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Role of the ubiquitin-proteasome system in the progression of oral squamous cell carcinoma

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ABSTRACT

The ubiquitin-proteasome system (UPS) controls the activity, subcellular localization, and stability of many cellular proteins that affect cellular homeostasis by regulating multiple signaling cascades. The activity of this system is associated with the emergence and progression of oral squamous cell carcinoma, since specific proteolysis of most intracellular proteins involved in the pathogenesis of cancer is implemented by this system.

The review article presents data on the characteristics of proteasomes and the process of substrate protein ubiquitination. The role of the ubiquitin-proteasome system in the pathogenesis of oral squamous cell carcinoma is shown, and the prospects of its use in precancerous diseases are described. The literature search was carried out in the search engines Medline, eLIBRARY, Scopus, The Cochrane Library, and RSCI.

Key words: ubiquitin-proteasome system, oral squamous cell carcinoma, proteasome, pathogenesis.

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Роль убиквитин-протеасомной системы в развитии плоскоклеточного рака полости рта

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РЕЗЮМЕ

Убиквитин-протеасомная система контролирует активность, субклеточную локализацию и стабильность множества клеточных белков, которые влияют на клеточный гомеостаз посредством регуляции сигнальных каскадов. Активность данной системы связана с возникновением и прогрессированием плоскоклеточного рака полости рта, так как специфический протеолиз большинства внутриклеточных протеинов, участвующих в патогенезе рака, происходит с помощью вышеупомянутой системы.

В обзорной статье представлены данные о характеристике протеасом и процессе убиквинтирования белков-субстратов. Показана роль убиквитин-протеасомной системы в патогенезе плоскоклеточного рака полости рта, приведены сведения о перспективах использования ее при предраке. Поиск литературы осуществлялся в поисковых системах Medline, Elibrary, Scopus, The Cochrane Library, РИНЦ.

Ключевые слова: убиквитин-протеасомная система, плоскоклеточный рак полости рта, протеасома, патогенез.

Конфликт интересов. Авторы декларируют отсутствие явных и потенциальных конфликтов интересов, связанных с публикацией настоящей статьи.

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INTRODUCTION

Oral squamous cell carcinoma (OSCC) is characterized by high mortality, early metastases, relapses, and a delay in seeking medical care in specialized institutions. The leading causes of death in patients with OSCC are metastases to the regional cervical lymph nodes and disease relapses. Doctors usually look at the prevalence of the tumor process to predict the course of the disease and choose an approach to treatment, however, the relationship between the prevalence of the tumor and the outcome of the disease and the treatment effectiveness is not always traced [1].

In some cases, clinical and morphological criteria are not very informative. According to statistics, about 25% of patients have latent metastases to regional lymph nodes at initial stages of the malignant process. The search for informative and reliable markers of squamous cell carcinoma is important for improving the prognosis and treatment of OSCC patients. [2]. Currently, the scientific literature describes many proteins that are involved in the pathogenesis of OSCC and control induction of angiogenesis, apoptosis, and metastasis. The ubiquitin-proteasome system (UBS) carries out specific proteolysis of most of these peptides [3].

CHARACTERISTICS OF THE UBIQUITIN-PROTEASOME SYSTEM

The ubiquitin-proteasome system (UPS) generates regulatory peptides, activates precursor proteins, provides intracellular protein hydrolysis, and is involved in preparing peptides for the class I major histocompatibility complex (MHC-1) [4]. The main components of UPS are proteasomes, ubiquitin molecules, and enzymes that activate and transport ubiquitin. The functional unit of this system is the proteasome.

Proteasomes are the main non-lysosomal multi-subunit proteases of eukaryotes; they hydrolyze up to 90% of cellular proteins. Proteasomes are multicatalytic complexes containing a cylinder-shaped 20S core particle, which consists of four heteroheptameric rings [5]. The two inner β-rings contain six proteolytic centers where substrates are cleaved; each ring has caspase-like (β1), trypsin-like (β2), and chymotrypsin-like (β5) activity [6].

The β-subunits (β1, β2, and β5) of the 20S proteasome particle can be completely or partially replaced by the immunosubunits LMP7 (β5i), LMP2 (β1i), and MECL1 (β2i), resulting in the immunoproteasome formation [7]. The main role of the immunoproteasome is to process antigens for presentation on MHC-

1 molecules. The immunoproteasome has higher chymotrypsin and trypsin activity and lower caspase activity than the standard 20S proteasome, which leads to alternative protein cleavage [8].

The two outer rings are composed of α-subunits that act as gatekeepers, controlling the access of substrates to the catalytically active β-chamber.

Proteasomes are not static complexes, and their activity can be modulated by binding of various proteasome activators (PAs), such as 19S, PA28, and PA200.

These proteasome regulators can symmetrically and asymmetrically bind to the α-rings of the 20S nucleus, forming proteasomes with a single or double cap. The binding of α-rings to regulatory particles leads to an increase in the proteasome activity by many times. Nevertheless, the free 20S proteasome unit remains a very common conformation in cells [9–12].

There are two types of proteasomes: 26S and 20S. The main hydrolyzing 26S proteasome consists of two subcomplexes: a catalytic 20S core particle and one or two 19S regulatory particles, which act as proteasome activators with a molecular weight of approximately 700 kDa (PA700). The 19S subcomplex recognizes ubiquitinated proteins, unfolds them, and moves inside the 20S core particle [13–15]. The immune forms of the 26S proteasome perform an important function: they produce immunogenic proteins for their further presentation by MHC-1 [15]. Regulatory particles implement specific substrate degradation. For example, if the PA28 protein complex acts as a regulatory particle, then the activated 20S proteasome will expose abnormal, small, and short-lived proteins to proteolysis [14].

UBIQUITINATION

The entry into the 20S core particle is usually closed by a regulatory particle acting as a gatekeeper. For penetration into the proteasome, the substrate protein must undergo polyubiquitination – attachment of a polyubiquitin chain (polyUb), which contains at least four monomers of ubiquitin (Ub). Then ATP-dependent activation of ubiquitin by a ubiquitin-activating enzyme (E1) and transfer of activated ubiquitin to a ubiquitin-conjugating enzyme (E2) take place, followed by formation of a peptide bond between ubiquitin and a substrate protein, catalyzed by a ubiquitin ligase (E3). The process is repeated several times in order to create a polyubiquitin chain through inter-ubiquitin bonds. During several cycles of protein ubiquitination, a build-up of a ubiquitin tag occurs, which is recognized by the 26S proteasome.

The substrate recognition by 26S proteasomes and their transfer to the proteolytic chamber occur due to the multi-subunit structure of the PA700 activator. After binding to the proteasome PA700 regulator, the ubiquitin chain is cleaved from the ubiquitinated substrate protein, the protein is unfolded and then transferred to the central chamber of the 20S proteasome, where it is degraded to short peptides, which then exit at the opposite pole of the proteasome.

When the proteolysis of the tagged molecule is complete, ubiquitin is released and tags another target. The proteasome is able to regulate both the amount and function of proteins: in some cases, the protein undergoes limited proteolysis (processing), which contributes to a significant change in the protein function (Fig. 1). Kinases, phosphatases, transcription and translation factors, cyclins, and inhibitors of cyclin-dependent kinases are processed or eliminated by the proteasome. This essential biological role of the UPS suggests that it is involved in the pathophysiology of inflammatory, viral, neurodegenerative, autoimmune, and oncological diseases [6].

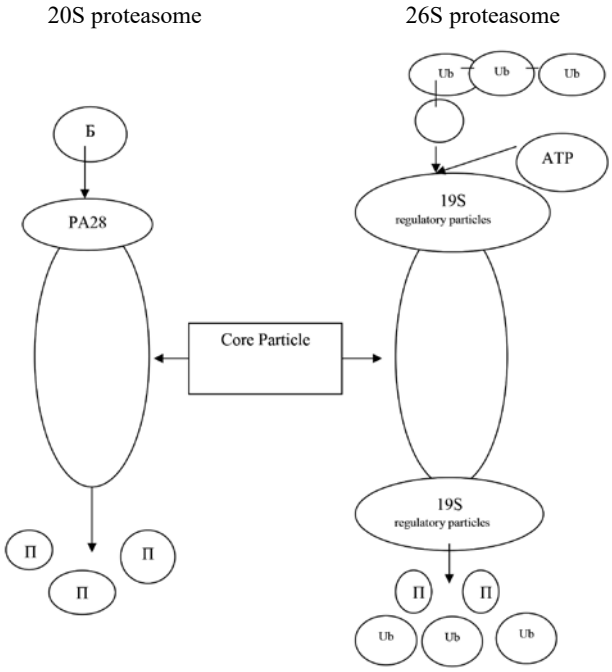

Fig. 1. Ubiquitination of proteins

CIRCULATING PROTEASOMES

Currently, circulating proteasomes are being actively investigated: the pathogenetic and prognostic value of these proteasomes, their biological signifi-

cance, and routes of exit into the extracellular space are being discussed.

Using the enzyme-linked immunosorbent assay (ELISA), 20S proteasomes were found in human serum. These proteasomes are now called circulating proteasomes or c-proteasomes. It was established that c-proteasomes are detected in the extracellular fluid of healthy people and patients with pathology [16].

According to the findings of quantitative iTRAQ-based proteomic analyses, it was revealed that the composition of the extracellular proteasome population included 19S regulatory particles and 20S core particles [17]. In addition, c-proteasomes obtained from blood plasma of healthy patients were similar in size, shape, subunit composition, and proteolytic activity to intracellular 20S proteasomes isolated from erythrocytes [18, 19]. Electron microscopy showed that purified c-proteasomes are intact 20S proteasome particles that are capable of hydrolyzing fluorogenic peptides [18, 20].

Taking into account the important role of the proteasome system in the pathogenesis of malignant neoplasms, it can be assumed that during tumor processes, proteasomes are capable of being secreted by cancer cells into the extracellular space or released into the circulation during breakdown of tumor cells [21].

Moreover, c-proteasomes can appear when the microparticles formed as a result of membrane blebbing are destroyed. This process is characterized by transfer of the contents of the plasma membrane to the membrane protrusions and subsequent formation of vesicles from the activated cells, which are microparticles of heterogeneous size (0.1–1 μm) with the corresponding content. The above-described structures, transporting various molecules, can act as messengers between cells [22, 23].

C-proteasomes can exist in a free, non-vesicular form. They are able to exist in the extracellular space, leaving exosomes. Exosomes are microscopic extracellular vesicles with a diameter of 30–100 nm, secreted by various cells and capable of carrying genetic information and protein markers, thus, being involved in intercellular communication [24]. It is believed that exosomes are involved in antigen presentation, non-classical secretion of proteins, and the pathogenesis of diseases associated with metabolic disorders, facilitate the immune response, and play a fundamental role in development of malignant tumors [25–27].

THE ROLE OF THE UPS IN THE MOLECULAR PATHOGENESIS OF OSCC

The 26S proteasomes play a significant role in the pathogenesis of malignant tumors, in particular, in the regulation of proliferation. Cyclins regulate progression of the cell through the cell cycle by sequential activation of cyclin-dependent kinases (CDK). These intracellular proteins are very unstable and exist for a short time. The number and presence of cyclins in the cell are regulated by proteasome-dependent degradation and transcription factors. UPS is involved in regulation of the stability of CDK inhibitors, as well as in hydrolysis of cyclins and their complexes [28].

The general scheme of interaction between cyclin and the 26S proteasome is the following: cyclin is polyubiquitinated and hydrolyzed by the proteasome after implementing its function, due to which the corresponding CDK becomes inactive, and the next cell cycle phase begins. For example, proteasome destruction of cyclin B leads to exit from mitosis [29]. When the cell passes through the restriction point located between the G1 phase and the S phase, proteasome-mediated destruction of cyclin A occurs. The anaphase stimulation complex (APC), which is a E3 ubiquitin ligase, ubiquitinates this cyclin [30]. The Skp1-Cul1-F-box(SCF)-containing and APC complexes are the key factors in cyclin degradation. At the same time, the SCF complex itself is regulated by APC through ubiquitination of the adapter protein Skp2, which suppresses SCF activity before the transition from the G1 phase to the S phase [31].

Studies reported that cyclin D1 is overexpressed in some primary human cancers, which confirms its role as an oncogene. In many tumors, genetic changes related to the cyclin *D1* gene often result in overexpression of the cyclin D1 protein. It was found that cyclin D1 also acts as a transcription modulator and regulates the activity of several transcription factors and histone deacetylase. The cyclin D1 protein is unstable with a short half-life of about 24 minutes. It is cleaved mainly by the 26S proteasome following the ubiquitin-dependent pathway. At the same time, cyclin D1 is an important proto-oncogene. Overexpression of cyclin D1 leads to shortening of the G1 phase and lower dependence on exogenous mitogens, which leads to abnormal cell proliferation, which, in turn, may contribute to additional genetic damage [32].

UPS plays an important role in maintaining the functional activity of cells, namely, in the regulation of the signaling systems, which are activated by inter-

action of growth factors with the corresponding receptors [33]. It was shown that proteasomes regulate the level of the transcription factor NF-κB, which is important for activation of gene expression in innate and adaptive immunity, inflammation, and stress responses. In cancer cells, NF-κB is involved in expression of the anti-apoptotic *IAP* gene family, as well as the pro-survival *BCL-2* genes [34]. Studies showed that proteasome activity in patients with head and neck cancers was higher than in the surrounding, relatively healthy tissue.

There is evidence that in patients with squamous cell carcinoma of the head and neck, the involvement of regional lymph nodes was accompanied by increased intracellular proteolysis. An increase in the total activity of proteasomes occurred along with an increase in the stage of cancer; however, a decrease in the expression of the LMP-2 proteasome subunit was observed. Changes in the expression of the transcription factor NFkappaBp50 and regression dependencies of the expression of the nuclear factor NF-kappaBp65 on the total activity of proteasomes were found [35].

The mechanisms of UPS involvement in the carcinogenesis include inhibition of vascular endothelial growth factor (VEGF)- and platelet-derived growth factor (PDGF)-mediated angiogenesis through degradation of platelet-derived growth factor receptor (PDGFR) and ubiquitination of vascular endothelial growth factor receptor (VEGFR) signaling pathway components, as well as proteasomal destruction of the α-subunit of the transcription factor HIF-1, which is impaired under hypoxic conditions. It subsequently leads to accumulation of HIF-1 in tumor cells and activation of transcription of genes involved in the angiogenesis [36, 37]. There are studies proving that proteasomes are involved in the post-translational modification of the p105 polypeptide, which is the precursor of NF-kappaBp50, which results in emergence of active forms of the transcription factor NF-kappaB. Moreover, a relationship between the level of HIF-1 production and the content of the transcription factor NF-kappaB was demonstrated. Most likely, it provides indirect involvement of NF-kappaBp50 in regulation of the VEGF level and neoangiogenesis in the tissue in head and neck squamous cell carcinomas [35]. This study showed that proteasome degradation of HEF-1 with the participation of PP-2A led to disruption of adhesive contacts with the extracellular matrix *in vitro* [37].

The UPS can play an important role in acquisition of immunity to antigrowth signals by transformed cells, degrading, along with caspases, the retinoblastoma (pRb) protein with the participation of a mouse double minute 2 (Mdm2) E3 ubiquitin ligase and destroying many components of the signaling pathway mediated by TGF-β [38]. In addition, the UPS is involved in regulation of apoptosis. Many nuclear proteins that implement programmed cell death are substrates for proteasomes: p53 tumor suppressor, transcription factors (c-Fos, c-Myc, AP-1), NFkB / IkB inhibitor, cell cycle regulators, caspase activity regulators (inhibitors of apoptosis (IAPs)), and proteins of the Bcl-2 family involved in proapoptotic signal transduction (cFLIP) [39].

UBIQUITIN-PROTEASOME SYSTEM IN OSCC

The accumulated data confirm that the UPS plays a key role in metabolism of proteins involved in regulation of many biological processes, such as cell cycle control, proliferation, apoptosis, neoangiogenesis, tumor progression, and metastasis [40].

Analysis of systematic literature reviews on PubMed (Ovid), EMBASE (Ovid), EBM (Ovid), and Web of Science (ISI) platforms by A. Villa et al. in 2018, aimed at identifying predictive biomarkers for stratification and long-term follow-up of progression of oral leukoplakia as an obligate precancer of OSCC, showed a correlation between the increased expression of genes associated with the proteasome system and a high risk of developing OSCC [41].

A study by J. Li et al. indicated that overexpression of the proteasome activator PA28γ was associated with a poor prognosis in patients with OSCC and promoted tumor progression. In addition, as a result of a study on a mouse xenograft model, it was found that the absence of PA28γ expression dramatically inhibited the growth and proliferation of cells in OSCC and slowed down tumor growth [42].

A proteomic study conducted by Z. Wang et al. to identify potential pathways for malignant transformation of oral leukoplakia into OSCC showed an increase in the expression of proteasome activators PA28a and PA28b, which confirms the clinical significance of proteasomes as a marker of early malignancy. This study demonstrated the role of proteasome degradation of proteins in the processing of intracellular antigens into peptides, which subsequently bound to MHC-1 molecules [43].

PA28γ-mediated mechanisms are of great importance for cancer therapy, especially in light of profoundly elevated levels of PA28γ in the tumor tissue. A significant increase in the level of PA28γ was ob-

served mainly in breast tumors, especially with a poor prognosis [44, 45], colorectal cancer [46], hepatocellular carcinoma [47], and OSCC [48].

The results of the studies by X. Feng et al. in 2016 showed that the activity of the proteasome activator PA28α was significantly higher in OSCC tissues compared to its activity in the healthy tissue. This study demonstrated that in immunohistochemistry, PA28α expression increased with the progression of dysplasia in the epithelium of the oral mucosa. It was found that after surgical treatment of moderately differentiated squamous cell carcinoma, no relapses occurred in the first two years. However, after radical treatment of a well-differentiated tumor, metastases to the cervical lymph nodes were diagnosed after two years, and the survival rate was four years.

The authors used reverse genetic approaches, which revealed that in oral squamous cells, along with a decrease in the PA28α expression, a consistent and statistically significant decrease in the ability to invade and migrate was observed. Invasion was reduced to 52% and migration – to 44%. Suppression of the PA28α expression led to a decrease in tumor growth of oral squamous cells *in vivo*. The volume of tumors decreased by 56% compared to tumors from the control group, while angiogenesis and apoptosis were not affected [49].

CONCLUSION

The UPS plays a key role in the pathogenesis of OSCC at the stages of malignancy onset and subsequent tumor progression. Recent studies on proteasome functioning in OSCC have demonstrated their key role in the molecular mechanisms of this disease. Further study of the proteasome system in the pathogenesis of OSCC will make it possible to find reliable markers for predicting the development of oral cancer from a precancer pathology and to assess the course of the disease.

REFERENCES

1. Choinzonov E.L., Spirina L.V., Kondakova I.V., Chizhevskaya S.Yu., Shishkin D.A., Kulbakin D.E. Prognostic value of determining the proteasome activity in the tissues affected by head and neck squamous cell carcinoma. *Bulletin of the Siberian Branch of RAS.* 2014; 34 (4): 103–108 (in Russ.).
2. Kakurina G.V., Kondakova I.V., Choinzonov E.L. Prognosis for head and neck squamous cell carcinomas. *Issues of Oncology*. 2012; 58 (1): 26–32 (in Russ.).
3. Voutsadakis I.A. Ubiquitination and the ubiquitin – proteasome system in the pathogenesis and treatment of squamous head and neck carcinoma. *Anticancer Research*. 2013; 33 (9): 3527–3542.
4. Lub S., Maes K., Menu E., Bruyne E., Vanderkerken K., Valckenborgh E. Novel strategies to target the ubiquitin proteasome system in multiple myeloma. *Oncotarget.* 2016; 7 (6): 6521–6537. DOI: 10.18632/oncotarget.6658.
5. Harshbarger W., Miller C., Diedrich C., Sacchettini J. Crystal structure of the human 20S proteasome in complex with carfilzomib. *Structure.* 2015; 23 (2): 418–424. DOI: 10.1016/j.str.2014.11.017.
6. Sorokin A.V., Kim E.R., Ovchinnikov L.P. Proteasome system of protein degradation and processing. *Advances in Biological Chemistry*. 2009; 49: 3–76 (in Russ.).
7. Sijts E.J., Kloetzel P.M. The role of the proteasome in the generation of MHC class I ligands and immune responses. *Cellular and Molecular Life Sciences.* 2011; 68 (9): 1491–1502. DOI: 10.1007/s00018-011-0657-y.
8. Guimaraes G., Gomes M., Campos P.C., Marinho F.V., de Assis N., Silveira T.N., Oliveira S.C. Immunoproteasome subunits are required for CD8+ T cell function and host resistance to brucella abortus infection in mice. *Infection and Immunity*. 2018; 86 (3): e00615–00617. DOI: 10.1128/IAI.00615-17.
9. Mao I., Liu J., Li X., Luo H. REGγ, a proteasome activator and beyond? *Cellular and Molecular Life Sciences.* 2008; 65 (24): 3971–3980. DOI: 10.1007/s00018-008-8291-z.
10. Savulescu A.F., Glickman M.H. Proteasome activator 200: the heat is on. *Molecular and Cellular Proteomics*. 2011; 10 (5): R110.006890. DOI: 10.1074/mcp.R110.006890.
11. Liu C.W., Jacobson A.D. Functions of the 19S complex in proteasomal degradation. *Trends in Biochemical Sciences.* 2013; 38 (2): 103–110. DOI: 10.1016/j.tibs.2012.11.009.
12. Cascio P. PA28αβ: the enigmatic magic ring of the proteasome? *Biomolecules.* 2014; 4 (2): 566–584. DOI: 10.3390/biom4020566.
13. Sharova N., Zakharova L. Multiple forms of proteasomes and their role in tumor fate. *Recent Patents on Endocrine, Metabolic Immune Drug Discovery*. 2008; 2: 152–161. DOI: 10.2174/187221408786241847.
14. Tanaka K. The proteasome: overview of structure and function. *The Proceedings of the Japan Academy, Series B*. 2009; 85: 12–36. DOI: 10.2183/pjab.85.12.
15. Sharova N.P., Astakhova T.M., Karpova Ya.D., Abaturova S.B., Lyupina Yu.V., Bogomyakova Yu.V., Abramova E.B., Erokhov P.A. Multiple forms of proteasomes as targets for development of new anti-cancer drugs. *Oncosurgery.* 2011; 3 (2): 37–42 (in Russ.).
16. Schliessmann S.S., Cicko S., Thomassen J., Anlasik T., Müller T., Idzko M., Zissel G., Sixt S.U. Acute smoke exposure decreases bronchial extracellular proteasome concentration. *The European Respiratory Journal*. 2011; 38: 737.
17. Zaykova Yu.Ya., Kulichkova V.A., Ermolayeva Yu.B., Bottrill A., Barlev N.A., Tsimokha A.S. Characteristics of extracellular proteasomes and proteins associated with them by iTRAQ-based mass spectrometry. *Cytology.* 2013; 55 (2): 111–122 (in Russ.).
18. Sixt S.U., Beiderlinden M., Jennissen H.P., Peters J. Extracellular proteasome in the human alveolar space: a new housekeeping enzyme? *American Journal of Physiology-Lung Cellular and Molecular Physiology*. 2007; 292 (2): 1280–1288. DOI: 10.1152/ajplung.00140.2006.

19. Bochmann I., Ebstein F., Lehmann A., Wohlschlaeger J., Sixt S.U., Kloetzel P.M., Dahlmann B. T lymphocytes export proteasomes by way of microparticles: a possible mechanism for generation of extracellular proteasomes. *Journal of Cellular and Molecular Medicine.* 2014; 18 (1): 59–68. DOI: 10.1111/jcmm.12160.
20. Zaykova Yu.Ya., Kulichkova V.A., Ermolayeva Yu.B., Gauze L.N., Tsimokha A.S. Comparative analysis of extra- and intracellular proteasomes in human K562 cell line. *Cytology*. 2011; 53 (6): 459–465 (in Russ.).
21. Van Zijl F., Krupitza G., Mikulits W. Initial steps of metastasis: cell invasion and endothelial transmigration. *Mutation Research*. 2011; 728 (1-2): 23–34. DOI: 10.1016/J.MRREV.2011.05.002.
22. Tang K., Liu J., Yang Z., Zhang B., Zhang H., Huang C., Ma J., Shen G.X., Ye D., Huang B. Microparticles mediate enzyme transfer from platelets to mast cells: a new pathway for lipoxin A4 biosynthesis. *Biochemical and Biophysical Research Communications.* 2010; 400 (3): 432–436. DOI: 10.1016/j.bbrc.2010.08.095.
23. Yuan A., Farber E.L., Rapoport A.L., Tejada D., Deniskin R., Akhmedov N.B., Farber D.B. Transfer of microRNAs by embryonic stem cell microvesicles. *PLoS One*. 2009; 4 (3): 4722. DOI: 10.1371/ journal.pone.0004722.
24. Yunusova N.V., Kondakova I.V., Kolomiets L.A., Molchanov S.V. Proteasomes and exosomes in ovarian cancer: association with the clinical course and prognosis. *Siberian Journal of Oncology.* 2014; (4): 53–59 (in Russ.).
25. Stamm T.A., Naryzhniy S.N., Landa S.B., Burdakov V.S., Artamonova T.O., Filatov M.V. Isolation and analysis of exosomes secreted by human malignant cells *in vitro*. *Cytology*. 2012; 54 (5): 430–438 (in Russ.).
26. Keller S., Konig A.-K., Marme F., Runz S., Wolterink S., Koensgen D., Mustea A., Sehouli J., Altevogt P. Systemic presence and tumor-growth promoting effect of ovarian carcinoma released exosomes. *Cancer Letters*. 2009; 278 (1): 73–81. DOI: 10.1016/j.canlet.2008.12.028.
27. Rupp A.-K., Rupp C., Keller S., Brase J.C., Ehehalt R., Fogel M., Moldenhauer G., Marmé F., Sültmann H., Altevogt P. Loss of EpCAM expression in breast cancer derived serum exosomes: role of proteolytic cleavage. *Gynecologic Oncology*. 2011; 122 (2): 437–446. DOI: 10.1016/j. ygyno.2011.04.035.
28. Tu Y., Chen C., Pan J., Xu J., Zhou Z-G., Wang C-Y. The ubiquitin proteasome pathway (UPP) in the regulation of cell cycle control and DNA damage repair and its implication in tumorigenesis. *International Journal of Clinical and Experimental Pathology*. 2012; 5 (8): 726–738.
29. Bassermann F., Eichner R., Pagano M. The ubiquitin proteasome system – implications for cell cycle control and the targeted treatment of cancer. *Biochimica et Biophysica Acta*. 2014; 1843 (1): 150–162. DOI: 10.1016/j.bbamcr.2013.02.028.
30. Borg N.A., Vishva M.D. Ubiquitin in cell-cycle regulation and dysregulation in cancer. *Annual Review of Cancer Biology.* 2017; 1: 59–77. DOI: 10.1146/annurev-cancerbio-040716-075607.
31. Skaar J.R., Pagano M. Control of cell growth by the SCF and APC/C ubiquitin ligases. *Current Opinion in Cell Biology*. 2009; 21 (6): 816–824. DOI: 10.1016/j.ceb.2009.08.004.
32. Alao J.P. The regulation of cyclin D1 degradation: roles in cancer development and the potential for therapeutic invention. *Molecular Cancer*. 2007; 6: 24. DOI: 10.1186/1476-4598-6-24.
33. Huang Q., Figueiredo-Pereira M.E. Ubiquitin/proteasome pathway impairment in neurodegeneration: therapeutic implications. *Apoptosis: an International Journal on Programmed Cell Death.* 2010; 15 (11): 1292–1311. DOI: 10.1007/s10495-010-0466-z.
34. Thibaudeau T.A., Smith D.M. A practical review of proteasome pharmacology. *Pharmacological Reviews*. 2019; 71 (2): 170–197. DOI: 10.1124/pr.117.015370.
35. Spirina L.V., Kondakova I.V., Choynzonov E.L., Chigevskaya S.Y., Shishkin D.A., Kulbakin, D.Y. Expression of vascular endothelial growth factor and transcription factors HIF-1, NF-kB expression in squamous cell carcinoma of head and neck; Association with proteasome and calpain activities. *Journal of Cancer Research and Clinical Oncology* 2013; 139 (4): 625–633 (in Russ.). DOI: 10.1007/s00432-012-1366-0.
36. Spirina L.V., Yunusova N.V., Kondakova I.V., Kolomiets L.A., Koval V.D., Chernyshova A.L., Shpileva O.V. Association of growth factors, HIF1 and NF-κB expression with proteasomes in endometrial cancer. *Molecular Biology Reports*. 2012; 39 (9): 8655–8662. DOI: 10.1007/ s11033-012-1720-y.
37. Rahimi N. The ubiquitin-proteasome system meets angiogenesis. *Molecular Cancer Therapeutics.* 2012; 11 (3): 538–548. DOI: 10.1158/1535-7163.MCT-11-0555.
38. Glasgow E., Mishra L. Transforming growth factor-beta signaling and ubiquitinators in cancer. *Endocrine-Related Cancer*. 2008; 15 (1): 59–72. DOI: 10.1677/ERC-07-0168.
39. Tsimokha A.S. Proteasomes: role in extracellular processes. *Cytology.* 2010; 52 (4): 277–300 (in Russ.).
40. Jang H.H. Regulation of protein degradation by proteasomes in cancer. *Journal of Cancer Prevention.* 2018; 23 (4): 153–161. DOI: 10.15430/JCP.2018.23.4.153.
41. Villa A., Celentano A., Glurich I., Borgnakke W.S., Jensen S.B., Peterson D.E., Delli K., Ojeda D. Vissink A., Farah C.S. World workshop on oral medicine vii: prognostic biomarkers in oral leukoplakia: a systematic review of longitudinal studies. *Oral Diseases*. 2019; 25 (Suppl. 1): 64–78. DOI: 10.1111/odi.13087.
42. Li J., Feng X., Sun C., Zeng X., Xie L., Xu H., Li T., Wang R., Xu X., Zhou X., Zhou M., Zhou Y., Dan H., Wang Z., Ji N., Deng P., Liao G., Geng N., Wang Y., Zhang D., Lin Y., Ye L., Liang X, Li L., Luo G., Jiang L., Wang Z., Chena Q. Associations between proteasomal activator PA28γ and outcome of oral squamous cell carcinoma: Evidence from cohort studies and functional analyses. *EBioMedicine*. 2015; 2 (8): 851–858. DOI: 10.1016/j.ebiom.2015.07.004.
43. Wang Z., Feng X., Liu X., Jiang L., Zeng X., Ji N., Li J., Li L., Chen Q. Involvement of potential pathways in malignant transformation from oral leukoplakia to oral squamous cell carcinoma revealed by proteomic analysis. *BMC Genomics*. 2009; 10: 383. DOI: 10.1186/1471-2164-10-383.
44. Wang X., Tu S., Tan J., Tian T., Ran L., Rodier J.F., Ren G. REG gamma: a potential marker in breast cancer and effect on

cell cycle and proliferation of breast cancer cell. *Medical Oncology*. 2011; 28 (1): 31–41. DOI: 10.1007/s12032-010-9546-8.
45. Chai F., Liang Y., Bi J., Chen L., Zhang F., Cui Y., Bian X., Jiang J. High expression of REGgamma is associated with metastasis and poor prognosis of patients with breast cancer. *International Journal of Clinical and Experimental Pathology*. 2014; 7(1): 7834–7843.
46. Arlt A., Bauer I., Schafmayer C., Tepel J., Sebens Muerkoster S., Brosch M., Roder C., Kalthoff H., Hampe J., Moyer M.P., Folsch U.R., Schäfer H. Increased proteasome subunit protein expression and proteasome activity in colon cancer relate to an enhanced activation of nuclear factor E2-related factor 2 (Nrf2). *Oncogene.* 2009; 28 (45): 3983–3996. DOI: 10.1038/onc.2009.264.
47. Kondo M., Moriishi K., Wada H., Noda T., Marubashi S., Wakasa K., Matsuura Y., Doki Y., Mori M., Nagano H. Up-regulation of nuclear PA28gamma expression in cirrhosis and hepatocellular carcinoma. *Experimental and Therapeutic Medicine*. 2012; 3 (3): 379–385. DOI: 10.3892/etm.2011.415.
48. Kontos C.K. Surrogate prognostic biomarkers in OSCC: The paradigm of PA28gamma overexpression. *EBioMedicine.* 2015; 2 (8): 784–785. DOI: 10.1016/j.ebiom.2015.07.032.
49. Xiaodong F., Yuchen J., Liang X., Lu J., Jing L., Chongkui S., Hao X., Ruinan W., Min Z., Yu Z., Dan H., Zhiyong W., Ning J., Peng D., Ga L., Ning G., Yun W., Dunfang Z., Yunfeng L., Qianming C. Overexpression of proteasomal activator PA28α serves as a prognostic factor in oral squamous cell carcinoma. *Journal of Experimental and Clinical Cancer Research*. 2016; 35: 35. DOI: 10.1186/s13046-016-0309-z.

Authors contribution

Mikhalev D.E. – conception and design, drafting of the manuscript. Baydik O.D. – conception and design, analysis of the article, critical revision of the manuscript for important intellectual content. Kondakova I.V., Sidenko E.A., Mukhamedov M.R., Sysolyatin P.G. – analysis of the article, critical revision of the manuscript for important intellectual content.

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