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REVIEWS

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Interaction of actin with plasminogen/plasmin system: mechanisms and physiological role

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In the present review, we have summarized and analyzed the literature data concerning cooperation between multifunctional proteins, the components of plasminogen/plasmin system and actin. The mechanisms underlying intermolecular interactions and the role of plasminogen kringle domains in protein-protein recognition are reviewed. A particular attention is paid to extracellular actin that serves as a surface protein of plasma membrane in various cells. A putative role of surface actin as the universal «non-hemostatic» center of plasminogen activation is discussed. The exposition of cytoskeletal actin on the outer surface of cellular membrane is thought to be a phenomenon, which is involved in both normal cell functioning and development of pathologies. In particular, the mechanism of plasminogen fragmentation on the surface of cancer cells mediated by actin, which results in generation of endogenous suppressors of tumor growth and metastazing (angiostatins), is described. It has been acknowledged that the plasminogen/plasmin system in concert with surface actin regulates releasing biologically active substances, e. g. catecholamines. The comprehensive assessment of plasminogen/plasmin system and surface actin exposition is proposed to be a criterion of functional status of cells and can be used as a diagnostic parameter at various pathologies.

Keywords: multifunctional proteins, plasminogen/plasmin sys- tem, actin.

The term “multifunctionality” is used to denote the ability of a protein to perform several alternative functions. The availability of a great number of multifunctional proteins, remarkable for multicompartmentation and “ubiquity”, brings up a reasonable issue of the interaction of such molecules and the physiological role of this cooperation. Countless studies show interacting ability for the whole spectrum of multifunctional proteins (cytoskeleton components, glycolytic enzymes, kinases, transcription factors, growth factors, chaperons, transmembrane proteins, proteins of extracellular matrix), previously considered to be functionally separate [1]. Among the proteins, “novel” functions of which are actively investigated, special attention is focused on actin and components of plasminogen/plasmin system (Pg/Pm). Until recently, the role of actin was limited to its participation in the functioning of cytoskeleton and contractile apparatus of cell [2]. However, there are rather unexpected reports about actin exposure by some cells on the external surface of the plasmatic membrane, though the functional significance of this phenomenon remains to be elucidated [3–5]. It is thought that actin of the cellular surface is capable of performing some receptor functions, interacting with the components of plasma or extracellular matrix [6]. Some of the proteins, which are recognized and bound by actin, are the components of Pg/Pm system [7]. Pg is a zymogene of serine proteinase Pm, the principal function of which is to cleave fibrin clots [8]. In addition to blood plasma, the components of Pg/Pm system are present in intracellular medium of tissues and involved in a number of normal and pathophysiological processes, regulating activity of various cells [9–10].

Special attention is paid to products of limited proteolysis of Pg, known as angiostatins (AS), which

demonstrate powerful angiostatic and anti-inflammatory effects, thus inhibiting pathological neovascularization and metastasis. Mainly the formation of AS in organism occurs on the surface of the plasma membrane of cells, including tumor cells [11, 12], and surface actin functions as a matrix for the Pg activation and further AS generation. Exposure of actin by malignant cells is considered to be the defense mechanism, aimed to inhibit tumor growth and metastasis [7]. The investigation of the molecular mechanisms of AS formation and the regulation of this process may be useful for elaboration of more efficient approaches to the therapy of malignant neoplasms and other disorders, associated with vascular pathologies.

There are reasons to assume a role of extracellular actin in normal physiological processes. The list of cells, exposing actin on the external surface of the plasma membrane, is rather long: B-lymphocytes [3], endotheliocytes [13], platelets [14], monocytes [4], fibroblasts [15], pericytes [16], smooth muscle cells [17], sperm cells [5]. There are considerable arguments that the exposure of outer surface actin depends on the physiological state of the cell. For instance, transformed or mitogen-stimulated lymphocytes expose much more surface actin than intact lymphocytes and the extracellular actin is only on B-, not T-lymphocytes [3]. It is assumed that platelets are capable of exposing actin during agonist-induced activation [14]. In contrast to abovementioned examples, resting monocytes U-937 expose surface actin, whereas their activation leads to the disappearance of this protein from the membrane surface.

Taking into consideration wide distribution of actin, one may predict that on the surface of cells this protein may serve as a universal modulator of the Pg/Pm system activity.

Using the model of catecholamine-producing cells, it was previously shown that extracellular actin mediates the formation of Pm and ensures the participation of proteinase in the regulation of hormone release [18].

The analysis of literature data indicates significance of the interaction between actin and proteins of the Pg/Pm system for the regulatory and compensatory mechanisms of organism. To the best of our knowledge, the present report is the first attempt to systemize the data about the molecular mechanisms of actin–Pg/Pm interaction, to generalize the physiological significance of this cooperation, and to define the perspectives of further studies and practical application of their results for diagnostics and therapy of a great number of pathologies.

Actin and components of the Pg/Pm system are multifunctional proteins. The name “actin” is used to denote the whole family of proteins, encoded by individual genes. One of the main peculiarities of actin is a high degree of structural conservatism. The differences between aminoacid sequences of various actins of the same species and diverse species are scarce: they are limited to about 25 aminoacid replacements. Actin is a “ubiquitous” protein, since it is synthesized by all eukaryotic cells, besides, it is the most common protein for their majority (10–15 % of the total amount of protein) [19].

It is well acknowledged that the principal functions of actin (contractile and cytoskeletal) are performed due to its existence in two forms, differing by physical and chemical properties and present in a cell in dynamic equilibrium. Globular actin (G-form) is a one-chain two-domain polypeptide with the molecular mass of about 42 kDa, the isoelectric point of about 5.4 and the length of 375 aminoacid residues. The G-actin polymerization leads to the formation of labile cytoskeleton structures – actin filaments, and polymerized actin in their composition is called F-actin.

At present, six actin isoforms have been identified in the cells of vertebrates, differing by the value of isoelectric point and the stability of filament structures. The synthesis of isoactins is tissue-specific and regulated at the level of expression of corresponding genes. For instance, the myofibrils of skeletal muscles, smooth muscles and heart contain different α-isoforms of actin, while the microfilaments of cytoskeleton of non-muscle cells contain β- and γ-actins. There are reasons to assume that the specificities of segregation and compartmentation of actin isoforms in the cells of different histotypes indicate their functional non-equivalence [20].

Recent studies proved that due to unique physical and chemical properties, specificities of synthesis and compartmentalization, actin is able to perform much wider spectrum of functions than it used to be considered before. Actin is involved in regulation of activity

of ion channels [21], transmission of intracellular signal to the genome [22], transport of mRNA and transcription [23], transfer of mediators [24], and regulation of enzyme activity [25]. Impairments of the processes of assembly–disassembly of the cytoskeleton actin structures lead to cell dysfunction and even their malignant transformation [26]. Some pathogenic bacteria use actin microfilaments to invade the host cell [27]. The variety of morphofunctional types of actin in cell is not limited to the monomer form, polymerized in filaments and associated with plasma membrane. There is a growing body of information describing exposure of different actin isoforms on the outer surface of the plasma membrane [1]. It is noteworthy that the surface actin maintains physical connection with the actin microfilaments of cell cytoplasm, and the translocation of actin onto the membrane surface depends on the functional state of a cell [28, 29].

Actin in both monomer form and polymerized state demonstrates high reaction ability to interact with a number of cell molecules: other actin subunits, actin-associated proteins, enzymes, nucleotides, ions, etc. [29]. It was established that actin binds to some proteins of blood plasma with high affinity; thereby, special attention is paid to the components of the Pg/Pm system [7, 30]. Pg is an inactive zymogene of serine proteinase Pm. Pg is a one-chain glycoprotein of 92 kDa, consisting of 792 aminoacid residues and containing about 2 % carbohydrates. Pg circulates in plasma in the form of native proenzyme with NH_2-terminal residue of glutamic acid (Glu-Pg), the concentration of which is about 0.15 g/l [30, 31]. Pm-mediated limited proteolysis of the latter yields partially degraded Pg molecule, or Lys-Pg, whose conformation is different from that of native Pg. It was determined that Lys-Pg is more readily transformed into Pm compared to Glu-form [32]. Pg becomes an active enzyme via the cleavage of peptide bond Arg560-Val561. The process of Pm transforming into Pg and the enzyme activity in the organism are under the strict control of a variety of activators and inhibitors. The conversion Pg > Pm in the organism is mainly performed by the tissue activator Pg (t-PA) and the activator of urokinase type (u-PA). There are also exogenous activators, streptokinase and staphylokinase being the most powerful ones. A specific endogenous inhibitor α2-antiplasmin is a negative effector for Pm. Also the formation of Pm from proenzyme is inhibited by Pg activators (PAI-1, PAI-2, PAI-3) [8, 33].

A remarkable feature of the Pg/Pm structure is the presence of five highly-homologous three-loop structures, so called kringles. Each of five domains consists of 80 aminoacid residues on average and is stabilized by three disulfide bonds. Kringles contain lysine binding sites (LBS), which mediate the interaction of Pg/Pm with other proteins, including fibrin, thus ensuring the orientation of the active enzyme center regarding some peptide bonds of the substrate molecule [34].

Liver is the main organ, in which Pg is synthesized and released to plasma. Besides hepatocytes, mRNA of Pg was discovered in the cells of adrenal glands, kidneys, brain, testis, heart, lungs, uterus, spleen, thymus, intestines [35].

A wide range of organism structures, the cells of which synthesize Pg, is the basis of the assumption that this protein plays the role of both the circulating predecessor of active protease of plasma, but also the signaling molecule, the functioning of which might be associated with some cellular or subcellular structures. The molecular mechanisms of Pg/Pm involvement in the processes of cellular signaling and realization of regulatory functions are extensively explored [36, 37]. Due to unique properties of kringle domains of Pg molecule and its capacity of conformational motility, the interaction of this protein with different ligands becomes possible. To date, a great number of potential plasminogen receptors (α-enolase, proteins of integrin family, anexin 2, cytokeratin 8, tetranectin, gangliosides) on the surface of plasma membranes of various cells (monocytes, macrophages, platelets, endotheliocytes, fibroblasts, sympathoadrenal system cells, sperm cells, etc.) have been identified [9, 38]. A special place in this list belongs to extracellular actin, since this protein plays a key role in modulating the activity of Pg/Pm system on the cell surface. The interaction of Pg with receptor molecules may have different consequences, leading either to signal transfer inside the cells, or to the activation of zymogene, however, this issue requires further studies.

The components of Pg/Pm system are involved in the pathogenesis of such pathologies as tumor growth [39], atherosclerosis [40], autoimmune diseases [41],

diabetes [42], Alzheimer's disease [43], inflammation [44], and the role of Pg/Pm system in the pathological processes is ambiguous. On the one hand, on the cell surface Pg may be converted into Pm, which stimulates monocytes and macrophages, thus activating the inflammatory reaction, and Pm-induced degradation of extracellular matrix proteins promotes angiogenesis, invasion of cancer cell and metastasis [45, 46]. On the other hand, Pm, anchored on the membrane binding site, may undergo autoproteolysis with the formation of products of limited hydrolysis – AS [47]. In contrast to Pg, AS inhibit the proliferation and migration of endotheliocytes, thus demonstrating their angiostatic activity, induce apoptosis in malignant cells and serve as anti-inflammatory agents [48, 49]. The authors of the study [50] established that surface β-actin is an exclusive center of Pg>Pm>AS conversion among all known receptors. Surface actin seems to mediate the realization of an important link of defense mechanisms of the organism, aimed to inhibit tumor growth. Further studies are required to investigate the presence of a positive correlative connection between the exposure of actin on the surface of tumor cells and the lifespan of patients with cancer. The investigations of the mechanisms of actin–Pg/Pm interaction and further generation of AS, the regulation of these processes, their role in pathologies associated with neovascularization, inflammatory reactions, tissue malignancy, are vital for the elaboration of novel diagnostic and therapeutic approaches.

Interaction between the Pg/Pm system and extracellular actin. Actin circulates in blood plasma of humans and animals; for different pathologies, accompanied by the damage of tissues and cell destruction, its concentration increases to several μM, therefore, damaged cells are considered to be the main source of circulating actin [50]. However, according to the data of the authors [51], some cultivated cell types, in particular, embryonic myocytes, secrete actin into the nutrient medium without loss of their viability. Similarly, the cells are assumed to secrete persistently the respective actin isoforms into the blood flow at normal functioning. The extracellular actin might play a role in the regulation of the activity of plasma proteins. The organism has a protein system that controls actin concentration in plasma. It includes gelsolin of plasma and vitamin D-binding protein, or Gc-globulin, that are necessary for defragmentation of actin filaments, binding of monomer polypeptides and, finally, removing actin from the circulation [52]. Some of plasma proteins, capable of interacting with actin with high affinity, are Pg/Pm and t-PA (according to the data of different researchers, the dissociation constant K_d of the actin-Pg complex is 70–140 nM, K_d of actin–t-PA – 0.55 μM) [7, 53]. However, the physiological significance of this interaction remains to be determined. The authors of [54] showed that the binding of monomer actin to Pm leads to inhibition of its catalytic activity and/or modification of substrate specificity and a decrease in the rate of fibrin clot cleavage. On the other hand, actin stimulates t-PA-mediated generation of Pm from both Glu- and (with even higher rate) Lys-Pg. In the presence of actin, a decrease in K_m value for Glu-Pg>Pm reaction, catalyzed by t-PA (enzyme, in turn containing two kringle domains) was obsereved. The mechanism of this process is the interaction of Pg and t-PA on the surface of actin (or actin filament), which is similar to the formation of so called triple complex on the surface of a fibrin matrix. The binding of Pg and t-PA to actin occurs via LSB of kringles of both proteins. A lysine analogue, 6-aminohexanoic acid (6-AHA), inhibits actin-mediated generation of Pm from Pg with the participation of t-PA, and this fact confirms the abovementioned mechanism. In contrast to polymerized fibrin, which is hydrolyzed by Pm activated on its surface, actin is not a "suitable" substrate for Pm. *In vitro* experiments on the hydrolysis of actin by pure Pm demonstrated that the enzyme is capable of limited proteolysis of actin by cleaving single peptide bond in the position Lys373-Cys374. Compared to the native molecule, a partially degraded actin binds Glu-Pg with higher affinity. It is assumed that this effect is possible due to the change in the actin molecule conformation after its hydrolysis by Pm, in particular, the orientation of lysine residues and representation of the additional binding center – C-terminal residue Lys, which effectively interacts with kringle domains of Pg molecule [53]. Based on these data, one may state that the circulating actin lowers the potential of the fibrinolytic system. Firstly, it binds Pg with high affinity and accelerates its transformation into Pm, thus depleting zymogene pool in plasma. Secondly, the

enzyme in the stable complex with actin appears to be inactive, interrupting its participation in the fibrinolytic process. It should also be noted that there are no literature data on any case of the autoproteolysis of Pm, formed from Pg in t-PA-dependent manner and bound to extracellular actin.

Appearance of actin in plasma in the amounts, exceeding the capacity of the defense system, may have deleterious consequences for the organism. It is especially harmful for the functioning of microvessels. The cases of rapid fibrin clot formation in microvessels in the presence of large amount of extracellular actin have been documented [54]. The significance of the interaction of two protein systems in the pathophysiological processes is emphasized due to their "ubiquity". The inflammation of any localization leads to the vascular endothelium cell death, accompanied by the release of actin and Weibel-Palade bodies and subsequent increase in the local concentration of t-PA in the damaged place [55]. In turn, the circulating actin is capable of enhancing the effects of inflammation mediators, and thus promoting the the vicious circle formation. Therefore, the actin-stimulated generation and further inactivation of Pm may make a considerable contribution into the hemostasis disorders, observed during sepsis, inflammation, and tissue damage. It will be demonstrated below that actin of plasma and extracellular actin, situated on the outer surface of plasma membrane, make different impact on both the system of Pg activation and Pm activity, including the capability of the latter to undergo autoproteolysis resulting in the formation of AS.

Interaction of Pg/Pm system with actin on the surface of cancer cells: the mechanism of AS generation. The components of cascade of Pg activation, first of all, u-PA and its receptor (u-PAR), are exposed in excessive amounts (in comparison with normal cells of the same histotype) on the outer surface of the plasma membrane of some malignant cells [47]. Participation of these proteins in the processes of tumor growth and metastasis has been proved experimentally [56]. It was established that parallel hyperexpression of u-PA and u-PAR correlates with the aggressiveness degree of breast cancer [57]. The comparison of the profiles of Pg binding to normal and neoplastic cells demonstrated that this protein is more efficient in binding to the membranes of malignant cells, especially the ones with high metastatic potential [58]. Key molecular events on the surface of malignant cells are highly efficient interaction of Pg, the change in its conformation and further conversion into Pm [47]. The proteins, exposing C-terminal residues of Lys, like cytokeratin 8 and α-enolase, were considered as possible candidates for Pg binding. However, these receptors appeared to be responsible for the binding of only 10 % Pg on the cell surface, which was demonstrated, for instance, in the experiments with lymphoma U937 cells [59]. Further u-PA-dependent activation of Pg leads to the appearance of membrane-associated active Pm, catalyzing the degradation of components of extracellular matrix, which is required for the invasion of malignant cells and metastasis. The pool of endothelial and malignant cells, containing membrane-associated Pm, has 3-fold higher invasive potential compared to Pm-negative cells [60]. It was demonstrated that the interaction of Pm with integrin receptor αvβ on the surface of endotheliocytes stimulates migration of these cells [44]. The alternative mechanism of realization of proangiogenic properties by Pm is the induction of angiogenesis factors, in particular, VEGF-C and VEGF-D, by means of activation of the corresponding propeptides [61]. There are a number of malignant or transformed cells, capable of exposing actin on the external surface of the plasma membrane, which may potentially serve as an additional center of Pg binding and activation [59].

Are cancer cells able to adsorb Pg via surface actin and what properties do they acquire due to the conversion of zymogene into Pm? These questions seem to be rather reasonable taking into consideration extremely important and somewhat unexpected observations. It turned out that 50 % of the total amount of Pg, bound to the surface of a malignant cell, are localized on actin [13]. Moreover, in cultivating conditions, plasmin activity on the surface of transformed cell, which are characterized by high density of extracellular membrane-bound actin, decreased by 75 % for a few minutes after Pg had been converted into Pm [59]. This observation may be explained by rapid inactivation of Pm by α2-antiplasmin and/or autoproteolysis of Pm, and, as a result, the dissociation of its complex with actin. However, it is well known that Pm, formed on the fibrin

clot or the cell surface, is protected from the α2- anti plasmin-induced inactivation [62]. Therefore, this inhibitor cannot have considerable influence on the inhibition of plasmin activity in the fraction of membrane-bound proteins. The second assumption proved to be true: the main cause of plasmin activity loss is physical dissociation of Pm from the surface of the plasmatic membrane due to autoproteolysis.

The data of the inhibitory analysis using lysine and its analogue (6-AGK) demonstrate that Pg/Pm interact with actin through LBS of kringle domains. In turn, lysine residues in the actin molecule are involved in the interaction with Pg/Pm in positions 61, 68 and 113, which are conservative and occur in all known actin isoforms. Residue Lys61 is the most important in the intermolecular recognition of Pg/Pm–actin, since it is situated on the surface of the actin globule. Failure of Pg/Pm binding to actin was observed when lysine residues in the positions 61 and 68 of actin molecule were substituted with arginine, while the synthetic peptide-analogue of the fragment of polypeptide actin chain that comprises aminoacid residues 55–69, inhibits the reaction of Pm autoproteolysis in the presence of actin in competitive manner.

The following observation is particularly important to understand the mechanism, by which actin is involved in Pm cleavage. As it turned out, the abovementioned short peptide-analogue of the actin chain in the cell-free medium is efficient in binding to Pm, but it does not stimulate the autoproteolysis reaction. Further experiments demonstrated that Pm self-cleavage requires an intact molecule of actin, which is important, firstly, as a matrix for conformational changes of Pm molecule, vital for further reaction and, secondly, as a reducing agent. The physicochemical properties of actin as well as the specificities of its spatial structure are ideal for optimal orientation of groups in the active center of Pm and its further cleavage. The analysis of the products of Pm autoproteolysis, bound to the surface β-actin on the membrane of three types of human malignant cells, PC-3 (prostate cancer), HT1080 (fibrosarcoma) and MDA-MB231 (breast cancer), revealed that the hydrolysis of the peptide bond in the position Arg530–Lys531 of Pm molecule leads to the releasing of the fragment with the truncated fifth cringle, i.e. K1-4 plus 85 % of cringle 5, often called K1-4,5 (AS 4,5). It implies that the intact cringle 5 is responsible for the interaction of Pg/Pm with actin, since the formed AS fails to interact with actin [7, 47]. The role of the fifth cringle in the intercellular recognition needs further experiments, because the reaction centers of cringle 5 have higher affinity to lysine residues, located inside the polypeptide chains compared to cringles 1, 2 and 4 [63]. After fragment K1-4,5 is released from the cell surface, it enters the systemic blood circulation [7]. It is known that, similarly to other proteolytic fragments of Pg/Pm-AS, containing different number of cringles (K1-3, K2-3, K1-4, K1-5), K1-4,5 has potent antiangiogenic activity, due to which it inhibits neovascularization and tumor metastasis. In addition, AS may cause direct antitumor activity, thus inducing apoptosis of cancer cells [11, 48, 49]. Therefore, the product of autoproteolytic degradation of Pm is characterized by physiological activity, which is opposite to that of the initial molecule.

The autoproteolysis of Pm with the formation of AS may be considered as a four-stage process, the main stages of which are illustrated in Fig. 1. It is noteworthy that the sequence of presented events could be possible only in case of simultaneous presence and close spatial location of all the required components of Pg activation: surface actin, u-PA and respective receptor (u-PAR). Current experimental data indicate the occurrence of these molecules in the amounts, required for AS generation, only on the surface of some malignant cells [47].

It is obvious that the metastasis potential of a malignant cell depends on specificities of the proteome, sorting and segregation of proteins and the possibility for the latter to realize a wide spectrum of intercellular interactions. On the one hand, the increased levels of u-PA and u-PAR on the membranes of malignant cells are considered to be negative prognostic parameters during cancer, since Pm induces angiogenesis and the invasion of cells through cleaving proteins of extracellular matrix and activating metalloproteinase. On the other hand, Pm is the source for AS (however, only in case of combined exposure of u-PA, u-PAR and actin on the cell surface).

Current literature data give reason to consider surface actin as an additional prognostic indicator, the level of which can be in positive correlation with the

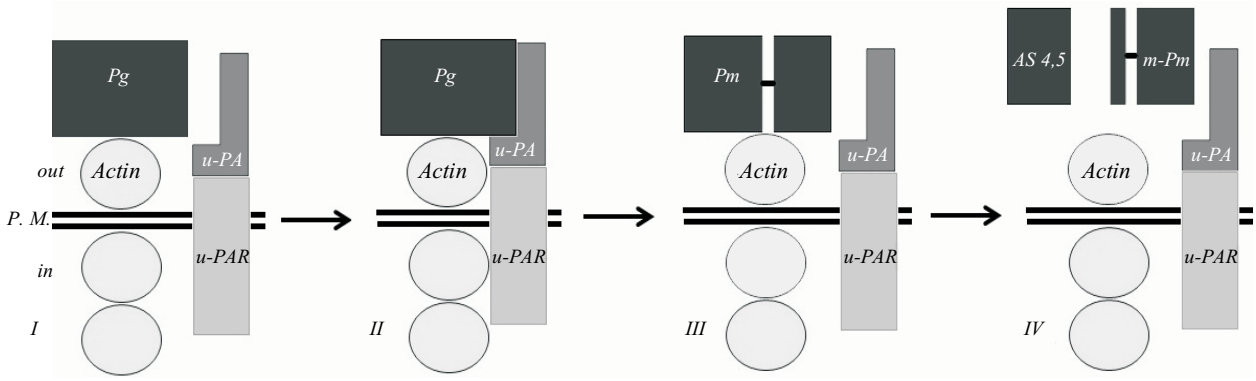

Fig. 1 The scheme of the formation of angiostatin K1-4,5 (AS 4,5) as a result of plasmin autoproteolisys (*Pm*), mediated by actin exposed on the outer surface of the plasma membrane (p.m.) of tumor cell: *I* – binding of plasminogen (*Pg*) with surface actin in the vicinity of activator (*u-PA*) and its receptor (*u-PAR*); *II*, *III* – recognition of Pg molecule by the activator and conversion of the latter into Pm; *IV* – Pm autoproteolisys followed by releasing of fragments: KI-4,5 (AS 4,5) and microplasmin (*m-Pm*) (according to the data of [7, 47])

suppression of tumor growth and prolonging the patients' lifespan.

Thus, the mentioned interaction between two multifunctional protein systems may to some extent explain the paradigm: why Pm as an inductor of angiogenesis causes local effects, while AS inhibit the angiogenesis systemically. Further study of the mechanisms of protein-protein interactions of the components of Pg/Pm system with membrane-associated ligands, including surface actin, should be carried out to understand the properties of the functioning of fibrinolytic system proteins in non-hemostatic processes.

Considering all the abovementioned, the elaboration of approaches, aimed at the actin exposure by malignant cells as an additional center of AS generation, endogenous inhibitors of tumor growth and metastasis, is believed to be a promising perspective.

Interaction of Pg/Pm system with actin on the surface of catecholamine-producing cells. The sympatoadrenal system regulates all processes in organism at different stages of its ontogenesis. The mediators of the sympathoadrenal system include catecholamines: noradrenaline (norepinephrine), adrenaline (epinephrine) and dopamine, mainly synthesized by chromaffin cells of adrenal medulla and adrenergic neurons of CNS and sympathetic ganglia. Their synthesis and secretion are the subject to strict control of the neuroendocrine system, implemented by a number of hormone-like substances of peptide nature [64]. All the inductors and inhibitors of catecholamine release are synthesized in the form of inactive prohormones, the mechanisms of processing of which are still obscured. It is known that protein chromogranin A (Cg A), present in many cells of the neuroendocrine system, is the precursor of a number of tissue-specific peptides that modulate secretory function of cells [65]. The initial studies of CgA processing revealed that it is the most active in the presence of chromaffin cells [66.] These researches supposed that the hormone-producing cells synthesize [the] substances that regulate levels of peptides, modulating the release of catecholamines via the negative feedback mechanism. However, the nature of enzymes in catecholaminergic cells, performing the limited proteolysis of CgA, remained unknown for a long time. It has been previously discovered that both bacterial endoproteinase LysC and trypsin (the enzymes that are not inherent for extracellular medium of chromaffin cells) are able to cleave CgA specifically at the hydrolysis site, which coincides with that for Pm, and Pm zymogene is present in extracellular matrix of these cells [67, 68]. This fact formed the basis of the hypothesis, which was experimentally confirmed later, that Pm is the most important protease, responsible for the inhibition of catecholamine release by means of CgA processing. The mechanism (Fig. 2) of CgA cleavage by Pm with the formation of physiologically active peptides, capable of inhibiting the secretion of catecholamines in response to acetylcholine stimulation of pheochromocytoma cells PC12 and chromaffin cells of bovine adrenal medulla [18, 67], has been recently discovered. At the first stage, Pg is bound to the cell surface in several sites, including high affine site

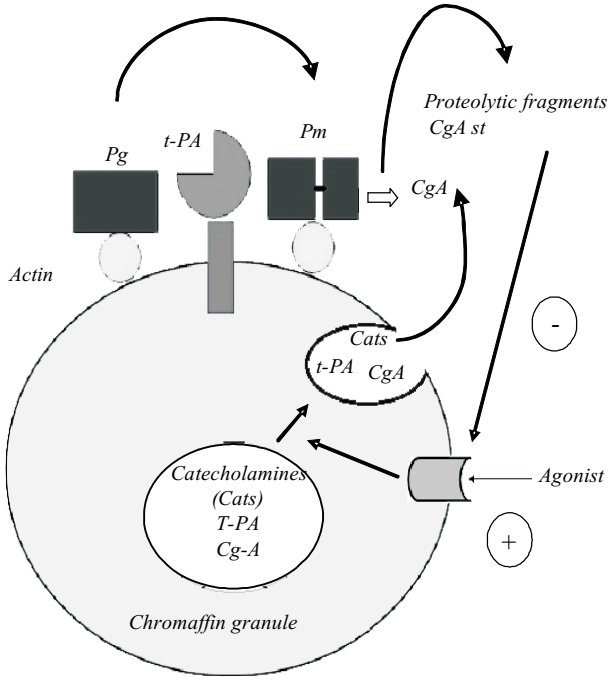

Fig. 2 The mechanism of chromogranin A processing (*CgA*) induced by plasmin (*Pm*) on the membrane of catecholamine-producing cells. Circulating plasminogen (*Pg*) is bound to surface actin and converted into plasmin with the participation of t-PA, located on the membrane receptor. CgA is accumulated in chromaffin granules together with t-PA and catecholamines and secreted by the cells in response to stimulation, and then fragmented by Pm. Proteolytic fragments of CgA inhibit secretion and releasing of catecholamines by virtue of negative feedback mechanism (according to the data of [18, 67]).

(K_d = 77 nM). The second stage of the process consists in activation of membrane-bound Pg with the participation of t-PA, which, in turn, is secreted by the chromaffin cells in response to stimulation.

The authors of these experiments established a crucial role of Pg interaction with the cellular membrane: firstly, this event is required for conformational changes in the zymogene molecule; secondly, as stated above, the membrane-bound Pm is protected from α2-antiplasmin-induced inhibition.

The nature of proteins, the potential receptors of Pg on the surface of catecholaminergic cells, was analyzed using two-dimensional electrophoresis, radioligand-blotting and tandem mass-spectrometry, established that the extracellular β- and γ-isomers of actin ($9.8 \cdot 10^4$ per cell in average) are responsible for Pg interaction with plasma membrane. Some experiments also demonstrated direct involvement of surface actin in protein processing and showed that incubation of chromaffin cell culture with anti-actin antibodies leads to parallel decrease in level of Pg activation and enhancement of norepinephrine release. The inhibitory effects of 6-AHA on Pg binding to actin in the cell culture support participation of LBS of Pg kringle domains and lysine residues in the actin molecule in intermolecular interaction. The treatment of cells with carboxypeptidase C was observed to decrease the Pg activation level by 90 %; this indicates that C-terminal lysine residues (most likely Lys373) in partially split actin are involved into interaction with Pg [18].

The functioning of complex of Pg activation system in concert with extracellular actin on the surface of catecholaminergic cells might play a key role in the modulation of secretion of biologically active compounds. It should be noted that Pg and the components of its activation system were also found in other neuroendocrine structures, including neurons of brain cortex, cerebellum, hippocampus as well as cells of peripheral nervous system [35, 69, 70]. It is possible that the described interaction of Pg/Pm system with surface actin could be a universal mechanism for the regulation of neurosecretory activity of adrenergic cells in both the central and peripheral nervous systems.

Conclusions. Plasminogen/plasmin and actin are the examples of multifunctional interoperable proteins. Taking into consideration the "ubiquity" of actin, it is assumed that this protein, located on the surface of the plasmatic membrane of cells, may serve as a universal modulator of the plasminogen/plasmin system activity. Actin induces conformational changes in Pm molecule, which modify the enzyme activity and substrate specificity. In the complex with the surface actin, plasmin is involved in processing of biologically active peptides or subjected to autoproteolysis with the formation of angiostatin, a powerful endogenous inhibitor of neovascularization and metastasis. Investigation of signaling mechanisms with the participation of the components of plasminogen/plasmin system and actin is considered to be a significant fundamental issue and a promising perspective for the elaboration of new approaches to diagnostics and therapy of various disorders, in particular, thrombotic states, cancer and pathologies of the nervous system.

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Взаємодія актину з плазміноген/плазміновою системою: механізми та фізіологічна роль

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Резюме

В огляді вперше проаналізовано та узагальнено літературні дані стосовно кооперації мультифункціональних білків: компонентів системи плазміноген/плазміну та актину. Розглянуто механізми, що лежать в основі міжмолекулярних взаємодій, та роль кринглових доменів плазміногену у міжбілковому розпізнаванні. Особливу увагу приділено екстрацелюлярному актину, який виконує функції білка поверхні плазматичної мембрани різних клітин. Обговорюється можлива роль поверхневого актину як універсального «негемостатичного» центра активації плазміногену, а також функціональне значення експонування цитоскелетного білка різними клітинами за нормальних фізіологічних умов та при розвитку па- тологій. Зокрема, описано механізм фрагментації плазміногену на поверхні ракових клітин за участі актину з утворенням ендогенних супресорів пухлинного росту та метастазування – ангіостатинів і залучення системи плазміноген/плазмін та поверхневого актину до регуляції релізингу біологічно активних речовин на прикладі катехоламінпродукуючих клітин. Комплексна оцінка стану плазміноген/плазмінової системи та актину пропонується як критерій функціонального статусу клітин, що може знайти застосування у діагностиці різних патологій.

Ключові слова: мультифункціональні білки, система плазміноген/плазмін, актин.

А. А. Тихомиров

Взаимодействие актина с плазминоген/плазминовой системой: механизмы и физиологическая роль

Резюме

В обзоре впервые проанализированы и обобщены литературные данные относительно кооперации мультифункциональных белков: компонентов плазминоген/плазминовой системы и актина. Рассмотрены механизмы, лежащие в основе межмолекулярных взаимодействий, и роль кринговых доменов плазминогена в межбелковом распознавании. Особое внимание уделено экстрацеллюлярному актину, выполняющему функции белка поверхности плазматической мембраны различных клеток. Обсуждается возможная роль поверхностного актина как универсального «негемостатического» центра активации плазминогена, а также функциональное значение экспонирования цитоскелетного белка разными клетками при нормальных физиологических условиях и развитии патологий. В частности, описан механизм фрагментации плазминогена на поверхности раковых клеток при участии актина с образованием эндогенных супрессоров опухолевого роста и метастазирования – ангиостатинов и вовлечение системы плазминоген/плазмин и поверхностного актина в регуляцию релизинга биологически активных веществ на примере катехоламинпродуцирующих клеток. Комплексная оценка состояния плазминоген/плазминовой системы и актина предлагается в качестве критерия функционального состояния клеток и может найти применение в диагностике различных патологий.

Ключевые слова: мультифункциональные белки, система плазминоген/плазмин, актин.

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