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Influence of urokinase gene knockout on level of prekallikrein and kallikreins 1 and 14 in mice with melanoma growth against the background of pain

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Abstract

The aim is to study the content of prekallikrein, kallikreins 1 and 14 (KLK-1 and KLK-14) in mice under the conditions of activation and inhibition of the B16/F10 inoculated melanoma growth.

Materials and methods

We produced a model of chronic neurogenic pain (CNP, bilateral ligation of sciatic nerves) and a model of the B16/F10 melanoma growth against the background of CNP using female/male mice of the C57BL/6 line (with normal genome, n=75) and the C57BL/6-Plautm1.1bugthisplaughfdhu/GFDhu line (with urokinase knockout (uPA), n=46). The ELISA method was used to determine the content of prekallikrein, KLK1 and KLK14 in the tumor and skin after 3 weeks of carcinogenesis against the background of CNP.

Results

The initially high content of prekallikrein, KLK-1 and KLK-14 in the skin of intact knockout mice was found. When inhibiting the growth and metastasis of melanoma in the skin of the knockout females, noted was a further increase in the initially high level of prekallikrein and KLK1, and in the tumor in the knockout mice of both genders recorded was a lower content of all the studied enzymes. When activating the growth of melanoma in the state of CNP in the skin of C57BL/6 mice, the level of KLK1 (female) decreased and the concentration of KLK14 (female and male) increased; in the knockout mice the content of prekallikrein and KLK14 decreased, and KLK1 changed multidirectionally. The tumor demonstrated a lower prekallikrein level in all C57BL/6 mice and multidirectional changes in KLK1 and KLK14 in females and males. The tumor in the knockout animals showed a lower content of all the studied enzymes, especially pronounced for KLK14.

Conclusion

The content of prekallikrein, KLK1 and KLK14 varies under activating and inhibiting the growth of melanoma, the changes are of the gender-specific type. It is probable that the functions of the studied enzymes in the skin change not only in case of CNP, but also in case of urokinase deficiency. The tumor itself adapts the performance of the kallikrein-kinin system depending on the metabolic features of the tumor-bearing organism.

Keywords

Mice, UPA knockout, B16/F10 melanoma, Chronic neurogenic pain, Kallikrein-kinin system, Skin, Tumor

Imprint

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Introduction

The study of pain has led to significant progress in understanding the mechanism of pain chronization, showing that damage to the peripheral nervous system, causing the mobilization of adaptive metabolic mechanisms, triggers the mechanisms of disorder in homeostasis when the mechanisms of pain resolution are disturbed or destroyed [1,2].

Previous studies have shown that in mice in the state of chronic neuropathic pain (CNP), observed is a change in the growth characteristics of the B16/F10 melanoma, indicating an increase in the aggressiveness of the tumor [3,4]. Stimulation of the growth of the tumor by CNP is also observed when using as a model mice having urokinase gene-knockout which itself causes inhibition of growth and metastasis of the tumor [5-7].

Urokinase plays an important role in the processes of the tumor growth and metastasis through the plas-

minogen activation and the pericellular proteolysis initiation, regulating the degradation of extracellular matrix proteins, promoting the release of growth factors and other biologically active molecules [8]. Lack of the urokinase activity can be compensated by other proteolytic pathways: in mice with uPA/tPA deficiency, the plasminogen activation, which is independent of these proteases, mediated by kallikrein is observed [9]. There is evidence appeared that there are complex proteolytic cascades between the uPA/uPAR families, kallikreins and metalloproteinases to modulate downstream cellular signaling pathways [10].

The tissue system of kallikrein-related peptidases (kallikreins KLKs), their precursors, kinins and their receptors plays an important role in homeostasis, and a disorder in the regulation of their expression, activity or localization is associated with various pathological conditions, including carcinogenesis [11]. The kallikrein-kinin system (KKS) is involved in the processes associated with pain and inflammation through the formation of kinins (endogenous algogenic peptides), activation of their receptors [12,13].

Previously, we had detected a change in the content of certain components of the kallikrein-kinin system when activating the growth of the B16/F10 melanoma against the background of CNP in the C57BL/6 female mice [14].

The aim is to study the content of prekallikrein, kallikreins 1 and 14 (KLK-1 and KLK-14) in mice under the conditions of activation and inhibition of the B16/F10 melanoma growth.

Materials and methods

The experimental study was conducted in the line C57BL/6 mice of both genders (uPA+/+, n=75) with an initial body mass of 21-23 g, received from by the Federal State Medical & Biological Institution "Research Center of Biomedical Technologies at the Federal Medical & Biological Agency", Branch Andreevka (Moscow region) and in the line C57BL/6-Plautm1. 1Bug This PlauGFDhu/GFDhu mice of both genders (urokinase gene knockout uPA-/-, n=46) with an initial body mass of females 24-26 g and males 31-33 g, received from the Scientific Production Enterprise "Laboratory Animals' Breeding Facility" at the Branch of the Institution of Bioorganic Chemistry named after Academy Members M.M.Shemyakin and Yu.A.Ovchinnikov (Pushchino); (Pushchino, Moscow region). The animals were kept under natural lighting conditions with no restrictions on access to water and food. The study was conducted in accordance with the "International Recommendations on Pursuance of Medical & Biological Research in Animals" and Order No.267 "Approval of Regulations on Laboratory Practice" dd. 19.06.03 issued by the Ministry of Health Care of the Russian Federation.

Experimental groups included the following cohorts: intact mice (10 males, 10 females of line C57BL/6, 8 males, 8 females of the knockout line), control group with animals to reproduce the model of chronic neurogenic pain (12 males, 14 females of line C57BL/6, 7 males, 7 females of knockout line), comparison group with animals with the growth of standard inoculated melanoma B16/F10 (15 males, 10 females of line C57BL/6, 7 males, 8 females of knockout line), main group of animals to reproduce the B16/F10 melanoma growth against the background of CNP (15 males, 14 females of ine C57BL/6, 8 males, 8 females of knockout line). Animals from the comparison group (growth of melanoma) were compared with the intact group, and mice from the main group were compared with the control group (with CNP).

The CNP model was produced by bilateral ligation of the sciatic nerves [3.14]. The cell suspension of mouse melanoma B16/F10, obtained from N.N. Blokhin National Medical Research Center of Oncology (Moscow) was simultaneously transplanted subcutaneously into all the animals of the comparison group, and, in to the main group animals, 2 weeks after ligation of the sciatic nerve [3,14]. Procedures of the sciatic nerves ligation, the B16/F10 melanoma inoculation, and the course of the experiment have been described previously [3,14]. The animals were decapitated 3 weeks after the tumor inoculation. The tumor and unaffected by malignant growth skin were isolated on ice immediately after decapitation. 10% cytosolic fractions prepared on 0.1 M potassium-phosphate buffer pH 7.4 containing 0.1% Tween-20 and 1% bovine serum albumin were obtained from the tissues, where concentrations of prekallikrein, KLK-1 and KLK-14 were determined using standard ELISA test systems. The result was recalculated per gram of tissue.

The obtained data were processed with the use of the Statistica 6.0 software. The results are presented as an average value ± standard error of the average. The Shapiro-Wilk criterion was used to test for fit to the normal distribution. The Mann-Whitney test was

Table 1
Content of prekallikrein and kallikreins in skin and tumors of female mice

Groups of animals	Prekallikrein, ng/g tissue	KLK1, pg/g tissue	KLK14, pg/g tissue
MICE C57BL/6	Skin uPA+/+		
Intact	5.6±0.4	380.1±27.9	15.6±1.2
Control (CNP)	11.6±0.8 p1=0.0000	189.5±13.6 p1=0.0000	14.8±1.1
Comparison group (melanoma B16F10)	13.3±1.2 p1=0.0000	304.1±25.8	10.3±0.9 p1=0.0312
Main group (CNP+melanoma B16F10)	16.7±1.3 p2=0.0266	83.7±5.6 p2=0.0000	32.6±2.2 p2=0.0000
	Tumor uPA+/+		
Comparison group (melanoma B16F10)	4.2±0.31 p3=0.0000	383.4±29.1	71.3±5.8 p3=0.0000
Main group (CNP+melanoma B16F10)	4.8±0.36 p3=0.0000	489.2±35.7 p3=0.0000	42.1±3.5 p3=0.0356
GENE KNOCKOUT MICE Skin	uPA-/-		
Intact	27.6±1.7	647.6±37.3	2783.5±151.3
Control (CNP)	23.8±2.8	441.5±39.8 p1=0.0127	3195.9±325.0
Comparison group (melanoma B16F10)	88.9±7.9 p1=0.0000	1425.1±153.5 p1=0.0000	2327.2±183.7
Main group (CNP+melanoma B16F10)	10.45±0.96 p2=0.0002	804.5±79.6 p2=0.0009	1028.9±84.4 p2=0.0000
	Tumor uPA+/+		
Comparison group (melanoma B16F10)	8.15±0.91 p3=0.0000	853.6±69.7 p3=0.0016	227.9±20.5 p3=0.0000
Main group (CNP+melanoma B16F10)	6.06±0.56 p3=0.0079	765.3±80.3	134.9±12.3 p3=0.0000

Note: Statistically significant variances as compared with the level as follows: p1 - in intact group, p2 - in control group, p3 - in the skin of the respective group.

used to evaluate the significance of variances between samples. The differences were considered significant at $p<0.05$ - $p<0.001$.

Results

It was found (see Table 1 herein) that intact uPA-/- females initially had significantly higher levels of prekallikrein, KLK1 and KLK14 in their skin than intact uPA+/+ females, by 4.9, 1.7 and 178.4 times, respectively (p=0.0000).

We have already shown that 3 weeks after melanoma inoculation in the uPA+/+ females, the tumor growth and metastasis occurred against the background of a 2.4-fold increase in prekallikrein content and a 34% decrease in the KLK14 content in the unaffected by tumor growth skin as compared to the intact skin. In the tumor, the level of precallicrein was 3.2 times lower, and the level of KLK14 6.9 times higher than in the skin of the same animals, while the level of KLK1 did not differ from the one in the intact group [14]. Previously, we found that the uPA-/- females showed significant inhibition of the melanoma growth (the growth dynamics was poorly pronounced) and metastasis 3 weeks after inoculation [5], while the skin showed an increase in the initially high level of prekallikrein by 3.2 times and KLK1 by 2.2 times and a high content of KLK14, compared to the indicators in the intact group of the knockout mice; in the uPA+/+ melanoma tissue, a considerably lower content of prekallikrein and KLK14, on average, by 10.6 times and KLK1 by 40.1% than recorded in the skin of the same animals.

As we have already shown, in case of CNP, in the skin of the uPA+/+ females, the content of prekallikrein increased by 2.1 times, and the concentration of KLK1 decreased by 2 times as compared to the level in the intact females [14]. In the uPA-/- females, the content of prekallikrein and KLK14 remained high, at the level of the indicators reported for the intact skin, and the content of KLK1 was lower by 31.8%.

3 weeks after the melanoma inoculation, in the UPA+/+ females we observed the stimulation of the tumor growth and metastasis, as shown earlier, against the background of CNP [3]. While in the skin found was a higher content of prekallikrein and KLK14,

Table 2
Content of prekallikrein and kallikreins in skin and tumors of male mice

Groups of animals	Prekallikrein, ng/g tissue	KLK1, pg/g tissue	KLK14, pg/g tissue
MICE C57BL/6	Skin uPA+/+		
Intact	15.25±1.85	323.8±29.4	47.13±2.68
Control (CNP)	22.26±1.52 p1=0.0038	1015.5±108.4 p1=0.0000	42.4±3.9
Comparison group (melanoma B16F10)	8.91±0.74 p1=0.0273	196.2±29.6 p1=0.0365	52.72±5.98
Main group (CNP+melanoma B16F10)	23.88±3.26	1062.1±145.4	59.92±5.42 p2=0.0106
	Tumor uPA+/+		
Comparison group (melanoma B16F10)	6.58±0.67	431.7±39.3 p3=0.0000	39.44±1.02
Main group (CNP+melanoma B16F10)	4.67±0.78 p3=0.0000	1001.5±153.8	29.42±3.14 p3=0.0004
GENE KNOCKOUT MICE	Skin uPA-/-		
Intact	37.2±1.8	1682.7±101.7	453.8±38.1
Control (CNP)	23.8±2.8	794.8±60.5 p1=0.0000	2674.8±210.9 p1=0.0000
Comparison group (melanoma B16F10)	9.7±0.5 p1=0.0000	470.9±31.3 p1=0.0000	2674.8±210.9 p1=0.0000
Main group (CNP+melanoma B16F10)	8.6±0.9 p2=0.0000	471.2±42.8 p2=0.0003	1092.7±88.4 p2=0.0000
	Tumor uPA+/+		
Comparison group (melanoma B16F10)	8.6±0.7	235.5±28.1 p3=0.0001	65.7±7.2 p3=0.0000
Main group (CNP+melanoma B16F10)	6.7±0.7	314.0±19.6 p3=0.0024	212.9±19.8 p3=0.0000

Note: Statistically significant variances as compared with the level as follows: p1 - in intact group, p2 - in control group, p3 - in the skin of the respective group.

by 44% and 2.2 times, respectively, under the lower KLK1 concentration decreased by 2.3 times than that reported in the skin of the CNP females, in the melanoma tissue the prekallikrein level was 3.5 times lower, and the levels of KLK1 and KLK14 were recorded to be 5.8 times and by 29.1% higher, respectively, than in the skin of these mice [14]. In a similar group of the uPA - / - females, stimulation of the melanoma growth and metastasis was also detected [5], while the direction of changes in the levels of the studied indicators was varying: the content of prekallikrein and the concentration of KLK14 was 2.3 and 3.1 times lower, but the KLK1 level was 1.8 times higher than in the skin of the knockout females with CNP. The levels of prekallikrein and KLK14 were lower by 42% and 7.6 times, respectively, than recorded in the skin of the same animals in the tumor tissue with the normal urokinase gene.

In the skin of the intact uPA - / - males, the content of all studied components of KKS, as well as in the females, was also significantly higher than those found in the intact uPA+ / + males: prekallikrein by 2.4 times, KLK1 5.2 times, KLK14 9.6 times (p=0.0000) (see Table 2 herein).

3 weeks after the melanoma inoculation, its active growth and metastasis in the uPA+/+ males occurred against the background of lower contents of prekallikrein and KLK1 in the unaffected by tumor growth skin as compared to the intact skin, by 41.6% and 39.4%, respectively. In the proper tumor, the level of prekallikrein did not show differences from that in the unaffected skin in the same animals and was 2.3 times lower than in the intact group; the level of KLK1 was 2.2 times higher than in the skin in the same animals. In the uPA-/- males, 3 weeks after the standard inoculation, noted were a quite active growth of melanoma (although the tumor volume at this time was less than that recorded in the uPA+/+ males) with the absence of metastasis [5], while in the skin unaffected by the tumor growth revealed was a decrease in the initially high-level of prekallikrein by 3.8 times and in the concentration of KLK1 by 3,6 times in comparison with the respective indicators in the skin in the intact uPA-/- males, the concentration of KLK14 remained

rather high. These changes were the opposite of those observed in a similar group of the uPA - / - females. In the melanoma tissue of the uPA+ / + males, recorded were considerably lower concentrations of KLK1 and KLK14 than in the skin of the same animals, by 2.0 and 5.2 times, respectively that was similar to the changes detected in the uPA-/- females in the corresponding group.

In the state with CNP, in the skin of the uPA+/+ males the content of prekallikrein increased by 46%, and the concentration of KLK1 by 3.1 times as compared to the level of the intact males. In the uPA-/- males, the prekallikrein content remained high, at the level of the indicators recorded in the intact skin, KLK1 was 2.1 times lower, and KLK14 was 5.9 times higher than the respective level in the intact skin.

3 weeks after the melanoma inoculation, we detected stimulation of the tumor growth and metastasis in the uPA+/+ males against the background of CNP [4]; at the same time, the skin showed a 41.3% higher content only of KLK14 than in the skin of the males with CNP (control group). In the melanoma tissue, the levels of prekallikrein and KLK14 were 5.1 times and by 50.1% lower, respectively, than reported in the skin in the same mice, and the level of KLK1 remained high and did not differ from the indicators in the skin of the same animals and the animals of the control group. In the similar group of the uPA - / - females, stimulation of the melanoma growth and metastasis was also observed [5], while the direction of changes in the level of the studied indicators was different: the contents of prekallikrein, KLK1 and KLK14 were 4.2 times, by 40.7% and 2,4 times lower, respectively, than found in the skin of the uPA-/- males with CNP. In the uPA+/+ tumor tissue, the level of prekallikrein did not differ from its level in the skin unaffected by the tumor growth and was 5.4 times lower than in the skin of the uPA-/- males with CNP. The content of KLK1 and KLK14 was lower by 33.3% and 5.1 times, respectively, than in the skin of the same animals and significantly lower than in the skin of uPA-/- males with CNP. The above changes were generally similar to what was observed in the skin and the tumor tissue in the uPA-/- females in the corresponding group, except for the content of KLK1.

Discussion and conclusions

The skin contains several members of this family, namely KLK1, KLK5, KLK7, KLK8, KLK11 and KLK14, which, in particular, are involved in the skin desquamation, inflammation, immune response, keratinocyte differentiation and wound healing. An analysis of the gene expression showed that the most expressed KLKs in the skin are KLK1 and KLK11, and KLK14 is the most skin-specific type [15,16]. The peculiarity of KLKs is that the members of this family function in the proteolytic cascade of self- and mutual activation and differ in a significant variety of substrates, which contributes to signal amplification due to positive feedbacks and adds complexity to the tissue proteolytic network [16].

So far the KLK1 tissue kallikrein is the only member in the above mentioned family that shows the substantial kininogenase activity in vitro and in vivo [17], so that as a result the pro-inflammatory kinin peptides are formed in the skin from kininogens, which are recognized as endogenous algogens [18]. In addition to KLK1, decomposition of kininogens and the release of bradykinin are performed by the plasma protease kallikrein, the precursor of which is prekallikrein, which is synthesized not only by hepatocytes, but also in other tissues, which may indicate the specific cellular functions of this enzyme [19]. The interaction of kinins and their metabolites with B1R or B2R receptors causes allodynia, hyperalgesia in acute and chronic pain models, activates cascade signaling systems (ERK1/2, PKC, cFos, NF-KB, PI3K/Akt, and transactivates the epidermal growth factor receptor (EGFR) and production of pro-inflammatory cytokines [12,13]. These data explain the results of our study, which shows that the level of prekallikrein in the skin was substantially increased in mice in the state of CNP, and the KLK1 level increased in males, while in females the level was reduced. Such peculiarities may be related to the identified gender differences in the processes and the signaling pathways activated during pain chronization [20,21]. The results presented in our work showed a change in the contents of KLK1 and KLK14 in the skin of mice unaffected by the malignant growth, and in this case, the gender differences should draw attention. It is known that KLKs gene expression is regulated by steroid hormones, including androgens, estrogens, and progestins, and synergistic hormonal regulation of KLKs gene transcription is identified as the main cause of dysregulation of their expression [22].

Activation of the signaling pathways that function at different stages of carcinogenesis under the conditions of chronic pain explains the formation of the

phenotype of the activation of the tumor growth and metastasis in mice. However, activation of some specific intracellular pathways depends both on the stimulus and the biological effect specific to each cell type. By initiating the specific intracellular signaling pathways, kinin peptides and various KLKs are involved not only in processes that spread inflammation and pain, but also participate as comitogenes in cell proliferation, may cause growth arrest, activation of keratinocyte differentiation and migration, remodeling of the extracellular matrix, regulate vascular permeability, and modulate angiogenesis [16,17,23].

In our study, 3 weeks of melanoma growth, increased levels of KLK1 were observed in C57BL/6 mice of both genders, and in the females recorded was also an elevated concentration of KLK14 in the tumor tissue both during normal growth and during activation of growth and metastasis by pain, and in the latter case the changes were more pronounced.

Many KLKs are known to be involved in tumor progression, especially in hormone-dependent cancers [24]. Strong correlations between the KLKs expression and the degree of aggressiveness of human melanoma, in particular with cell migration and depth of invasion, have been established [25]. Interestingly, the expression of each KLK is regulated differentially depending on the specific type of cancer, a significant increase in the expression in some KLK and a decrease in others are observed [11]. KLK1 (like a number of other KLKs) participates in the regulation of the tumor growth by increasing the level of the active form of epidermal growth factor EGF and activating its EGFR receptor and ERK1/2 cascade, as well as through degradation of the protein binding insulin-like growth factors (IGFsBP), providing their release [26]. In addition, the increased KLK1 expression mediates the proinflammatory pathway through activation of protease-activated receptors (PAR) [27]. Activation of PAR1 and subsequent transactivation of EGFR lead to enhanced wound healing in rats, as well as migration and invasion of prostate cancer cells [28]. In this case, cell proliferation was stimulated through the kinin B2R receptor. In other words, the signaling pathways, which are stimulated by KLK1 during physiological proliferation and during migration/invasion of cancer cells, are different [11,22].

Currently, the physiological role of KLK14 is not clearly defined, although dysregulation of its expression has been revealed in various localization of the malignant process. Participation of KLK14 in the regulation of skin desquamation and desmoglein degradation involves it in the processes of intercellular adhesion of tumor cells, and, consequently, in the process of invasion and metastasis [15]. Most of the identified KLK14 substrates are involved in the regulation of cell adhesion, migration and morphology, regulate the androgen-independent growth and metastasis of prostate cancer cells, participate in the modulation of the extracellular matrix and control the activity of other proteases, in particular matriptase, which has been reported to be an important mechanism of invasion [29].

Another important actor in the degradation of the extracellular matrix and regulation of migration/invasion of tumor cells is the uPA/uPAR urokinase-type plasminogen activator system, which regulates cell proliferation, survival and adhesion involving multiple cell receptors. The uPAR receptor is involved in all these processes, which can also occur in the absence of proteolytically active uPA [30]. In addition to the membrane-bound form of the receptor, another active form, a soluble suPAR, the effects of which do not depend on the presence of urokinase, was detected [31].

In the present study, mice with urokinase gene knockout showed rather initially recorded higher levels of kallikreins and prekallikrein in the skin, with males having the particularly elevated KLK1 concentration and females having the KLK14 elevated level. It allows suggesting that in case of urokinase deficiency in the organism, compensatory changes occur in the system of tissue kallikreins. It is also noteworthy that the direction of changes in the concentrations of the KKS components in the UPA-/- mice varies both when the growth of melanoma is inhibited (female mice from the comparison group) and when growth is stimulated in the state of CNP (females and males) in comparison with changes in the uPA+/+mice. In this case, the tendencies in the tumor tissue with normal urokinase, also demonstrate changes. Interestingly, among the many substrates of KLKs, components of the urokinase system are found, the activity of which can be modulated by tissue kallikreins, decomposing pro-uPA and its uPAR receptor. A number of KLKs have the ability to cleave the D1 domain from the receptor molecule, regulating, in particular, its adhesive function [10,32]. In addition, it has been found that in some cases KLKs can also demonstrate antitumor effects, inhibiting proliferation, invasion, and angiogenesis, which highlights the possible dependence of

the action of these enzymes on the type of tumor cells and the metabolic state of their environment [11,33].

Thus, chronic neurogenic pain contributes to changes in the metabolism of KKS during inhibition and activation of the tumor growth and metastasis that has gender specificity. The functions of the skin's KKS components may change against the background of chronic neurogenic pain, not only in the normal organism, but also in an organism with a deficit of components of other proteolytic systems (for example, urokinase), which may have a compensatory character. A malignant tumor adapts the performance of its kallikrein-kinin system depending on the metabolic characteristics of the tumor-bearer organism. The complex interaction of various external and internal factors in the organism leads to a dynamic change in the functioning of the proteolytic network and ultimately affects the phenotypic manifestations, namely inhibition/activation of the tumor growth and metastasis.

Abbreviations

EGF – epidermal growth factor
EGFR – epidermal growth factor receptor
KKS – kallikrein-kinin system
KLKs – kallikrein-related peptidases
KLK1 – kallikrein 1
KLK14 – kallikrein 14
PAR – protease-activated receptors
PAR1 – protease-activated type 1 receptor
tPA – tissue plasminogen activator
uPA – urokinase-type plasminogen activator
uPAR – urokinase-type plasminogen activator receptor
uPA+ / + female / male mice line C57BL/6
uPA - / - females/males - mice line C57BL/6-Plautm1.1BugThisPlauGFDhu/GFDhu (the urokinase knockout line)
CNP – chronic neuropathic pain

Statement on ethical issues

Research involving people and/or animals is in full compliance with current national and international ethical standards.

Conflict of interest

None declared.

Author contributions

The authors read the ICMJE criteria for authorship and approved the final manuscript.

References

1. Yakhno NN, Kukushkin ML. Chronic pain: medico-biologic and sotsio-economic aspects. Annals of the Russian academy of medical sciences. 2012;67(9):54-8. [in Russian]
2. Price TJ, Ray PR. Recent advances toward understanding the mysteries of the acute to chronic pain transition. Current Opinion in Physiology. 2019; (11):42-50. https://doi.org/10.1016/j.cophys.2019.05.015
3. Kit OI, et al. Some mechanisms of increasing malignancy of B16/F10 melanoma in female mice with chronic pain. Russian Journal of Pain. 2017;2(53):14-20. [in Russian]
4. Kit OI, et al. Influence of chronic neuropathic pain on the course of malignant B16/F10 melanoma in male mice. University news. North-Caucasian region. Natural sciences series. 2019;1(201):106-11. [in Russian]
5. Frantsiyants EM, et al. Effect of urokinase gene-knockout on growth of melanoma in experiment. Siberian Scientific Medical Journal. 2019;39(4):62-70. https://doi.org/10.15372/SSMJ20190408 [in Russian]
6. Almholt K, et al. Reduced metastasis of transgenic mammary cancer in urokinase-deficient mice. Int J Cancer. 2005;113(4):525-32. https://doi.org/10.1002/ijc.20631.
7. Breuss JM, Uhrin P. VEGF-initiated angiogenesis and the uPA/uPAR system. Cell Adh. Migr. 2012;6(6),535-540. https://dx.doi.org/10.4161%2Fcam.22243
8. Dergilev KV, et al. Multifaced Roles of the Urokinase System in the Regulation of Stem Cell Niches. Acta naturae. 2018;10(4):19-32.
9. Lund LR, et al. Plasminogen activation independent of uPA and tPA maintains wound healing in gene-deficient mice. EMBO J. 2006; 25(12):2686-2697. https://doi.org/10.1038/sj.emboj.7601173
10. Dong Y, et al. Activation of membrane-bound proteins and receptor systems: a link between tissue kallikrein and the KLK-related peptidases. Biol. Chem. 2014; 395(9): 977–90. https://doi.org/10.1515/hsz-2014-0147
11. Kryza T, et al. The kallikrein-related peptidase family: dysregulation and functions during cancer progression. Biochimie. 2016; 122:283-99. https://doi.org/10.1016/j.biochi.2015.09.002
12. Yam MF, et al. General Pathways of Pain Sensation and the Major Neurotransmitters Involved in Pain Regulation. Int J Mol Sci. 2018; 19(8):2164. https://doi.org/10.3390/ijms19082164

13. Brusco I, et al. Kinins and their B1 and B2 receptors are involved in fibromyalgia-like pain symptoms in mice. Biochemical Pharmacology. 2019; 168: 119-32. https://doi.org/10.1016/j.bcp.2019.06.023
14. Kit OI, et al. Influence of neurogenic chronic pain on indicators of kallikreinkinin system in skin of female mice in dynamics of B16/F10 melanoma development. Medical Herald of the South of Russia. 2018; 9(2):51-60. https://doi.org/10.21886/2219-8075-2018-9-2-51-60 [in Russian]
15. Borgoño CA, et al. Expression and functional characterization of the cancer-related serine protease, human tissue kallikrein 14. J. Biol. Chem. 2007; 282: 2405-22. https://doi.org/10.1074/jbc.M608348200
16. Nauroy P, Nyström A. Kallikreins: Essential epidermal messengers for regulation of the skin microenvironment during homeostasis, repair and disease. Matrix Biology Plus. 2020; 6–7: 100019. https://doi.org/10.1016/j.mbplus.2019.100019
17. Matus CE, Bhoola KD, Figueroa CD. Kinin B1 Receptor Signaling in Skin Homeostasis and Wound Healing. Yale J Biol Med. 2020;93(1):175-85.
18. Koumandou VL, Scorilas A. Evolution of the plasma and tissue kallikreins, and their alternative splicing isoforms. PLoS One. 2013; 8(7): e68074.
19. Fink E, et al. Cellular expression of plasma prekallikrein in human tissues. Biol Chem. 2007; 388(9):957-63. https://doi.org/10.1515/BC.2007.104
20. Mogil JS. Sex differences in pain and pain inhibition: multiple explanations of a controversial phenomenon. Nat Rev Neurosci. 2012;13(12):859-66. https://doi.org/10.1038/nrn3360
21. Mapplebeck JC, Beggs S, Salter MW. Sex differences in pain: a tale of two immune cells. Pain. 2016;157 Suppl 1:S2-6. https://doi.org/10.1097/j.pain.0000000000000389
22. Kalinska M, et al. Kallikreins–the melting pot of activity and function. Biochimie. 2016; 122:270-82. https://doi.org/10.1016/j.biochi.2015.09.023
23. Pavlopoulou A, et al. Evolutionary History of Tissue Kallikreins. PLoS One. 2010; 5(11): e13781. https://doi.org/10.1371/journal.pone.0013781
24. Fuhrman-Luck RA, et al. Proteomic and other analyses to determine the functional consequences of deregulated kallikrein-related peptidase (KLK) expression in prostate and ovarian cancer. Proteom. Clin. Appl. 2014; 8(5–6): 403-15. https://doi.org/10.1002/prca.201300098
25. Delaunay T, et al. Aberrant expression of kallikrein-related peptidase 7 is correlated with human melanoma aggressiveness by stimulating cell migration and invasion. Mol Oncol. 2017;11(10):1330-47. https://doi.org/10.1002/1878-0261.12103
26. Stefanini AC, da Cunha BR, Henrique T, Tajara EH. Involvement of Kallikrein-Related Peptidases in Normal and Pathologic Processes. Dis Markers. 2015;2015:946572. https://doi.org/10.1155/2015/946572
27. Yiu WH, et al. Tissue kallikrein mediates pro-inflammatory pathways and activation of protease-activated receptor-4 in proximal tubular epithelial cells. PloS One. 2014; 9(2): e88894. https://doi.org/10.1371/journal.pone.0088894
28. Gao L, Chao L, Chao J. A novel signaling pathway of tissue kallikrein in promoting keratinocyte migration: activation of proteinase-activated receptor 1 and epidermal growth factor receptor. Exp. Cell Res. 2010;316(3): 376-89. https://doi.org/10.1016/j.yexcr.2009.10.022
29. Kryza T, et al. The molecular function of kallikrein-related peptidase 14 demonstrates a key modulatory role in advanced prostate cancer. Mol Oncol. 2020;14(1):105-28. https://doi.org/10.1002/1878-0261.12587
30. Smith HW, Marshall CJ. Regulation of cell signalling by uPAR. Nat. Rev. Mol. Cell. Biol. 2010; 11(1):23–36. https://doi.org/10.1038/nrm2821
31. Klimovich PS, Semina EV. Mechanisms of Participation of the Urokinase Receptor in Directed Axonal Growth. Mol. Biol. 2020; 54: 89–98. https://doi.org/10.1134/S0026893320010094
32. Beaufort N, et al. Interplay of human tissue kallikrein 4 (hK4) with the plasminogen activation system: hK4 regulates the structure and functions of the urokinase-type plasminogen activator receptor (uPAR). Biological Chemistry. 2006; 387(2):217-22.
33. Figueroa CD, Molina L, Bhoola KD, Ehrenfeld P. Overview of tissue kallikrein and kallikrein-related peptidases in breast cancer. Biol Chem. 2018;399(9):937-57. https://doi.org/10.1515/hsz-2018-0111