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BIOMEDICAL APPLICATION OF K5 PLASMINOGEN FRAGMENT

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Aim. Plasminogen kringle 5 is an endogenous angiogenic inhibitor. The purpose of the present review was to highlight the potential biomedical application of kringle 5 in the regulation of angiogenesis and tumor growth.

Methods. Angiogenesis is a complex process that involves endothelial cell proliferation, migration, basement membrane degradation, and neovessel organization. Since the uncontrolled growth of new blood vessels causes the progression of many common diseases, first of all, oncological diseases, autoimmune disorders, neovascular damage of the eye, the use of angiostatins can be a promising pharmacotherapeutic approach to the prevention and adjuvant therapy of these pathological conditions. The advantages of angiostatins application are their non-toxicity even at high doses, non-immunogenicity, lack of tolerance of target cells to their action. Angiostatins comprise a group of kringle-containing proteolytically-derived fragments of plasminogen/plasmin, which act as potent inhibitory mediators of endothelial proliferation and migration. Among all known angiostatin species, isolated K5 plasminogen fragment was shown to display the most potent inhibitory activity against proliferation of endothelial cells via triggering multiple signaling pathways, which lead to cell death and resulting angiogenesis suppression.

Results. Current literature data suggest that in addition to expressed and highly specific cytotoxicity in relation to endotheliocytes and some types of tumor cells, the kringle domain 5 of human plasminogen has other advantages as an antiangiogenic and antitumor regulator, including its specific inhibitory activity, which affects only activated, proliferating endothelial cells, and therefore is non-toxic to other types of normal cells. As an endogenous protein, which is formed in the human organism, K5 does not provoke an immune response. K5 as a small polypeptide molecule with a stable structure can be obtained as a recombinant protein in *E. coli* cells, and can also be used in pharmacokinetic systems of targeted delivery and sustained release.

Conclusions. The prospect of successful use of K5 as a therapeutic agent to manage pathological processes associated with dysregulation of angiogenesis makes it necessary to develop and improve methods of its production and to further test its plausible pleiotropic biological activities.

Key words: angiostatins; plasminogen fragment kringle 5; angiogenesis; endothelial cells; neovascular diseases; tumor growth; retinopathy.

Angiogenesis is the process of outgrowth of new blood vessels from pre-existing ones. It plays an important role in development, regeneration, and repair. However, pathological angiogenesis occurs not only in tumor formation, but also in a number of non-neoplastic diseases, which can be classified together as “angiogenesis-dependent diseases”. Viewing the process of angiogenesis as an “organizing principle” in biology can provide intriguing insights into the molecular mechanisms of seemingly unrelated

phenomena. This has important implications for the clinical use of angiogenesis inhibitors and drug discovery, not only to optimize cancer treatment, but perhaps also to develop therapeutic approaches for different, otherwise unrelated diseases [1].

Normally, in an adult organism, the formation of new blood vessels processes occur with low intensity due to the maintenance of a balance between pro- and antiangiogenic factors and are activated only during regenerative processes. Dysregulation of new vascular formation and associated pathological angiogenesis are indicated for age-related changes in tissues, oncological processes, atherosclerosis, diabetes, peptic ulcers, some autoimmune diseases, Alzheimer's disease, and a number of developmental pathologies. Neovascularization plays a significant role in tumor development and metastasis [1]. Among many physiological inhibitors of angiogenesis, fragments of various kringle-containing proteins, including plasminogen, urokinase, tissue type plasminogen activator, hepatocyte growth factor (HGF), play special roles. Proteolytic fragments of the glycoprotein protein — plasminogen, containing varying amounts of its kringle domains, are considered one of the most powerful suppressors of angiogenesis and are called angiostatins [1]. Angiostatins are most intensively generated by primary tumor cells due to dysregulation of the activity of a number of proteinases and can be markers of tumor growth. They effectively suppress the proliferative activity and migration of endothelial cells, trigger the processes of their apoptosis, preventing the formation of new blood vessels and thereby inhibiting the development of metastases. Angiostatins are involved not only in processes associated with oncogenesis, but also modulate angiogenesis in other disorders accompanied by activation of inflammatory reactions, for example, in diabetes mellitus. The role of angiostatins in the development of diabetes-associated angiopathy, as well as the its involvement in the development of diabetic complications such as coronary heart disease and retinopathy, is a complex and controversial issue that needs to be further explored.

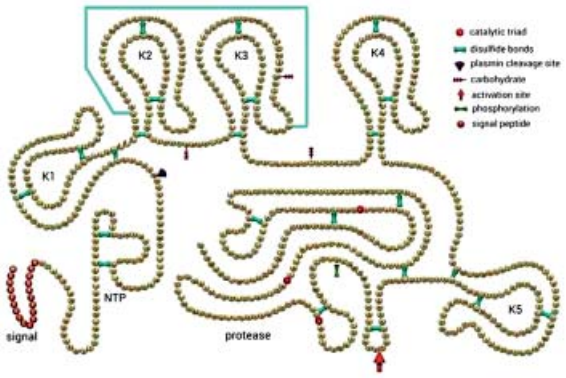

Fig. 1. **Domain structure of a native human plasminogen molecule [3]**

Plasminogen/plasmin system is involved in normal (physiological) and pathological angiogenesis. Physiological angiogenesis (from the development of a fetus and the birth to formation of normal vessels in a grown up organism) proceeds with moderate intensity and accelerates during a number of processes, including regeneration of injured tissues, recanalization of thrombi, and scarring. In contrast to normal vascular network, pathological angiogenesis (i.e., in the course of growth and metastasis of a tumor, myocardial infarction, wound healing, chronic inflammatory diseases, etc.) proceeds abnormally. In these conditions, vessels are heterogeneous, irregularly branched, have multiple fenestrations, and are hyperpermeable for plasma proteins [2].

Angiostatins: general information. Products of limited proteolysis of plasminogen, containing different amounts of its kringle domains (K1-3, K2-3, K1-4, K1-4.5, K1-5, K5), known under the general name "angiostatins", perform the functions of endogenous inhibitors in the body neovascularization and vessel growth (Fig. 1) [4, 5].

The biological effects of angiostatins are related to their ability to specifically inhibit the proliferation of activated endothelial cells, induce apoptosis, and inhibit cell migration. It has been established that various variants of angiostatins suppress tumor-associated angiogenesis, thus restraining the growth of the primary tumor and the progression of metastases [6]. They play an important role in the pathogenesis of neovascular eye diseases. Angiostatins maintain the angiogenic balance in the retinal tissue, preventing its excessive vascularization, in particular during vision correction using laser photocoagulation [7]. Suppression of the production of angiostatins in the retina can be one of the triggers for the development of diabetic retinopathy [8]. Angiostatins are among the proteins of the tear fluid proteome, forming on the surface of the cornea of the eye, thus preventing the formation of blood vessels in it, which is important for maintaining its optical transparency [9]. The anti-adhesive and anti-inflammatory effects of angiostatins have been

shown [10, 11]. Traditionally, angiostatin is considered as a structure corresponding to the kringle domain 1–3 fragment (K1–3) or kringle domain 1–4 (K1–4) fragment of plasminogen/plasmin molecule. Each kringle consists of 80 amino acids held together by three disulfide bonds and formed in loops. Later, by proteolysis of plasminogen or autolysis of plasmin, angiostatin K1–3, containing the first three kringles, and angiostatin K1–4.5, containing kringles 1–4 and 85% kringle 5 of plasminogen, were obtained. It has been shown that angiostatin K1–3 is a weaker inhibitor of endothelial cell proliferation than angiostatin K1–4 [12]. Angiostatin K1–4.5 inhibited angiogenesis and tumor growth at a dose 50 times less than K1–4 [13]. Comparative studies of plasminogen fragments (angiostatin, K1, K3, K2-3, etc.) have shown that kringle 5 (K5) exerts the greatest inhibitory activity [14].

Studies of the therapeutic effects of angiostatins is divided into two groups depending on the nature of the studied proteins, either native or recombinant. It has been suggested that one of the mechanisms of therapeutic action of laser retinal photocoagulation, which is aimed to avoid vision loss in retinopathy is the induction of the formation of endogenous pool of angiostatins [15]. Results of another study has indicated possibility of pharmacocorrection of diabetes-induced retinopathy by modulating angiostatin levels in the injured retina. It has been shown that inhibitors of proapoptotic enzyme PARP-1 are able to restore production of angiostatins in retinas of diabetic rats near to control levels [16]. The prospect of delivery of a genetically engineered construct containing an angiostatin-coding sequence (rAAV-AS K1–4) to retinal tissue in diabetic retinopathy has been declared [17].

Pearce et al. have shown that angiostatins under a normal physiological state are characterized by a wide localization in the structures of the eye. Using immunochemical methods, angiostatins were detected in the nerve fiber layer, ganglion cells, inner and outer plexiform layers, and photoreceptor layer of the retina of the eye of the cat, cow, dog, and rat, while in the retina of the eye of horses and pigs, additional immunostaining of angiostatins was shown in the matrix of the inner nuclear layer [18]. At present, the populations of astroglial and some other retinal cells responsible for the generation of angiostatin in this eye structure remain unknown, and their identification requires additional efforts.

Since the uncontrolled growth of new blood vessels causes the progression of many common diseases, first of all, oncological diseases, autoimmune disorders, neovascular damage of the eye, the use of angiostatins can be a promising pharmacotherapeutic approach to the prevention and adjuvant therapy of these pathological conditions. The advantages of using angiostatins are their non-toxicity even at high doses, non-immunogenicity, lack of tolerance of target cells to their action.

The results of a number of experimental works support the effectiveness of the use of exogenous angiostatins or the corresponding genetic engineering structures that encode them, with the aim of suppressing proangiogenic signaling in diabetic retinopathy. The authors of the paper [19] used a recombinant adenosine virus vector (rAAV) corresponding to the sequence of the K1–4 fragment of human plasminogen for the expression of angiostatin in the retina of rats with STZ-induced hyperglycemia. It was shown that expression of rAAV-AS significantly reduced capillary permeability in the retina of hyperglycemic rats. The use of the proposed gene delivery system has significant prospects for the therapy of eye diseases, since rAAV-AS is characterized by high stability, the ability for long-term expression, which allows achieving a significant therapeutic effect even after a single injection. It is assumed that native angiostatins are promising as agents that normalize vascularization in the retina. It was shown that a single injection of angiostatin K1–4 into the vitreous body at a dose of 7.5 μg per eye significantly reduced the degree of retinal capillary permeability with oxygen- and streptozotocin-induced diabetic retinopathy [20].

Although most of the work aimed at studying the effects of angiostatins in diabetic retinopathy has been conducted with the use of fragments of plasminogen consisting of the first three or four kringle domains, the special role of isolated K5 is increasingly becoming the subject of research. During the first trials, K5 proved itself as a promising potential therapeutic agent for the treatment of diabetic retinopathy. It was shown that the delivery of the gene encoding the K5 sequence to retinal cells reduced capillary permeability, inhibited VEGF overexpression, and inhibited retinal neovascularization under conditions of ischemia [21]. The results of the work [22] demonstrate that K5 is able to exert a direct effect on Müller glia cells, which are the main source of pro-inflammatory and pro-angiogenic

factors, including VEGF, in the retina and play a key role in maintaining neovascularization under conditions of hyperglycemia. Data were obtained that other angiostatins, including K1–4, do not interact with the K5 binding site on the surface of Müller cells, which indicates the specific nature of the association of K5 with cells. Moreover, it has been shown that K5 in the retina enhances the production of an endogenous inhibitor of angiogenesis — a pigment epithelial derived factor (PEDF) [23]. It is assumed that the physiological effects of this plasminogen fragment are realized due to its ability to bind specifically and with high affinity to the potential-dependent anion channel (VDAC1), as it occurs in endotheliocytes [24].

It is known that any disturbances in the trophism and functioning of the retinal pigment epithelium lead to indirect damage to photoreceptors, which leads to a malfunction of the entire visual apparatus. Dysfunction of retinal pigment epithelial (RPE) cells is the main reason for the development of such disorders as Stargardt's disease, Best's macular dystrophy, retinitis pigmentosa, rod-cone retinal dystrophy, age-related macular degeneration, etc. [25]. The new data from our laboratory indicate the absence of cytotoxic properties of angiostatins in relation to RPE cells. These data are of great practical importance in the context of the possibility of safe use of these angiogenesis inhibitors for the purpose of targeted inhibition of the activity of vascular endothelial cells in the treatment of various eye diseases associated with retinal neovascularization. Its antiproliferative effect is several times higher than that of angiostatin, as well as that of any single kringle domain. This may be due to the fact that the anti-endothelial effect of K5 and other kringle domains is realized by different mechanisms. For example, electro-dependent anion channel (VDAC1) may play a role of the K5 receptor on the surface of endothelial cells. K5 binding to endothelial cells induces a decrease in intracellular pH and hyperpolarization of the mitochondrial membrane [26]. ATP synthase associated with the cytoplasmic membrane of endothelial cells, and integrin $\alpha v\beta 3$ has been reported to be angiostatin receptors [27].

It is concluded from these *in vitro* studies [47] that the ranking order of endothelial cell inhibition is K5 > K1, K2, K3 > K1, K2, K4 > K1 > K3 > K2 > K4. However, these *in vitro* data have not been directly translated into antiangiogenic activity *in vivo*. For example, K5 has been found to be less active than angiostatin in suppression of angiogenesis in the chick chorioallantoic membrane assay and the mouse corneal angiogenesis model [28, 29]. Insufficient suppression of *in vivo* angiogenesis by K5 is mainly due to its relatively short half-life *in vivo*. Thus, the antiangiogenic effect of a given compound must be tested in *in vivo* angiogenesis models and not only in *in vitro* endothelial cell cultures [30].

Kringle 5: structure and biological properties. Among all known variants of angiostatins, K5 (Fig. 2) attracts a special attention. K5 has a molecular weight of ~15 kDa and exists as a compact structure with the distinct globular type of hydrophobic core, stabilized by three disulfide bonds. Amino acid analysis of the NH_2-terminal region of K5 revealed two elastase cleavage

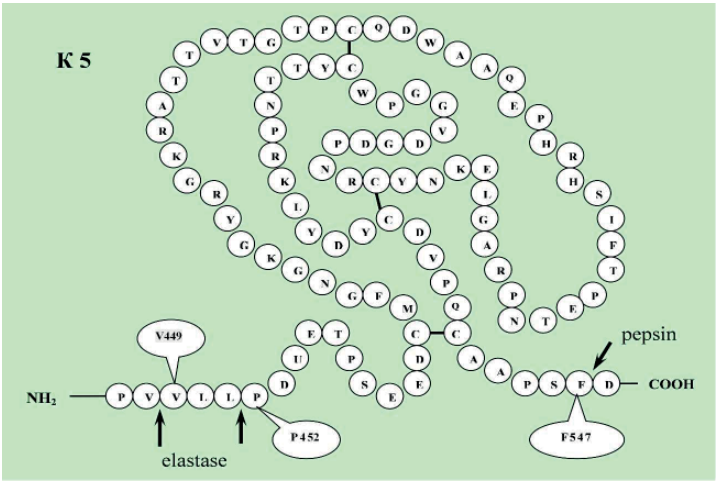

Fig. 2. **The structure of kringle 5 (K5) domain of plasminogen molecule [31]**

sites located both between Val448 and Val449, and between Leu451 and Pro452, and pepsin cleavage site at the position between Phe547 and Asp548. According to the result of the electrophoresis, K5 runs as two bands with molecular mass of 14.9 and 15.7 kDa, correspondingly K5Pro452-Phe547 and K5Val449-Phe547 (Fig. 3).

The antiproliferative and antimigratory effects of K5 in relation to endothelial cells are several times higher of those of other angiostatins, as well as of any individual kringle domain. The high angiostatic potential of K5 is realized due to its ability to specifically interact with a number of alternative receptor molecules exposed by endotheliocytes and some other types of cells, including oncotransformed ones. Today, special attention is paid to the mechanisms of antiangiogenic properties of the K5 fragment of plasminogen. It is believed that the main target of K5 is the protein VDAC1, which was first identified on the outer membrane of mitochondria, and later identified on the plasma membranes of some cells.

The primary function of this pore-forming protein is thought to be regulation of ATP release, transport of ions and metabolites, maintenance of mitochondrial volume, and regulation of redox balance. By interacting with pro- and anti-apoptotic factors, VDAC1 plays a role as a regulator of mitochondria-mediated signaling pathways, which may determine cell death or survival. Thus, VDAC1 is involved in carcinogenesis and the development of neurodegenerative conditions [32]. Amino acid homology between plasminogen activator streptokinase and VDAC1 is shown. It turned out that the binding site of K5 in the streptokinase molecule is located between residues Tyr252-Lys283, and is a homologous site in the primary structure of VDAC1 Tyr224-Lys255. Antibodies against these sequences interact with VDAC1 and recognize this protein on the plasma membrane of human endothelial cells. K5 binds with high affinity (K_d = 28 nM) to endothelial cells, and this interaction is blocked by specific antibodies. Purified VDAC1 binds to K5, but exclusively in the liposomal form. It is suggested that K5 disrupts the mechanisms that control intracellular Ca^{2+} levels precisely through interaction with VDAC1.

The binding of K5 to endothelial cells also induces a decrease in the intracellular pH value and the amount of hyperpolarization of the mitochondrial membrane. However, the exact role and underlying mechanisms of VDAC1 in K5-induced endothelial cell apoptosis remain to be elucidated. In the study [33], authors showed that K5 increased protein level of VDAC1, which initiated the mitochondrial apoptosis pathway of ECs. They also showed that K5 inhibited the ubiquitin-dependent degradation of VDAC1 by promoting the phosphorylation of VDAC1, possibly at Ser-12 and Thr-107. The phosphorylated VDAC1 was attenuated by the AKT agonist, glycogen synthase kinase (GSK) 3β inhibitor, and siRNA, suggesting that K5 increased VDAC1 phosphorylation via the AKT-GSK3β pathway. Furthermore, K5 promoted cell surface translocation of VDAC1, and binding between K5 and VDAC1 was observed on the plasma membrane. HKI protein blocked the impact of K5 on the AKT-GSK3 pathway by competitively inhibiting the interaction of K5 and cell surface VDAC1.

Moreover, K5-induced EC apoptosis was suppressed by VDAC1 antibody. Thus it was demonstrated that K5-induced EC apoptosis is mediated by the positive feedback loop of "VDAC1-AKT-GSK3β-VDAC1", which may provide new insights on the mechanisms of K5-induced apoptosis [33]. It is important to note that annexin II and ganglioside GM1 bind to the plasminogen molecule through the LBS located in K1, while VDAC1 interacts via a

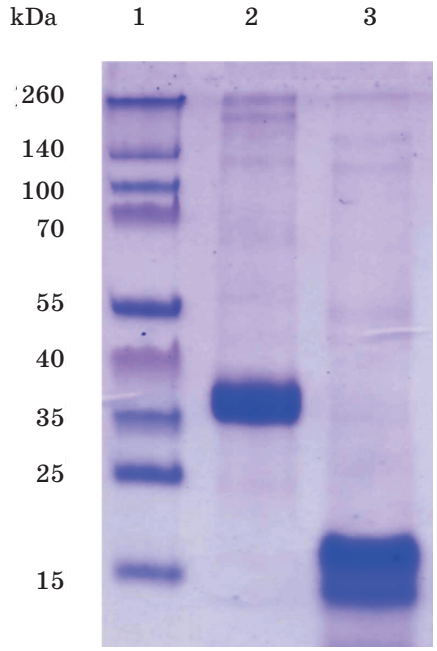

Fig. 3. **Electrophoregram of a K5 fragment obtained by limited proteolysis of plasminogen by pepsin:**
1 — molecular weight markers,
2 — mini-plasminogen, *3* — K5

site located in K5 [26, 33]. In addition, both the zymogen molecule via its K5 and t-PA via the finger domain bind to VDAC1 exposed on the cell surface. This mediated interaction of zymogen and its activator leads to a decrease in the value of K_m and an increase in the value of K_{max} for the reaction of converting plasminogen to plasmin [34].

K5 promoted an increase in the ratio of Bak/Bcl_{xl} on the mitochondrial membrane, which led to its depolarization, leakage of cytochrome c, and activation of caspases 7, 8, and 9 in endothelial cells, which led to their apoptosis [35]. It is possible that the pro-apoptotic properties of K5, which are realized through its binding to VDAC1, determine antitumor activity of this plasminogen kringle, as it was shown in some types of gastric cancer [36]. It was shown that K5 domain of the plasminogen molecule is a substrate for the NADH-dependent reductase activity of VDAC1. It turned out that such a ternary complex is an effective proteolytic machine that removes β-peptide deposits in the brain, as well as disposes of cellular debris from damaged tissue [34]. In particular, one of the receptors for K5 on the surface of endothelial cells is the potential-dependent anion channel (VDAC1). It is assumed that K5 disrupts the mechanisms that control intracellular Ca^{2+} homeostasis precisely through interaction with the VDAC1 molecule [26].

The therapeutic effect of K5 is due to its suppressive effect on the cell cycle of endothelial cells and the subsequent initiation of apoptosis [37]. Unlike other types of angiostatins, K5 has been shown to exert cytotoxic effects directly on cancer cells. The pro-apoptotic properties of K5, which are realized through its binding to VDAC1, determine the antitumor activity of this kringle domain of plasminogen, as shown in some types of gastric cancer [36]. The antiangiogenic and cytotoxic effects of K5 fused to galectin-3 (PK5-RL-Gal-3C) were described in a model of hepatocellular carcinoma [38]. We recently found that isolated K5, obtained by pepsinolysis of mini-plasminogen, halved the invasive/migratory potential of the highly invasive mouse mammary adenocarcinoma cell line 4T1 [unpublished data]. Suppression of the migratory activity of tumor cells by kringle domain 5 opens up prospects for its use as an antimetastatic agent.

Despite the fact that most of the studies aimed at studying the effects of angiostatins in diabetic retinopathy were conducted using fragments of plasminogen consisting of the first three or four kringle domains, the special role of isolated K5 has paid a peculiar attention [19]. During the first experiments, K5 proved to a promising therapeutic agent for the treatment of diabetic retinopathy. It was shown that the delivery of the gene encoding the K5 sequence to retinal cells contributed to a decrease in the degree of capillary permeability, inhibited the overexpression vascular endothelial growth factor (VEGF), and inhibited retinal neovascularization under ischemia conditions [24]. At the same time, the therapeutic effects of K5 in the retina were not accompanied by a cytotoxic effect on the cells of the pigment epithelium.

Inhibition of tumor angiogenesis has an important role in antitumor therapy. However, a recent study indicates that antiangiogenesis therapy may lead to glucose-related protein 78 (GRP78) associated antiapoptotic resistance [36]. Fang and co-authors discovered the dual effects of plasminogen kringle 5 (K5) on tumor angiogenesis and apoptosis induction by targeting hypoxia-inducible factor 1α (HIF-1α) and GRP78. K5 promoted the sumo/ubiquitin-mediated proteasomal degradation of HIF-1α by upregulating von Hippel-Lindau protein under hypoxia, resulting in the reduction of vascular endothelial growth factor and thus suppressing tumor angiogenesis. Furthermore, K5 decreased GRP78 expression via downregulation of phosphorylated extracellular-regulated protein kinase, leading to caspase-7 cleavage and tumor cell apoptosis.

Blocking voltage-dependent anion channel abrogated the effects of K5 on both HIF-1α and GRP78. K5 significantly inhibited the growth of gastric carcinoma xenografts by inhibiting both angiogenesis and apoptosis (Fig. 4) [36]. Gastric cancer is an aggressive malignancy that is frequently diagnosed at an advanced stage with poor prognosis. Although surgery and/or a combination of chemotherapy improve the survival rates, the 5-year relative survival rates of the patients receiving these treatments remains low at 30% and that of the patients with advanced disease is < 1 year [39, 40]. Therefore, it is necessary to develop more effective therapeutic strategies. The dual effects suggest that K5 might be a promising bio-therapeutic agent in the treatment of gastric cancer.

HIF-1α pathway has been proposed as a suitable target for future anticancer therapy [41–44]. Some previous research confirmed that K5 reduced the HIF-1α levels in the retina of retinopathy model and the retinal

capillary endothelial cells [23]. Another study of the same authors demonstrated that HIF-1α was expressed apparently both in nuclear and cytoplasmic compartments of LLC cells induced by hypoxic conditions, and K5 significantly down-regulated HIF-1α expression *in vivo* and *in vitro*. The protein level of intracellular HIF-1α is determined mainly by its rate of proteasomal degradation [45]. Briefly, HIF-1α is hydroxylated by prolyl hydroxylases (PHDs) under normoxic conditions. This modification allows the binding of the tumor-suppressor protein von Hippel-Lindau (VHL) to HIF-1α, and then promotes the formation of E3 ubiquitin ligase complex. The VHL protein mediates polyubiquitination of the HIF-1α subunit at three lysine residues, which results in its degradation by the proteasome. Thus, PHDs and VHL may be the candidate target molecules for the stabilization of HIF-1α during hypoxia. It has been confirmed earlier that K5 promoted the ubiquitin-proteasomal degradation of HIF-1α by inducing VHL, resulting in the decreased protein level of intracellular HIF-1α. Authors demonstrated that K5 treatment significantly reduced the amount of HIF-1α in cytoplasm and lead to a more marked reduction of HIF-1α in nucleus, suggesting that K5 not only down-regulated the protein level of HIF-1α, but also inhibited HIF-1α nuclear accumulation. The rapid nuclear translocation of HIF-1α represents an efficient way to escape from degradation and is the essential steps for HIF-1α in the transactivation of hypoxia-responsive genes [46]. The anti-metastasis effect of K5 is likely to be mediated by suppressing the protein stabilization and nuclear accumulation of HIF-1α, consequently inhibited the HIF-1α transcriptional activity that could be responsible for decreasing gene expression of VEGF and CXCR4, resulting in the inhibition of angiogenesis and tumor chemotaxis movement which are indispensable steps in the progression of metastasis (Fig. 5) [41].

K5 up-regulates VHL and consequently promotes ubiquitin-proteasome medicated protein degradation of HIF-1α. Moreover, K5 decreased HIF-1α protein stabilization, reduced nuclear HIF-1α accumulation and then inhibited transcriptional activation. Consequently, K5 down-regulated the gene expression of CXCR4 and VEGF, which were the downstream genes of HIF-1α pathway. VEGF and CXCR4 play key roles in angiogenesis and chemotaxis migration which both are requisites to metastasis promotion. This may be responsible for the dual inhibitory effects of K5 on tumor metastasis.

Advantages K5 application. In addition to pronounced and highly specific cytotoxicity in relation to endotheliocytes and some types of tumor cells, other advantages of kringle domain 5 of human plasminogen as an antiangiogenic and antitumor regulator are also obvious. Firstly, K5 shows its inhibitory activity specifically, affecting only activated, proliferating endothelial cells, and therefore is non-toxic to other types of normal cells, including the predecessors of endothelial cells and epitheliocytes [47]. Secondly, plasminogen is an endogenous protein present in the human body, and therefore K5 does not provoke an immune response. Thirdly, K5

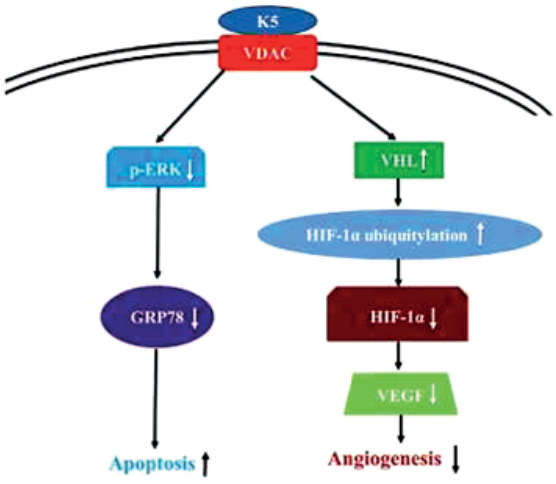

Fig. 4. **The schematic diagram of the signalling pathway** affected by K5 **in the regulation of tumor angiogenesis and tumor cell apoptosis (from [36])**

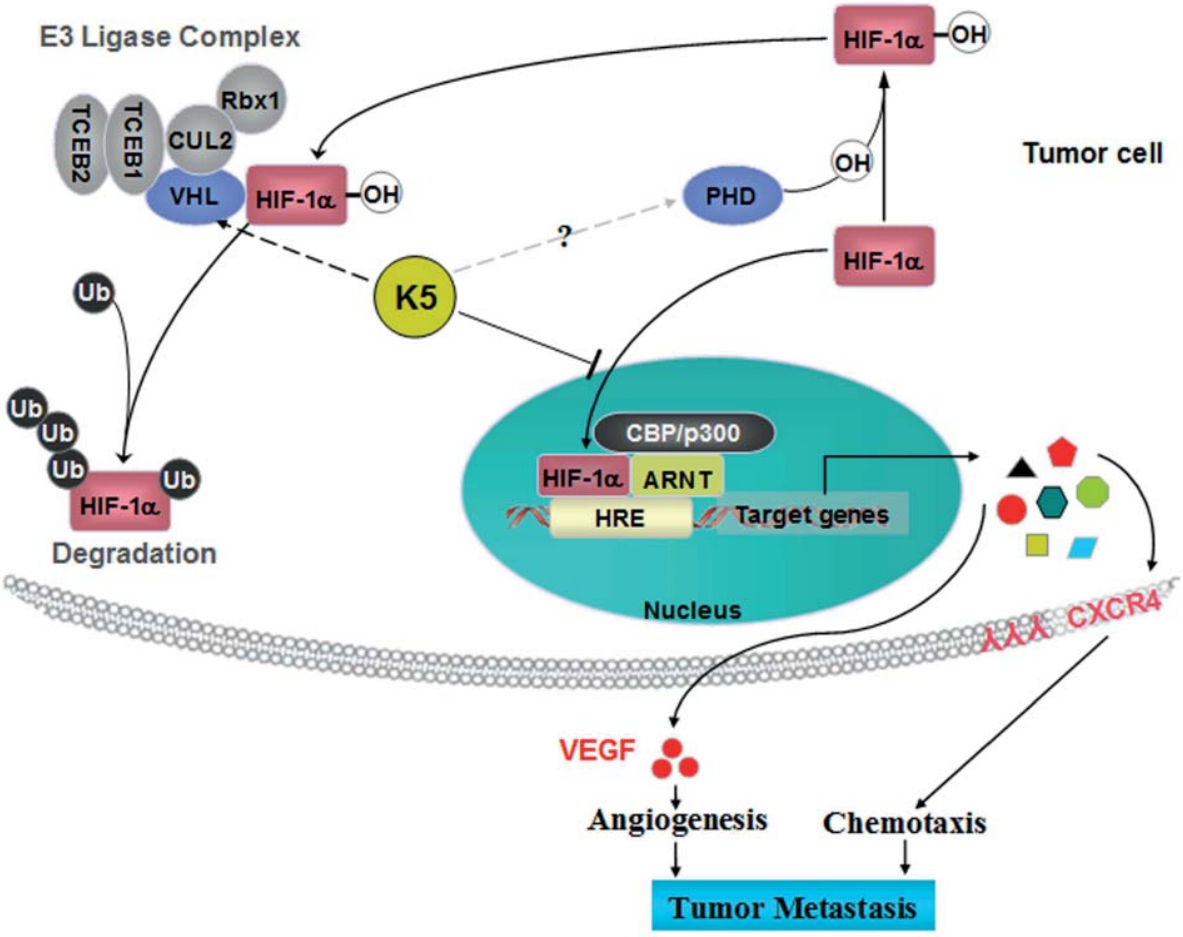

Fig. 5. **A schematic overview of the potential mechanism involved in K5-mediated inhibition of HIF-1α in tumor cells [41]**

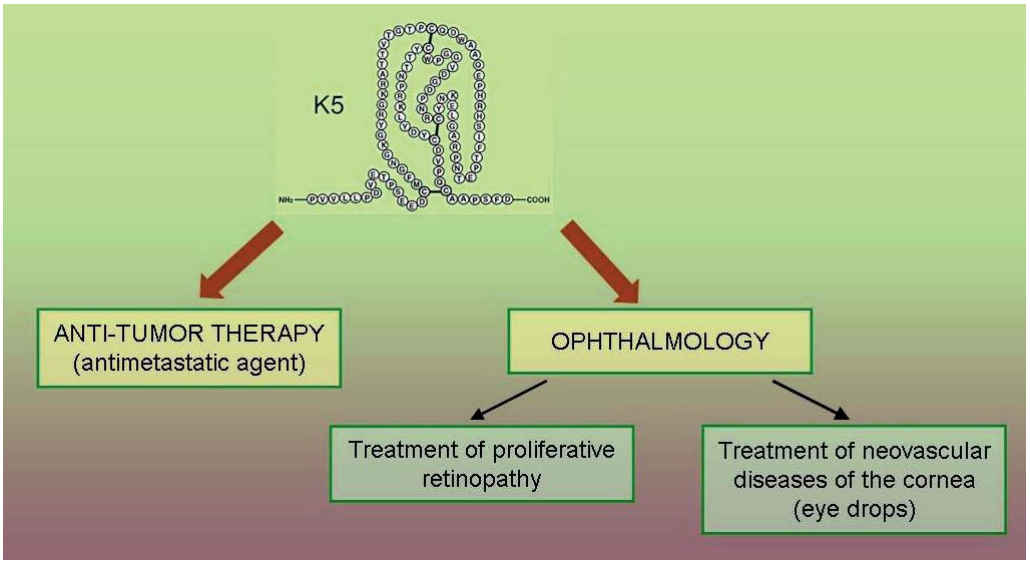

Fig. 6. **Directions of potential pharmacological application of rK5**

as a small polypeptide molecule with a stable structure can be obtained as a recombinant protein in *E.coli* cells, and can also be used in pharmacokinetic systems of targeted delivery and sustained release. In contrast to K5, it has proved extremely difficult to obtain soluble forms of higher molecular weight angiostatins with qualifications that meet the requirements for clinical application (Fig. 6) using expression systems based on *E.coli,* baculoviruses, yeast and mammalian cells.

Usually, the expression of such genetic constructs in *E. coli* resulted in the formation of insoluble protein aggregates of uncertain composition, unsuitable for further use, and in other expression systems the yield of the target protein was extremely low. Also, considering the high cost of native K5 preparations, which are obtained from blood plasma plasminogen, producing a genetically engineered form of this protein in order to create new drugs is expedient and profitable from an economic point of view.

Conclusions

Thus, the prospect of successful use of K5 as a therapeutic agent in pathological processes associated with dysregulation of angiogenesis makes it necessary to develop methods of obtaining it for the creation of new drugs with an antiangiogenic therapeutic effect.

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БІОМЕДИЧНЕ ЗАСТОСУВАННЯ ФРАГМЕНТА ПЛАЗМІНОГЕНУ К5

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Мета. Висвітлити потенційне біомедичне застосування кringла 5 у регуляції ангіогенезу та розвитку пухлини.

Методи. Ангіогенез є складним процесом, який включає проліферацію ендотеліальних клітин, міграцію, деградацію базальної мембрани та організацію нових судин. Оскільки неконтрольований ріст нових кровоносних судин є причиною прогресування багатьох поширених захворювань, насамперед, онкологічних та аутоімунних захворювань, неоваскулярних уражень ока, застосування ангіостатинів може бути перспективним фармакотерапевтичним підходом до профілактики та допоміжної терапії цих патологічних станів. Перевагами застосування ангіостатинів є їхня нетоксичність навіть у високих дозах, неімуногенність, відсутність толерантності клітин-мішеней до їхньої дії. Ангіостатини включають групу протеолітичних фрагментів плазміногену/плазміну, що містять кринглові структури, які діють як потужні інгібувальні медіатори ендотелійної проліферації та міграції. Показано, що серед усіх відомих видів ангіостатинів ізольований фрагмент плазміногену К5 демонструє найпотужнішу інгібіторну активність проліферації ендотелійних клітин через запуск багатьох сигнальних шляхів, які призводять до загибелі клітин і, як наслідок, пригнічення ангіогенезу.

Результати. Сучасні наукові дані літератури свідчать про те, що, окрім вираженої та високоспецифічної цитотоксичності по відношенню до ендотеліоцитів та деяких типів пухлинних клітин, кringловий домен 5 плазміногену людини має інші переваги як антиангіогенний та протипухлинний регулятор: він виявляє інгібіторну активність, зокрема, впливає лише на активовані ендотеліальні клітини, що проліферують, і тому не є токсичним для інших типів нормальних клітин; як ендогенний протеїн, присутній в організмі людини, К5 не провокує імунну відповідь; і К5 у вигляді невеликої поліпептидної молекули зі стабільною структурою може бути отриманий у вигляді рекомбінантного білка в клітинах *E. coli*, а також може бути використаний у фармакокінетичних системах спрямованого доставляння та пролонгованого вивільнення.

Висновки. Перспектива застосування К5 як ефективного засобу терапії при патологічних процесах, пов'язаних з порушеннями регуляції ангіогенезу, зумовлює як необхідність розроблення та вдосконалення методів його отримання, так і подальшого тестування його ймовірної плейотропної біологічної активності.

Ключові слова: ангіостатини; фрагмент плазміногену кringл 5; ангіогенез; ендотелійні клітини; неоваскулярні захворювання; розвиток пухлини; ретинопатія.