme) or SPC1 (Subtilisin-like Proprotein Convertase 1); PC2 (Proprotein Convertase 2 or SPC2—Subtilisin-like Proprotein Convertase 2, EC 3.4.21.94); PC1/3 (SPC2, EC 3.4.21.93); PACE4 (EC 3.4.21.B25); PC4 (SPC5, EC 3.4.21.B24); PC5/6 (SPC6-A, EC 3.4.21.B26); LPC (Lymphoma Proprotein Convertase, PC7 or PC8—SPC7, EC 3.4.21.B27); SKI-1 (Subtilisin/Kexin-Isozyme-1 or SIP-1—Site-1 Proteinase, EC 3.4.21.112); PCSK9 (Proprotein Convertase Subtilisin-like/Kexin type, EC 3.4.21.112). PCs to which furin belongs share similar specificity towards substrates and inhibitors, their active sites are evolutionarily conservative and highly homologous. PCs demonstrate affinity to basic amino acids and their specificity is associated with nonpolar amino acid residues [3, 4]. PCs exhibit important regulatory functions required for development of various biological processes under normal and pathological conditions. Among PCs furin is the most studied enzyme.

1. FURIN STRUCTURE AND FUNCTIONAL ROLE OF ITS DOMAINS

Furin has been found in all tissues and cell lines cells, as evidenced by detection of the *fur* gene which encodes the protein product furin (and thus gives the name to this enzyme). In humans furin is synthesized as a precursor with molecular mass of 104 kDa, comprising of 794 amino acid residues. Molecular mass of active furin including several domains is 98 kDa. Furin belongs to transmembrane proteins. Figure 1 schematically shows the structure of furin [1, 3, 5], which

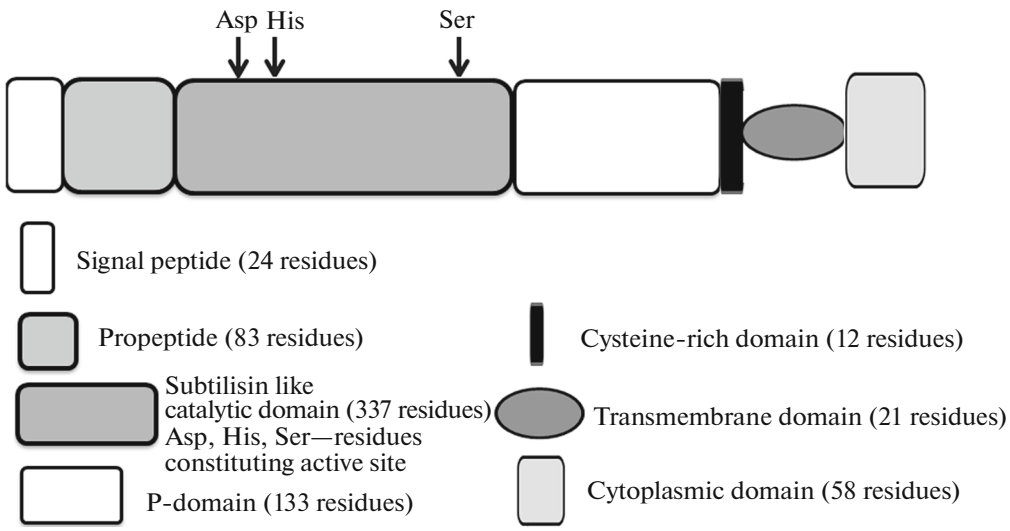

Fig. 1. Domain structure of human furin.

includes a signal peptide (24 residues), propeptide (83 residues), subtilisin-like catalytic domain (337 residues), P-domain required for catalytic activity (133 residues), cysteine-rich domain, containing 12 Cys residues, C-terminal transmembrane helix domain (21 residues) and C-terminal cytoplasmic domain consisting of 58 residues. Each site of the furin zymogen polypeptide chain performs a specific function, aimed at the "maturation" of the enzyme molecule and the manifestation of its catalytic activity. The signal peptide determines the localization of furin in the endoplasmic reticulum. After its removal, furin glycosylation and formation of two disulfide bonds in this enzyme occur; this provides formation of certain structure of the protein molecule required for the first autocatalytic cleavage at Arg^{107}. The propeptide is important for activation and also for regulation of catalytic activity and transport of furin. The propeptide acts as a chaperone; it is crucial for folding of the enzyme molecule, which is a multistep process and depends how, at which particular peptide bond, and in which cell compartment propeptide cleavages/cuts do occur. This process includes two cuts (rapid and slow ones), which occur in different cell compartments [6, 7]. The first cut including autocatalytic hydrolysis of the propeptide after Arg^{107} (in the sequence Arg^{104}–Thr^{105}–Lys^{106}–Arg^{107}) occurs in endoplasmic reticulum in the neutral pH environment (Fig. 1). After this the propeptide remains associated with the enzyme molecule and forms a complex propeptide-furin where it acts as an inhibitor. This rapid stage with $t_{1/2}$ of 10 min is necessary for furin folding and translocation. After furin translocation to the Golgi apparatus the second cut of the propeptide is made at the peptide bond Arg^{75}–Ser^{76} (in the sequence Arg^{71}–Gly–Val–Thr–Arg^{75}–Ser^{76}). This slow stsge with $t_{1/2}$ of 2 h occurs in the mildy acidic pH. The catalytic domain of furin contains amino acid residues characteristic for serine proteases: Ser^{368}, His^{194}, Asp^{153} (Fig. 1). The Ser residue acts as a nucleophile, His is responsible for deprotonation of the Ser hydroxyl group, while the Asp residue stabilizes the His residue. The P domain is important for stabilization of the catalytic domain, and also for manifestation of furin activity depending on pH and the presence of calcium ions. In subtilisin (to which family furin belongs) this domain is absent. The cytoplasmic domain is responsible for the enzyme localization not only in the Golgi apparatus, but in other compartments of the cell, where it can activate different preproteins. Intracellular furin trafficking is controlled by phosphorylation/dephosphorylation of Ser residues located in this domain. The two-step removal of propeptides is a commonfeature of PC activation. The first cut determines intramolecular folding and translocation of PC, while the second cut occurs when the enzyme is destined to its cell compartment. The presence of furin in various compartments of the endosomal system can be explained by the furin role in activation of many different substrates [1, 3, 5].

Furin belongs to intracellular proteases, which are normally absent in biological body fluids. However, after removal of the C-terminal transmembrane and cytoplasmic domains in model systems the truncated recombinant form becomes soluble. This form exhibit full proteolytic activity and is widely used to study enzymatic, kinetic, and other properties of this enzyme [8]. In 2003, a soluble form of mouse furin was crystallized in a complex with an irreversible inhibitor. The X-ray analysis of this complex has shown that such furin consists of two domains: the catalytic domain and the P domain, which are connected by an interdomain linker and can effectively interact with each other [5]. In 2014, the X-ray analysis of the solu-

ble form of human furin (the sequence Asp^{23}–Ala^{574}) in a complex with a competitive inhibitor resulted in estimation of coordinates of its active site and the sites binding three Ca^{2+} ions and Na^{+} ion, required for the enzyme activity. These studies are an important prerequisite to create effective, highly specific furin inhibitors of a new generation [9].

2. SUBSTRATE SPECIFICITY OF FURIN

Most of furin is accumulated in the Golgi apparatus, where it performs its principal function, activation of precursor proteins. Furin is an enzyme with well defined narrow substrate specificity [10–12]. Its commonly known name PACE means that this enzyme cleaves the protein substrates at a paired basic amino acid site. It cleaves protein substrates after the amino acid sequence — Arg-X-Lys/Arg-Arg (RXK/RR) [11]. Importantly, P_1 and P_4 [13] positions should be occupied by Arg (table). Furin can also cleave the peptide bond in the sequence Arg-X-X-Arg- [11], although less effectively. It should be noted that furin is active in a wide range of pH (5.0–8.0) in dependence of the substrate structure. Furin is a calcium-dependent enzyme. Its maximum activity requires the presence of 1 mM Ca^{2+} and the deacylation step requires the presence of potassium ions (20 mM) [14].

3. FURIN INHIBITORS

Considering important biological functions of furin and its role in the development of many pathological conditions, it should be noted that much attention is paid to research and development of inhibitors of this enzyme. Major results on furin and other PC inhibitors have been summarized in reviews [15–17]. In the context of chemical structure the furin inhibitors can be divided into protein, peptide, peptide-like or pseudopeptide and synthetic (nonpeptide) inhibitors [18–21]. Three protein inhibitors of furin are currently known. These include (1) endogenous inhibitor serine proteases (PI8)—serpin of the ovalbumin type (K_i = 53.8 pM); (2) inter-alpha-inhibitor protein (IαIp) of blood plasma (exhibits activity against anthrax); (3) serpin Spn4A from the fruit fly *Drosophila melanogaster* (K_i = 13 pM). For other PCs three protein inhibitors with rather narrow specificity of action are known: neuroendocrine-granule associated pro7B2 protein inhibiting PC2 (K_i = 6.7 nM); specific PC2 inhibitor CRES (cystatin-related epididymal spermatogenic) (K_i = 25 nM); selective PC1 inhibitor known as the neuroendocrine protein proSAAS (IC_{50} = 590 nM). Based mainly on natural inhibitors of serine proteases (serpins) several recombinant protein inhibitors of some PC and furin have been created by using the site directed mutagenesis method. Several mutant proteins have been created on the basis of α1-antitrypsin; one of them, so-called Portland-α1-PDX (K_i = 1.4 nM) exhibits antibacterial and antiviral effect, especially pronounced against HIV [21]. Some furin inhibitors demonstrating different inhibitory portency against the target protein (K_i values ranged from 1.6 nM to 330 pM) have been created on the basis of eglin. Some peptide inhibitors of PCs have been developed on the basis of proteins and peptides. These include the C-terminal fragment of the PC protein inhibitor, pro7B2 (K_i values ranged from μM to nM); the C-terminal fragment of the PC protein inhibitor, proSAAS (K_i values ranged from μM to nM); polypeptides derived from PC propeptides (K_i values within the nanomolar range), cyclic peptides and psuedopeptides PC (K_i values ranged from μM to nM); peptide chloromethyl ketones (K_i = 3.4 nM); polyarginine peptides containing (4 to 10 Arg residues, K_i = 40–74 μM) which exhibit antibacterial, antiviral and anticarcinogenic activity [22–24], and others.

Various compounds other than proteins and peptides also decrease furin activity. For example, EGTA chelating calcium ions decreases activity of this enzyme, while Zn^{2+} and Hg^{2+} cations inhibit furin activity completely. Furin activity is also sensitive to inhibition by zinc- and copper-containing complexes of pyridine derivatives (IC_{50} = 5–10 μM), and 2,5-dideoxystreptamine derivatives (K_i = 6–812 nM) dicumarol derivatives (K_i = 1–185 μM) flavonoids (K_i = 5–230 μM), and others [16].

Numerous studies of PC inhibitors performed in vitro and in vivo demonstrate existence of certain potential for their use as therapeutic agents, as shown by 17 patents obtained from 1994 to 2013 [17]. However, PC inhibitors have not been introduced into clinical practice yet. The most advanced in the context of clinical applications are the studies on the use of furin and other PC inhibitors as antibacterial and antiviral agents and also as agents for treatment of cancer [15, 22, 23]. Major difficulties in the implementation of the PC inhibitors into clinical practice are associated with their penetration into cells because many studies have been carried out using the soluble form of furin; the other problem consists in limited data on toxicity profiles of the tested inhibitors, possible consequences of their actions on health, effects on immune tolerance and recognized lethality in mice embryos [16, 23, 25]. The search for new inhibitors and studies of their actions at different levels are in progress [17, 26].

4. BIOLOGICAL FUNCTIONS OF FURIN

Biological functions of furin are closely related to activation of cell protein precursors, which are converted by furin and furin-like PCs into functionally active forms. Such proteins include enzymes (matrix metalloproteinases (MMP)—MT1,2,3,5-MMP, stromelysin-3, ADAM), hormones (parathyroid hormone endothelin, natriuretic peptides), growth/differentiation and factors (TGFb, IGF-1, VEGF-C, PDGF-

Amino acid sequences at furin cleavage site during activation of biologically active preproteins

Substrate	Cleavage site										
	P_7	P_6	P_5	P_4	P_3	P_2	P_1	↓	$P_{1/}$	$P_{2/}$	Reference
Growth factors and hormones											
Human transforming growth factor beta (TGF-β1)	Gln	Ser	Ser	Arg	His	Arg	Arg	–	Ala	Leu	135 136
Human insulin like growrh factor 1 (IGF1)	Lys	Pro	Ala	Lys	Ser	Ala	Arg	–	Ser	Val	65
Human vascular endothelial growth factor (VEGF-C)	Val	His	Ser	Ile	Ile	Arg	Arg	–	Ser	Leu	76
Human platelet-derived growth factor (PDGF-A)	Leu	Pro	Ile	Arg	Arg	Lys	Arg	–	Ser	Ile	76
Human parathyroid hormone	Gly	Lys	Ser	Val	Lys	Lys	Arg	–	Ser	Val	137
Human atrial natriuretic peptide (ANP)	Ala	Leu	Leu	Thr	Ala	Pro	Arg	–	Ser	Leu	108 115
Human brain natriuretic peptide (BNP)	Tyr	Thr	Leu	Arg	Ala	Pro	Arg	–	Ser	Pro	108 115
Human C-type natriuretic peptide (CNP)	Tyr	Lys	Gly	Ala	Asn	Lys	Lys	–	Gly	Leu	108
Cell surface receptors											
Human insulin receptor	Arg	Pro	Ser	Arg	Lys	Arg	Arg	–	Ser	Leu	138
Hepatocyte growth factor (HGF) receptor	Thr	Glu	Lys	Arg	Lys	Lys	Arg	–	Ser	Thr	139
Human integrin alpha 3	Ser	Pro	Gln	Arg	Arg	Arg	Arg	–	Gln	Leu	140
Human integrin alpha 6	His	Asn	Ser	Arg	Lys	Lys	Arg	–	Glu	Ile	140
Human IGF1 receptor	Arg	Pro	Glu	Arg	Lys	Arg	Arg	–	Asp	Val	66 67
Matrix metalloproteinases											
Human stromelysin 3	Ala	Arg	Asn	Arg	Gln	Lys	Arg	–	Phe	Val	141
Human MT1-MMP	Ala	Asn	Val	Arg	Arg	Lys	Arg	–	Tyr	Ala	37–39
Human MT2-MMP	Leu	Arg	Arg	Arg	Arg	Lys	Arg	–	Tyr	Ala	37
Human MT3-MMP	Phe	His	Ile	Arg	Arg	Lys	Arg	–	Tyr	Ala	37
Human MT5-MMP	Arg	Arg	Arg	Arg	Asn	Lys	Arg	–	Tyr	Ala	37
Viral envelope glycoproteins											
Human immunodeficiency virus (HIV-1gp160)	Val	Val	Gln	Arg	Glu	Lys	Arg	–	Ala	Val	142 143
Avian influenza virus hemagglutinin	Ser	Lys	Lys	Arg	Glu	Lys	Arg	–	Gly	Leu	144
Measles virus Fo	Ser	Ser	Arg	Arg	His	Lys	Arg	–	Phe	Ala	145
Karelian fever virus	Ser	Ser	Gly	Arg	Ser	Lys	Arg	–	Ser	Val	146
Bacterial exotoxins											
Anthrax toxin protective antigen	Ser	Asn	Ser	Arg	Lys	Lys	Arg	–	Ser	Thr	147
Diphtheria toxin	Ala	Gly	Asn	Arg	Val	Arg	Arg	–	Ser	Val	148
Pseudomonas exotoxin A	Thr	Arg	His	Arg	Gln	Pro	Arg	–	Gly	Trp	146

A,B), receptors (of insulin, IGF1, HGF), adhesion proteins (integrin chains—α3,4,5,6 and αV), plasma proteins (albumin, coagulation factors VII, IX, X) (table). Furin and furin-like PCs play an important role in maintenance of homeostasis, in embryogenesis and other biological processes, and also in the development of various pathologies. Furin is involved in processing of some of viral and microbial toxins (table). Maturation of the protein envelope of such viruses such HIV, avian influenza virus, F_0 measles, Ebola hemorrhagic fever requires cleavage of envelope-proteins of these viruses by furing or furin-like proteases (table) [10]. Furin is needed for activation of such exotoxins, as the anthrax toxin, diphtheria toxin, *Pseudomonas* exotoxin. Only after their intracellular cleavage the toxins become active (table) [10]. Furin and other PCs play an important role in the development of such diseases as cancer [25], atherosclerosis [27], neurodegenerative diseases, as well as metabolic dysfunctions, for example in hypercholesterolemia, which is the cause of cardiovascular diseases. Because of the important furin role in the development of various pathological processes, much attention is paid to the development of therapeutic agents based on furin inhibitors [17]. Below we consider involvement of furin in the development of such pathologies as cancer and cardiovascular diseases.

5. PARTICIPATION OF FURIN IN THE DEVELOPMENT OF CANCER

The role of furin in the development of cancer processes is associated with activation of precursors of membrane-bound matrix metalloproteinases (pro-MT-MMP) and some growth factors (GFs), which trigger a cascade of reactions inducing/enhancing the development of malignant tumors [25, 28, 29]. Furin is mainly in the Golgi apparatus, it has been found in all tissues and in most cell lines. Furin expression in normal tissues and cells is significantly lower than in most tumor tissues and tumor cell lines. High expression of furin and furin-like PCs results in activation of various biomolecules functionally important for transformation of cells and promotes tumor progression [25, 28, 30]. For example, high furin expression has been found in tumor tissues and in cell lines of breast cancer [31]. In ovarian cancer high expression of this enzyme was associated with decreased survival [32]. Furin expression was absent in non-metastatic head and neck carcinomas, while in the case of metastasis its expression was at a high level [33]. The expression of this enzyme correlated with the degree of development of squamous cell carcinoma of the tongue. The highest furin expression was detected in the most aggressive carcinoma cell lines, whereas in less aggressive cell lines furin expression was significantly lower [30, 33]. Thus, increased expression of furin is accompanied by increased tumor aggressiveness and promotes the formation of metastases, which reduce the life expectancy of cancer patients. The development of cancer is significantly affected by furing-dependent activation of growth factors functionally important for this process: platelet derived growth factor (PDGF), insulin-like growth factor (IGF), transforming (TGF) and tumor necrosis (TNF) factors, which promote growth, development and invasiveness of tumors [33–35]. Furin-dependent activation of such enzymes as a membrane-bound matrix metalloproteinases (MT-MMPs) leads to increased cell motility and also increased destructive potential of the tumor and the development of the invasive and metastatic potential [37–39].

5.1. Furin, as the Factor That Triggers a Cascade of Reactions Associated with Activation of Membrane-Bound Matrix Metalloproteinase (MT-MMP)—Key Enzymes of Invasion and Metastatic Process of Tumors

Furin is responsible for activation of MT-MMPs (MT1-MMP, MT2-MMP and MT3-MMP), which are located on the cell surface in active form, and their activation by furin and other PC occurs intracellularly in the Golgi apparatus (table, Fig. 2). The fragment of the polypeptide chain containing a sequence specific for furin action is located between pro- and catalytic domain of pro-MT-MMs: —Arg–Arg–Lys–Arg–Tyr–Ala– (table, Fig. 2) [37–39]. Active MT-MMPs having pronounced substrate specificity, are involved not only in the degradation of connective tissue matrix (CTM) at the site of the localization on the cell surface, they can also activate other MMPs, thus triggering a cascade of proteolytic reactions, initiating and potentiating CTM degradation [37].

By activating MT-MMP, furin triggers a complex cascade process that plays a key role in the development of invasion and metastasis. What are the consequences of the activation of MT-MMPs? MT-MMPs are located in the pericellular space. They trigger hydrolysis of fibrillar collagens, which form the connective tissue barrier that prevents the development of destructive invasive processes; these enzymes also participate in hydrolysis of basement membrane collagen (via MMP-2), thus promoting metastasis [40–42]. Currently six human MT-MMPs are known [37, 43]. The most studied is MT1-MMP (Fig. 3). This enzyme specifically hydrolyzes type I–III fibrillar collagens, and a number of matrix adhesion molecules, such as fibronectin, laminin, vitronectin, and other biologically active molecules; this results in disruption of cell adhesive properties, degradation of matrix, and the development of invasive process [37]. However, this enzyme does not hydrolyze directly type IV collagen, the main basement membrane component, but it activates proMMP-2, which in turn hydrolyzes type IV collagen and thus provides the development of the metastatic process [44–46]. MT1-MMP is also responsible for activation of proMMP-13 (collagenase 3), which has a broad substrate specificity and

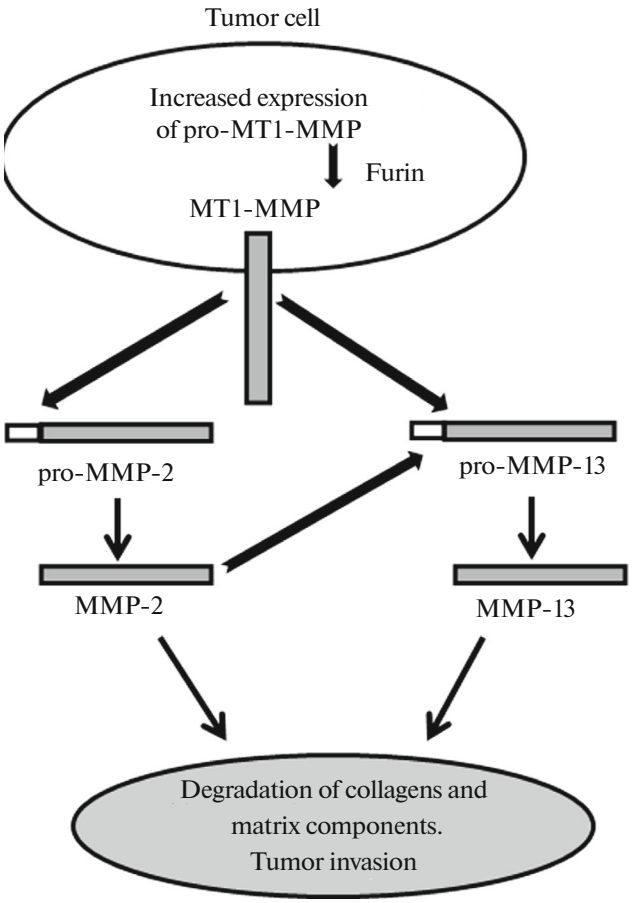

Fig. 2. Triggering by furin of the activation cascade: pro-MT1-MMP → MT1-MMP → pro-MMP-2 and pro-MMP-13 → MMP-2 and MMP-13 → tissue degradation.

hydrolyzes fibrillar collagens, type IV collagen, and a number of CTM components; this enzyme participates in the development of cancer [43, 46, 47]. The mechanism of activation of this enzyme remains unknown [24]. MT2-MMP also hydrolyzes fibrillar type I collagen, but it is 100-fold less efficient than MT1-MMP [48]. MT3-MMP hydrolyzes type III collagen, but not type I collagen [37].

Thus, the increase in active forms of MT-MMPs (particularly MT1-MMP) is accompanied by the development of a number of important biological processes, both normal and pathological conditions. MT1-MMP is responsible for degradation of the fibrillar collagens in the pericellular space, and thereby provides local tissue destruction and development of invasion and metastasis [37]. This enzyme contributes to the growth of tumors [37], it is also involved in processes of epithelial morphogenesis [49], angiogenesis [50], inflammation [51]; it is a key factor in the development of brain glioblastoma and medulloblastoma and in association with MMP-2 and MMP-9 this protease functions at the invasive front of the tumor [52–54]. MT1-MMP plays an important role in the development of ovarian cancer [55, 56], cervical cancer [57], cervical carcinoma [58–60], prostate cancer [61], pituitary adenoma [62], and nasopharyngeal cancer [63].

Thus, furin triggers a cascade of reactions, which are accompanied by activation of a number of MMPs and other biologically active molecules; this leads to the development of a number of (patho)physiological processes in normal and pathological tissues.

5.2. Involvement of Furin in Activation of Growth Factors and Regulation of Cancer Development

The regulation of the development of malignant tumors involves a number of growth factors. Table and Fig. 4 summarize some data on the participation of furin and other PCs in activation of growth factor precursors and their receptors, associated with oncogenesis.

5.2.1. Insulin-like growth factor 1 (IGF1). IGF1 belongs to the family of insulin-like growth factors, also including the IGF2, proinsulin, and relaxin [64]. IGF1 is structurally and functionally similar to insulin. In the body, it is synthesized as two precursors, proIGF1A and proIGF1B, which are activated by furin (table) [65]. Polypeptide chains of precursors comprise four identical domains (which subsequently form mature IGF1) and also the C-terminal region, differed by the polypeptide chain length and containing a unique motif KXXKXXR^{71}-XXRXXR, which undergoes proteolytic cleavage by furin. The mature form of IGF1 containing 70 residues is stabilized by three intramolecular disulfide bridges [65]. IGF1 is produced mainly in the liver and muscles; it is involved in endocrine, paracrine, and autocrine regulation of growth processes, development, and differentiation of cells and tissues. The effects of IGF1 are mediated by IGF1 receptor (IGF1R) exhibiting tyrosine kinase activity; IGF1R is synthesized as a precursor protein of 1367 residues. Activation of this receptor by furin includes cleavage at the site containing the sequence RKRR, characteristic for the specific action of furin; in the polypeptide chain this site is located in the region of 740 residues (table) [66, 67]. IGF1 binding to IGF1R stimulates cell proliferation and promotes cell survival. There is evidence that IGF1-mediated signaling plays a pathogenic role in cancer development. It has been shown that the increased expression of IGF1 and IGF1R in cancer cells is accompanied by increased cell growth [65, 70] and increased invasion, possibly via induction of MMP. Conversely, inhibition of IGF1 and IGF1R expression decreases invasiveness [71–74]. Inhibition of furin activity reduces the IGF1R level; this results in decreased IGF1-mediated proliferation [30, 68, 69]. Results of clinical studies suggest that blockade of IGF1-signaling may increase the effects of various chemotherapeutic drugs and prevent adaptive resistance to IGF1R antagonists [73].

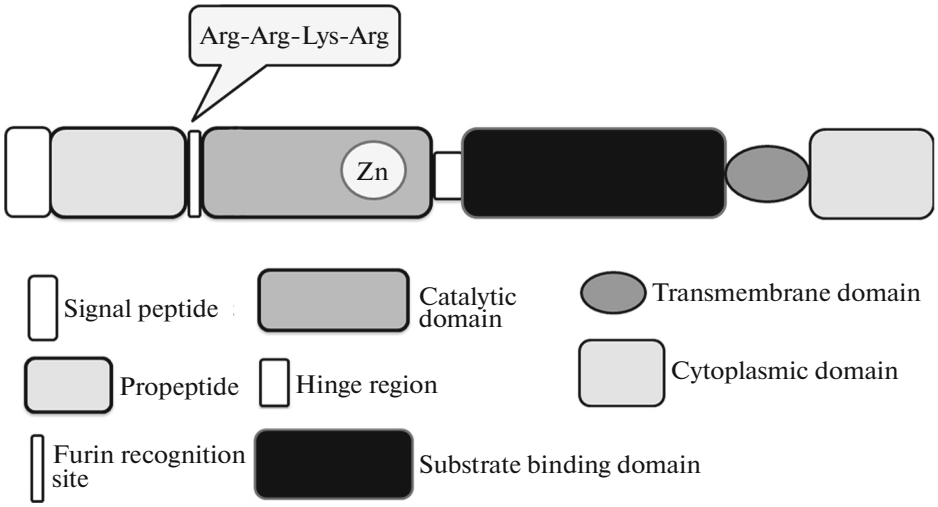

Fig. 3. Domain structure of human MT1-MMP.

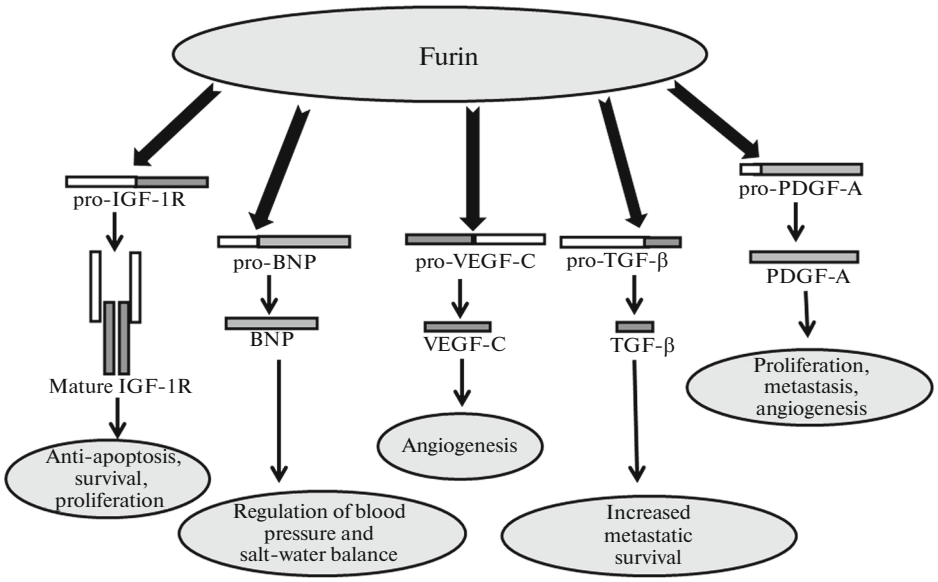

Fig. 4. Activation of growth factors precursors (pro-VEGF, pro-TGF, pro-PDGF), receptor (pro-IGF-1R), and pro-BMP by furin.

5.2.2 Vascular endothelial growth factor (VEGF-C). VEGF-C belongs to the VEGF family. Its main function is associated with angiogenesis in lymphatic vessels [75]. VEGF-C is synthesized as a precursor, pro-VEGF-C, consisting of 399 residues. Activation of proVEGF-C by furin occurs via proteolytic cleavage of the peptide bond between the amino acid residues Arg^{227} and Ser^{228} at the site specific for furin action and containing the RXXR motif (table) [76]. The active form of VEGF-C has a molecular mass of 23 kDa. VEGF-C is one of the main angiogenic growth factors that play an important role in the development of malignant tumors. In vivo experiments revealed a link between furin expression, VEGF-C processing, and angiogenesis of lymph vessels [30, 77]. VEGF-C effects (like effects of other members of the VEGF family) are realized via interaction with receptors possessing tyrosine kinase activity; these receptors are characterized by a specific extracellular part [78, 79]. VEGF-C and its receptors, VEGF-R2 and VEGF-R3, are present on endothelial cells of lymph capillaries. Expression of the "lymphogenic" VEGF-C and its receptor promotes the proliferation and survival of tumor cells and regulates many key stages of tumor development, first of all, formation of vessels, including proliferation of endothelial cells of blood and lymphatic vessels. Stimulation or inhibition of signaling systems associated with these molecules influence the

intensity of angiogenesis in the tissue [80]. This suggests that VEGF-C and its receptor may serve as markers for assessing the state of the vascular system and activity of angiogenesis in the tissue. These parameters are crucial for prognosis of the development of tumors and for search of approaches that can change the intensity of angiogenesis in the tissue due to stimulation or inhibition of signaling systems associated with these molecules [80].

5.2.3. Platelet derived growth factor (PDGF). PDGF is synthesized, processed, and then accumulated in granules of bone marrow cells, megakaryocytes, which are precursors of platelets. PDGF synthesized as a precursor, pro-PDGF, mainly exists as a heterodimer composed of two polypeptide chains A and B. There are five different isoforms of PDGF: A (PDGFA), B (PDGFB), C (PDGFC), D (PDGFD) and the heterodimer AB, which differ in their functional properties [76, 81–84]. PDGFs induce a cell response via two different receptors, α- and β-types. The PDGF receptors (PDGFR) are receptors with tyrosine kinase activity; they can bind only dimeric forms of PDGF [81–84]. The PDGF precursors are activated by furin. Human pro-PDGF with molecular mass of 22 kDa consists of 211 amino acid residues; furin cleaves the peptide bond formed by Arg^{86} and Ser^{87}. The active form of PDGF has a molecular mass of 14.3 kDa. PDGF plays an important role in angiogenesis, proliferation, embryonic development, and carcinogenesis. Expression of PDGF and PDGFR increases in different types of human oncologic diseases [81, 85–89]. Mutations in the recognition site of PC and PC propeptides inhibit PDGF processing and cell proliferation [30, 83, 90, 91].

5.2.4. Transforming growth factor beta (TGF-β). TGF-β is synthesized by many cell types. Three isoforms of this protein are currently known: TGF-β1, TGF-β2 and TGF-β3. These isoforms are synthesized as precursors (proTGF-β), share similar structure, but differ in the length of the polypeptide chain: proTGF-β1 contains 390 residues, while proTGF-β2 and proTGF-β3 contain 412 residues [92]. ProTGF-β can be activated in various ways, including reactive oxygen species, pH, integrins. Some of them are cell or tissue specific; in most tissue proTGF-β precursors are activated by furin [92]. ProTGF-β activation by furin occurs via cleavage of peptide bonds at furin specific motifs RXXR and RXK/RR in the polypeptide sequences of precursors. In proTGF-β1, containing the RHRR-motif furin cleaves the peptide bond between Arg^{278} and Ala^{279} (table) [92]. The main function of TGF-β in most cells and tissues is associated with the control of proliferation and differentiation [92–95]. In normal epithelial cells as well as in early stages of tumor progression (premalignant or well-differentiated cancer cells) TGF-β acts as an antiproliferative factor inhibiting carcinogenesis, invasive and metastatic processea [95, 96]. However, at later stages of cancer it maintains tumor growth and metastasis [92–94, 97–101]. TGF-β can also act as a neuroprotector in various lesions of the brain, including cerebral ischemia [102].

6. PARTICIPATION OF FURIN IN REGULATION OF THE CARDIOVASCULAR SYSTEM

The endocrine function of the heart is associated with the action of natriuretic peptides. Their discovery is considered as one of the most important achievements in fundamental and practical cardiology of the second half of the 20th century. The interest in their role in cardiology remains unchanged. Below we consider the mechanisms of activation of these peptides performing hormonal functions in the regulation of the cardiovascular system [103–105].

6.1. Influence of Furin on the Cardiovascular System

Furin participates in activation of precursors of natriuretic peptides (NPs), which are involved in the regulation of water-salt metabolism, blood pressure, as well as proliferation and growth of cells [106–108]. The NP family includes three main members: atrial natriuretic peptide (ANP), brain natriuretic peptide (BNP), and C-type natriuretic peptide (CNP) [106, 107, 109]. They are expressed mainly in the atria, the ventricles of the heart and in brain vascular endothelial cells, respectively. Certain evidence exists that NPs are also synthesized in some other organs and tissues [107, 109–111]. Biological effects of NPs are mediated via receptors localized on cell plasma membrane. Three types of NP receptors have been found in mammals: NPR-A, NPR-B, and NPR-C [107, 109–111]. Each peptide is encoded by its own gene. In humans ANP, BNP, and CNP are synthesized pre-propeptide consisting of 151, 134, and 126 residues, respectively. After cleavage of the signal (pre) peptide during movement in the Golgi apparatus, NPs become shorter (and their sequences contain 126, 108 and 103 residues, respectively) (Fig. 5). Activation of proNPs occurs in the Golgi apparatus and involves protease pro-peptide cleavage by furin or other furin-like PCs. Cleavage of the pro-peptide yields active forms of NPs (Fig. 5), performing various functions and having the general structural organization, which is based on the 17-membered ring, stable by a single disulfide bond. Eleven amino acids of this structure are identical in all members of the NP family, while terminal fragments are variable [112–114].

6.2. Activation of Atrial Natriuretic Peptide (ANP)

The ANP precursor, proANP, is synthesized primarily in right atrial cardiomyocytes and represents a peptide consisting of 126 residues. Proteolytic cleavage of pro-ANP by furin and furin-like PCs results in for-

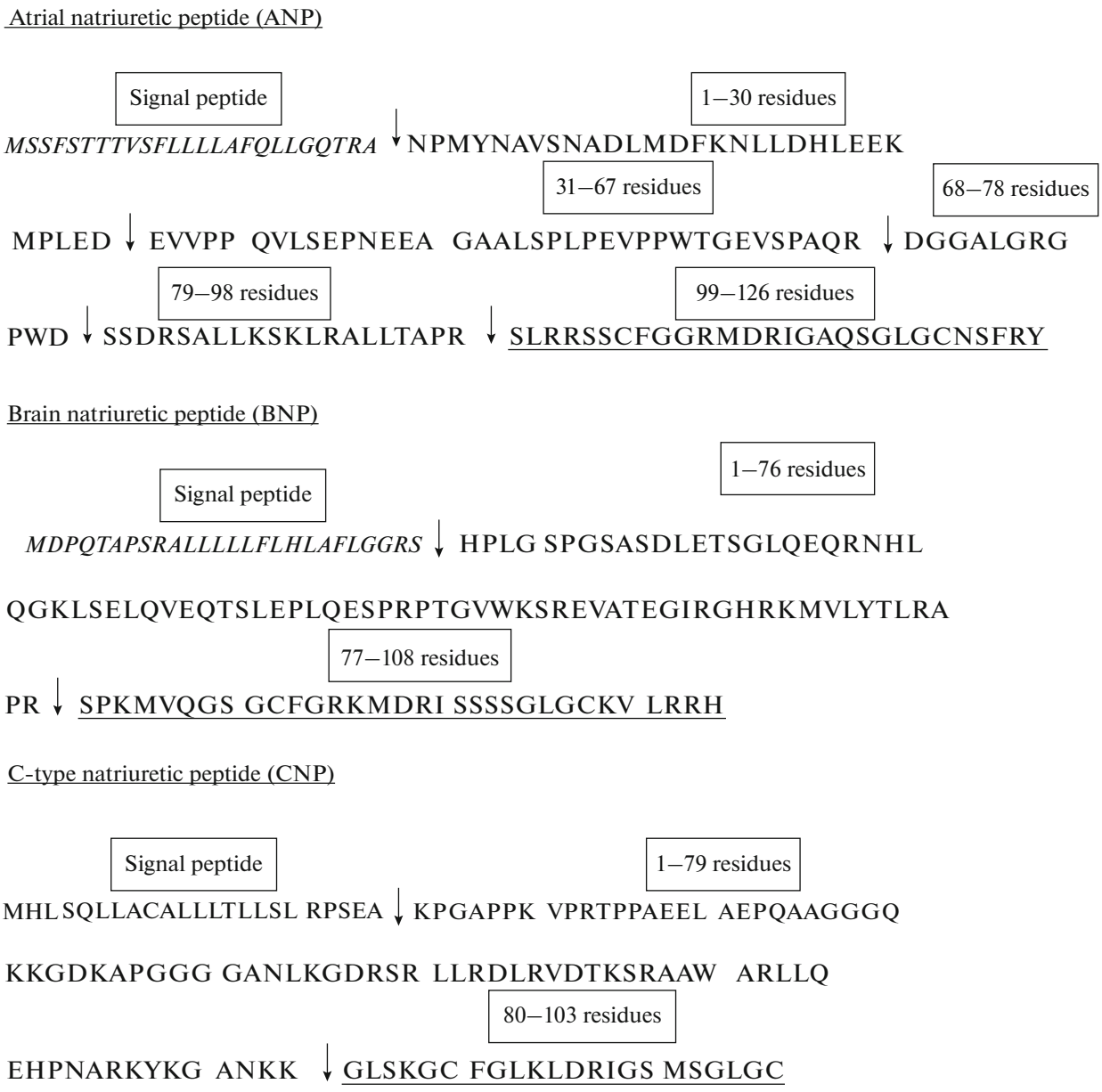

Fig. 5. The action of furin on of natriuretic peptide precursors.

mation of active C-terminal ANP (including residues 99–126) and inactive N-terminal ANP (N-ANP or N-proANP, including residues 1–98) (Fig. 5) [108, 115]. Both peptides are formed simultaneously in equimolar amounts in response to hypervolaemia or increased heart rate. ANP has its receptor on (NPR-A) cell surfaces [108, 115, 116]. ANP is rapidly eliminated from the blood circulation (its half-life time is 3–4 minutes), while the half-life of proANP is much longer 60–120 minutes, so its concentration in blood is much higher (50 times) than that of ANP; this means that measurement of proANP may more accurately reflect the level of ANP secretion [117, 118]. ANP is found in various areas of the brain, kidneys, lungs, but, basically, it is contained in the auricles [110]. ANP increases excretion of sodium ions and water; it inhibits renin and aldosterone secretion, reduces vascular tone, i.e. exhibits vasodilatory action, which is opposite to the vasoconstrictor action of angiotensin II. ProANP (residues 1–98) is cleaved by endoproteinases into three peptide circulating in the blood and performing some functions. Numerous data suggest that all four peptides (including ANP), formed from proANP, participate in carcinogenesis as agents reducing or suppressing the development of cancer process [119–121]. Certain evidence exists that the N-terminal fragment, preproANP, can be used as a biomarker of myocardial infarction [122].

6.3. Activation of Brain Natriuretic Peptide (BNP)

The BNP precursor, proBNP, is synthesized primarily in left ventrical cardiomyocytes and represents a peptide consisting of 108 residues. Its proteolytic activation by furin (Fig. 5) [116] results in formation of equimolar amounts of two fragments: inactive proBNP (residues 1–76) and active BNP (residues 77–108) comprising of 32 amino acid residues (Fig. 5). Although both peptides circulate in plasma, proBNP has a longer half-life than BNP, and therefore its detection is considered to be more informative as compared with BNP determination. Both peptides have been recognized as biomarkers of heart failure. They are used for the diagnosis of myocardial dysfunction; they directly reflect the load on the myocardium; they are markers of acute coronary insufficiency and left ventricular dysfunction, and also serve to assess the risk of cardiovascular complications [110, 123, 124]. In the case of left ventricular dysfunction and congestive heart failure, increased plasma content BNP is detected earlier as compared with signs of these pathologies detected by instrumental studies, including an echocardiogram [110, 123, 124]. This makes BNP determination in blood virtually indispensable in the early diagnostics of these pathologies [106, 107]. This marker is becoming the standard in the early diagnostics of heart failure worldwide. In 2001, it was recommended for this purpose by the European Society of Cardiology for the diagnostics and treatment of heart failure [125]. At the Congress of the European Society of Anesthesiologists (Munich, 2007) BNP was included in the list of parameters of preoperative laboratory monitoring, suitable in the practice of anesthesiologists and intensive care [126]. The key role of furin in the formation of the biologically active natriuretic peptides is also emphasized by the simultaneous increase in expression of genes encoding furin and BNP, which is observed, for example, in myocardial infarction [127].

6.4. Activation of C-Type Natriuretic Peptide

The CNP precursor, proCNP, is synthesized in the brain and vascular endothelium, as well as in the epithelial cells of renal tubules, bone (but not in cardiomyocytes) and represents a peptide consisting of 103 residues. The peptide is not accumulated in cell granules and therefore its functioning requires CNP synthesis de novo. Activation proCNP involves furin and/or furin-like PCs (Fig. 5) [108]: C-terminal proteolytic cleavage of proCNP yields active CNP (including residues 80–103), which consists of 22 amino acid residues. Although the second active form of CNP containing 53 residues also exists, the process of its formation has not been studied yet. CNP is rapidly eliminated from the body, its concentration in plasma is extremely low (0.65 pmol/L) [128]. CNP acts as a local regulatory factor in the tissues, where its secretion has been recognized. Although CNP acts complementary BNP and ANP, it plays even more important role in the regulation of vascular tone than these NPs.

The main effects of NPs are aimed at the cardiovascular and excretory systems, their actions are also related to hormonal functions of the body and the central nervous system. ANP and BNP are regulators of water-salt metabolism in the body and are important regulators of blood pressure. These processes also involve CNP. NPs are antagonists of the aldosterone/renin-angiotensin system, they inhibit the secretion of renin, aldosterone, angiotensin II, which contributes to high blood pressure and retention of sodium ions in the bloodstream [130]. NPs stimulate excretion of sodium, potassium and water by the kidneys and thereby contribute to reduction in the blood pressure [116]. Their hypotensive action is also associated with the ability to dilate blood vessels, reduce cardiac output, and antagonize the action of hormones that increase blood pressure, such as aldosterone [129]. NPs circulating in the blood can not penetrate the blood-brain barrier, but they can (to a greater or lesser extent) be synthesized in the brain (particularly CNP) and influence behavioral responses [131]. There is evidence that NPs participate in inhibition of growth and proliferation of cardiomyocytes thus reducing compensatory myocardial hypertrophy [130]. Products of proNP activation, particularly proBNP, are used in the diagnostics [132]. The level of blood proBNP increases in patients with acute coronary syndrome; it is an important parameter indicating the development of heart failure and the degree of an increase in its blood concentrations in these patients, determines the prognosis of the disease in the long-term period [132, 133].

CONCLUSIONS

The enzyme furin belongs to intracellular serine Ca^{2+}-dependent endopeptidases of the subtilisin family, also known as proprotein convertases (PC). It is characterized by narrow substrate specificity: it hydrolyzes peptide bonds at the site of paired basic amino acids and is active in a wide range of pH (5.0-8.0). Cleaving specific sites in polypeptide chains of functionally important molecules, furin triggers the cascade processes accompanied by activation of inactive precursors and appearance of biologically active molecules; their action via limited proteolysis and receptor mediated signaling is directed to realization of certain biological functions.

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