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The mRNA expression levels of calpains and their activity in malignant and dysplastic epithelium of the upper respiratory tract

Sidenko E.A., Kakurina G.V., Cheremisina O.V., Spirina L.V., Shashova E.E., Korshunov D.A., Kondakova I.V.

Cancer Research Institute, Tomsk National Research Medical Center of Russian Academy of the Sciences (TNRMC RAS)
5, Kooperativny Str., 634050, Tomsk, Russian Federation

ABSTRACT

Background. The calpain proteolytic system plays an important role in the development of cancer. Detection of early cancer in the upper respiratory tract is often challenging, as symptoms are largely non-specific, and most cases are diagnosed at an advanced stage.

Aim. To identify candidate markers of transition from premalignant lesions to invasive carcinoma, we studied mRNA expression levels of CAPN1 and CAPN2 and the total activity of calpains in the tumor tissues of patients with head and neck squamous cell carcinoma (HNSCC) and in the epithelial dysplasia-affected tissues of patients with chronic diseases of the upper respiratory tract.

Materials and methods. The study included 32 patients with HNSCC (T1-3N0-1M0) and 12 patients with chronic diseases of the upper respiratory system associated with epithelial dysplasia. The expression levels of CAPN1 and CAPN2 were assessed using real-time polymerase chain reaction (PCR). The calpain activity was determined by hydrolysis of the fluorogenic Suc-LLVY-AMC oligopeptide.

Results. The mRNA expression levels of CAPN1 and CAPN2 were, respectively, 3 and 4 times higher in the tumor tissue of patients with HNSCC than in the tissue of patients with endothelial dysplasia in the upper respiratory tract. The level of calpain activity was 4.4 times higher in patients with HNSCC than in patients with epithelial dysplasia of different severity.

Conclusion. The elevated mRNA expression levels of CAPN1 and CAPN2 and their activity in the tumor tissues of patients with HNSCC compared to patients with chronic respiratory diseases associated with epithelial dysplasia are likely to characterize a high potential for transition from precancerous lesion to cancer. To clarify the role of calpains in the carcinogenesis of HNSCC, further studies of intact tissues using animal models are required.

Key words: head and neck squamous cell carcinoma, epithelial dysplasia, calpain activity, mRNA expression of CAPN1 and CAPN2.

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✉ *Sidenko Evgeniya A.,* e-mail: sidenkoevgeniyaaleksandrovna@gmail.com.

Экспрессия мРНК кальпаинов и их активность в злокачественном и диспластически измененном эпителии верхних дыхательных путей

Сиденко Е.А., Какурина Г.В., Черемисина О.В., Спирина Л.В., Шашова Е.Е., Коршунов Д.А., Кондакова И.В.

Научно-исследовательский институт ((НИИ) онкологии, Томский национальный исследовательский медицинский центр (НИМЦ) Российской академии наук
Россия, 634050, г. Томск, пер. Кооперативный, 5

РЕЗЮМЕ

Актуальность. Кальпаиновая протеолитическая система играет важную роль в развитии злокачественных опухолей. В настоящее время диагностика рака верхних дыхательных путей на ранних стадиях проводится редко, заболевание часто характеризуется запущенностью опухолевого процесса.

С **целью** поиска кандидатных маркеров перехода предопухолевых заболеваний в злокачественные изучали уровни экспрессии мРНК *CAPN1*, *CAPN2* и общей активности кальпаинов в опухолевой ткани пациентов с плоскоклеточным раком головы и шеи (ПРГШ) и в диспластически измененном эпителии верхних дыхательных путей.

Материалы и методы. В исследование были включены 32 пациента с ПРГШ ($T_{1-3}N_{0-1}M_0$), группу больных с предопухолевой патологией составили 12 пациентов с хроническими заболеваниями верхних отделов дыхательной системы, ассоциированными с диспластическими изменениями эпителия различной степени. Уровень экспрессии мРНК *CAPN1* и *CAPN2* оценивался с помощью полимеразной цепной реакции в режиме реального времени. Активность кальпаинов определяли по гидролизу флуорогенного олигопептида Suc-LLVY-AMC.

Полученные **результаты** показали увеличение уровня экспрессии мРНК *CAPN1* и *CAPN2* (в 3 и 4 раза соответственно) в опухолевой ткани у пациентов с ПРГШ в сравнении с диспластически измененным эпителием верхних дыхательных путей. Также отмечен высокий уровень активности кальпаинов у больных ПРГШ, который в 4,4 раза превышал показатели, полученные для пациентов с диспластическими изменениями эпителия различной степени.

Заключение. Вероятно, увеличение уровня мРНК *CAPN1* и *CAPN2* и общей активности кальпаинов в опухолевых тканях пациентов с ПРГШ в сравнении с пациентами с хроническими заболеваниями, ассоциированными с диспластическими изменениями различной степени, может играть важную роль в процессе перехода предрака в рак. Для полного установления роли кальпаинов в канцерогенезе ПРГШ необходимо дальнейшее проведение подобных исследований в интактной ткани, что возможно только на экспериментальных моделях.

Ключевые слова: плоскоклеточный рак головы и шеи, дисплазия эпителия, активность кальпаинов, экспрессия мРНК *CAPN1* и *CAPN2.*

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INTRODUCTION

Head and neck squamous cell carcinoma (HNSCC) is the sixth most common malignancy worldwide, with more than 500,000 new cases diagnosed each year [1, 2]. Most head and neck squamous cell carcinomas arise from the mucosal surfaces of the larynx and hypopharynx [3]. Despite the availability of visual and instrumental studies, diagnosis of the upper respiratory tract cancer at early stages remains challenging, as symptoms are largely non-specific, and most cases are diagnosed at an advanced stage [4]. The mechanism of epithelial dysplasia progression to squamous cell carcinoma is not well understood. This mechanism is thought to represent a stepwise process in which genetic damage is followed by morphological changes in squamous epithelium [5]. The presence of dysplasia in the mucous membrane of the larynx, laryngopharynx, and oropharynx indicates the increased risk of developing cancer [6].

Proteolytic systems that can regulate many molecular and cellular processes have a great influence on tumor transformation. The calpain system, which is involved in cancer development and progression, is an important system of specific intracellular proteolysis [7]. Calpains are cytoplasmic cysteine proteases exhibiting Ca^{2+}-dependent proteolytic activity. Proteolysis implemented by calpains is partial; it does not degrade protein but only changes its structure. Therefore, they are called "modulating proteases" [8].

In the calpain family, there are ubiquitously expressed isoforms, such as μ-calpain (calpain 1) and m-calpain (calpain 2), and tissue-specific isoforms, such as calpain 9, which is found in the digestive tract [9]. Although many of the functions of calpains and mechanisms controlling proteolytic activity remain to be analyzed, experimental studies demonstrated the apparent role of calpains in a number of important cellular processes, including proliferation, differentiation, DNA repair, and apoptosis [10, 11]. Moreover, calpains play an essential role in cancer progression [12–14].

Despite the active study of the calpain system, there are currently not enough data showing the changes in the expression level and activity of calpains in patients with HNSCC and dysplasia-associated chronic diseases. Therefore, the aim of the study was to assess mRNA expression levels of CAPN1 and CAPN2 and their activity in the tumor tissues of patients with HNSCC ($T_{1-3}N_{0-1}M_0$) and in the epithelial dysplasia-affected tissues of patients with chronic diseases of the upper respiratory tract.

MATERIALS AND METHODS

The study included 32 patients with HNSCC ($T_{1-3}N_{0-1}M_0$) and 12 patients with chronic respiratory disease associated with histologically verified epithelial dysplasia (DI-II), who were treated at the Department of Head and Neck Cancer of the Cancer Research Institute (TNRMC RAS, Tomsk, Russia). HNSCC was histologically verified in all patients, who had not previously received any special treatment. The average age of the patients was (56.3 ± 7.2) years.

The study was carried out in compliance with the principles of voluntariness and confidentiality in accordance with the "Fundamentals of the legislation of the Russian Federation on the protection of citizens' health" (Decree of the President of the Russian Federation No. 2288 of 24.12.1993). The permission of the Biomedical Ethics Committee of the Institute was obtained.

Biopsy samples of both cancerous and healthy tissues obtained during videolaryngoscopy served as a study material. The expression levels of calpains (CAPN1, CAPN2) were analyzed using the real-time PCR (RT-PCR) with the intercalating dye SYBR Green I (BioMaster HS-qPCR SYBR Blue (2×); Biolabmix, Novosibirsk). The total RNA pool was isolated from the tissue samples using the LIRA reagent (Biolabmix, Novosibirsk). The concentration and quality of the isolated RNA were evaluated using a NanoDrop 2000C spectrophotometer (ThermoScientific, USA). To obtain cDNA from mRNA, a reverse transcription reaction was performed using the OT M-MuLV-RH reaction mix (Biolabmix, Novosibirsk). Primers for RT-PCR were selected using specialized programs Vector NTI Advance 11.5 and the NCBI database (Table 1).

The expression levels of the target genes were calculated using the $2\Delta\Delta Ct$ equation [15] and expressed in arbitrary units. The housekeeping gene of the GAPDH enzyme was used as the reference gene, and the expression level of each target gene was normalized with respect to the expression of GAPDH.

Table 1

The sequence of the studied gene primers

Gene	Primers
CAPN1 NM_001198868.2	F 5′- AGAGCCTGGGTTACAAG -3′ R 5′- TGTCGTTGAGAGTGAGG -3′
CAPN2 NM_001146068.1	F 5′- ATGCTAGATTCGGACGGGAG-3′ R 5′- TGGAGTTGACAGGGCATCTT-3′
GAPDH NM_001256799.3	F 5′- GGAAGTCAGGTGGAGCGA-3′ R 5′-GCAACAATATCCACTTTACCAGA-3′

Note. NM – RNA sequence number in the National Center for Biotechnology Information (NCBI); F – forward primer; R – reverse primer.

The calpain activity was determined in clarified tissue homogenates by hydrolysis of the fluorogenic Suc-LLVY-AMC oligopeptide (Sigma, USA). The reaction mix containing 3mM Suc-LLVY-AMC and 5 µl supernatant was incubated at 25 °C for 30 min in the presence or absence of 10 mM $CaCl_2$ and 5 mM N-acetyl-Leu-Leu-norleucinal inhibitor (Sigma, USA). The resulting product was recorded with the Hitachi-850 fluorimeter (Japan) at an excitation wavelength of 380 nm and emission of 440 nm. The calpain activity was determined in samples with 10 mM $CaCl_2$ and with an inhibitor. The unit of activity was the amount of the enzyme at which 1 nmol of Suc-LLVY-AMC is hydrolyzed for 1 min. The specific activity was expressed in units of activity per 1 mg of protein. The protein content was determined by the Lowry method.

For statistical analysis, the Statistica 10.0 software package was used. The results shown in the table are presented as the median with the interquartile range *Me* (Q_1–Q_3). Using the Kruskal — Wallis test, statistically significant differences were found between the groups under investigation. For further pairwise comparison, the nonparametric Mann – Whitney test for multiple comparisons (with Bonferroni correction) was applied. The differences were considered statistically significant at $p < 0.05$.

RESULTS AND DISCUSSION

A significant difference in the mRNA expression levels of CAPN1 and CAPN2 between patients with epithelial dysplasia (DI-II) and patients with HNSCC ($T_{1-3}N_{0-1}M_0$) was found (Table 2). CAPN1 and CAPN2 expression levels were, respectively, 3 and 4 times higher in the tumor tissue of patients with HNSCC than in the dysplastic epithelium of the upper respiratory tract. In the tumor tissues of patients with stages $T_2N_{0-1}M_0$ and $T_3N_{0-1}M_0$, the CAPN1 expression level was, respectively, 2 and 4 times higher than that observed in patients with epithelial dysplasia (DI-II). The CAPN2 expression level was 4 times higher in patients with stages $T_1N_{0-1}M_0$ and $T_3N_{0-1}M_0$ than in patients with epithelial dysplasia of varying degree. The highest expression levels for both CAPN1 and CAPN2 were observed in patients with stage $T_3N_{0-1}M_0$. The CAPN1 level increased along with the tumor size. It should be noted that in the malignant epithelium of the upper respiratory tract, the expression level of CAPN2 was higher than that of CAPN1.

The findings of our study are consistent with other studies that indicate that the components of the calpain system are involved in the pathogenesis of head and neck tumors [10, 16, 17]. Calpains are implicated in processes crucial for cancer development, such as impaired intercellular adhesion, actin cytoskeletal rearrangement, morphological transformation, and cell migration, since calpains degrade proteins involved in these processes [8, 10].

Table 2

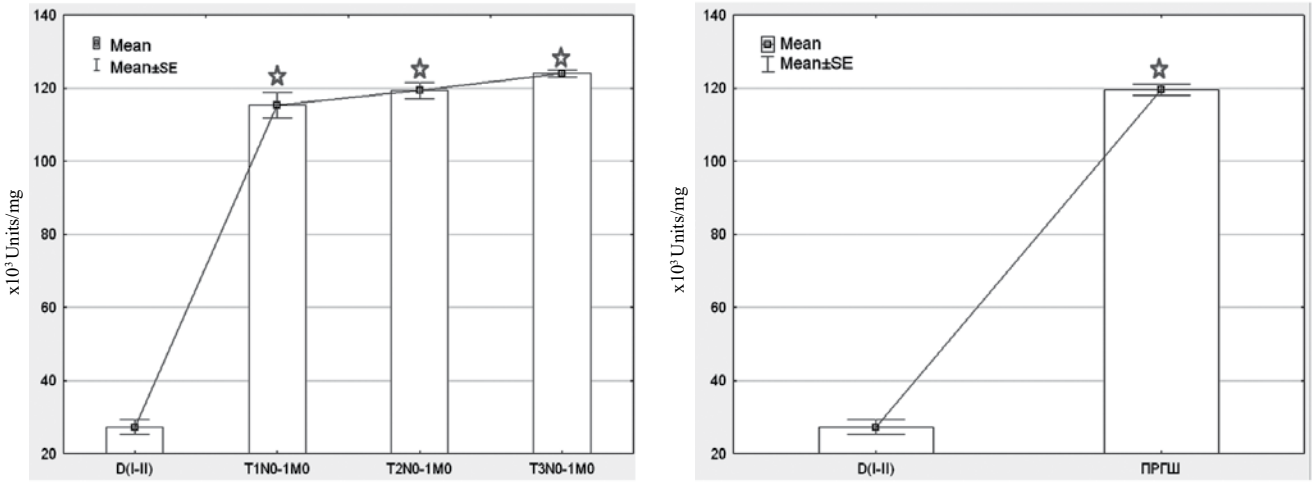
mRNA expression levels of *CAPN1* and *CAPN2* in the tissues of malignant and dysplastic epithelium of the upper respiratory tract

Group	Expression level, conventional units	
	CAPN1	CAPN2
Epithelial dysplasia (DI-II), $n = 12$	0.5 (0.08–0.86)	0.5 (0.06–0.95)
HNSCC ($T_{1-3}N_{0-1}M_0$), $n = 32$	1.58 (0.25–10.98) $p = 0.021$	1.94 (0.25–4.12) $p = 0.045$
$T_1N_{0-1}M_0$, $n = 10$	0.59 (0.25–5.0)	2.09 (0.51–5.68) $p = 0.043$
$T_2N_{0-1}M_0$, $n = 11$	1.0 (0.25–11.88) $p = 0.041$	0.52 (0.19–2.27)
$T_3N_{0-1}M_0$, $n = 11$	1.92 (0.53–10.98) $p = 0.021$	2.14 *(0.28–5.13)* $p = 0.046$

Note. Significance level of differences in the parameters compared to the group "Epithelial dysplasia (DI-II)" – *p*.

Analysis of the total activity of calpains in the biopsy samples showed a significant difference between HNSCC and dysplasia (DI-II) groups (Figure). Diagram A showed changes in the activity of calpains depending on the tumor spread in comparison

with dysplastic changes in the epithelium of the upper respiratory tract. Patients with stage $T_3N_{0-1}M_0$ had the highest rate of calpain activity. Additionally, this diagram showed a tendency towards a rise in the activity of calpains along with the increasing size of the tumor. Diagram B demonstrated that the total activity of calpains in patients with HNSCC was 4.4 times higher (125.7×10^3 units / mg of protein) than that observed in patients with dysplastic epithelial changes.



a *b*

Figure. Calpain activity in the biopsy samples of patients with epithelial dysplasia of the upper respiratory tract (DI-II) and HNSCC (T1-3N0-1M0): significance of differences compared to the group "Epithelial dysplasia (DI-II)", $p < 0.05$

Our data were consistent with the recent studies conducted by V.D.Koval et al., who revealed that the activity of calpains was nearly 12 times higher in patients with endometrial hyperplasia than in patients with endometrial cancer [18]. Compared to the healthy tissue, the increased activity of the calpain system components was reported in many cancers, such as meningioma, renal cell carcinoma, colorectal adenocarcinoma, endometrial cancer, gastric cancer, and breast cancer [10, 12, 13, 19]. Involvement of calpains in development of malignant tumors is determined by their essential role in many physiological cellular processes. Calpains potentially recognize more than 200 substrates, as confirmed by *in vitro* studies [11].

Among the proteins identified as calpain substrates, there are transcription factors, transmembrane receptors, signaling pathway components, and cytoskeletal proteins. The calpain proteases and proteasomes function in a coordinated manner. In this case a complex develops in the malignant tissue which is called the cancer degradome and represented by enzymes of various types of catalysis [7]. The components of the cancer degradome provide effective proteolysis during tumor progression, including invasion and metastasis.

CONCLUSION

Our results show significant differences in the expression levels and activity of the calpain system components between malignant and dysplastic epithelium of the upper respiratory tract. The elevated mRNA expression levels of CAPN1 and CAPN2 and overall calpain activity in the tumor tissues of HNSCC patients (compared to patients with chronic respiratory diseases associated with epithelial dysplasia) are likely to characterize a high potential for transition from a precancerous lesion to cancer. However, to fully establish the role of calpains in HNSCC carcinogenesis, it is necessary to conduct similar studies in the intact tissues using animal models. The data obtained indicate that the calpain system is directly involved in the development of head and neck cancer. Further development of criteria for potentially premalignant respiratory lesions posing a high risk of

malignant transformation is promising for successful prevention of HNSCC.

REFERENCES

1. Leemans C.R., Braakhuis B.J.M., Brakenhoff R.H. The molecular biology of head and neck cancer. *Nature Reviews Cancer.* 2011; 11 (1): 9–22. DOI: 10.1038/nrc2982.
2. Alsahafi E., Begg K., Amelio I., Raulf N., Lucarelli P., Sauter T., Tavassoli M. Clinical update on head and neck cancer: molecular biology and ongoing challenges. *Cell Death Dis.* 2019; 10 (8): 540. DOI: 10.1038/s41419-019-1769-9.
3. Kaprin A.D., Starinskiy V.V., Petrova G.V. Malignant neoplasms in Russia in 2018 (morbidity and mortality). Moscow: P. Hertsen Moscow Research Oncology Institute – branch of FSBI "NMRRC" of the Ministry of Health of the Russian Federation; 2019: 250 (in Russ.).
4. Frolova I.G., Choinzonov E.L., Goldberg V.E., Chizhevskaya S.Yu., Chernov V.I., Goldberg A.V., Belevich Yu.V. Imaging techniques for the detection of lymph node metastasis in patients with laryngeal and hypopharyngeal cancer. *Siberian Journal of Oncology.* 2018; 17 (3):101–108 (in Russ.). DOI: 10.21294/1814-4861-2018-17-3-101-108.
5. Trivedi S., Rosen C.A., Ferris R.L. Current understanding of the tumor microenvironment of laryngeal dysplasia and progression to invasive cancer. *Curr. Opin. Otolaryngol. Head Neck Surg.* 2016; 24 (2): 121–127. DOI: 10.1097/MOO.0000000000000245.
6. Cheremisina O.V., Choinzonov E.L., Pankova O.V., Menshikov K.Yu. Chronic hypeplastic laryngitis as a criterion for defining groups at high risk of laryngeal cancer. *Russian Otorhinolaryngology.* 2013; 63 (2): 84–89 (in Russ.).
7. Kakurina G.V., Kondakova I.V., Choinzonov E.L. Degradome components in progression of squamous cell carcinoma of the head and neck. *Annals of the Russian Academy of Medical Sciences.* 2015; 70 (6):684–693 (in Russ.). DOI: 10.15690/vramn563.
8. Sorimachi H., Hata S., Ono Y. Calpain chronicle – an enzyme family under multidisciplinary characterization. *Proc. Jpn. Acad. Ser. B Phys. Biol. Sci.* 2011; 87 (6): 287– 327. DOI: 10.2183/pjab.87.287.
9. Macqueen D.J., Wilcox A.H. Characterization of the definitive classical calpain family of vertebrates using phylogenetic, evolutionary and expression analyses. *Open Biol.* 2014; 4 (4): 130219. DOI: 10.1098/rsob.130219.
10. Moretti D., Del Bello B., Allavena G., Maellaro E. Calpains and cancer: Friends or enemies? *Archives of Biochemistry and Biophysics.* 2014; 564: 26–36. DOI: 10.1016/j.abb.2014.09.018.
11. Suzuki K., Hata S., Kawabata Y., Sorimachi H. Structure, Activation, and Biology of Calpain. *Diabetes.* 2004; 53 (suppl 1): S12-S18. DOI: 10.2337/diabetes.53.2007.S12.
12. Ivanova E.V., Kondakova I.V., Spirina L.V., Afanas'ev S.G., Avgustinovich A.V., Cheremisina O.V. Chymotrypsin-Like Activity of Proteasomes and Total Calpain Activity in Gastric and Colorectal Cancer. *Bull. Exp. Biol. Med.* 2014; 157 (6): 781–784. DOI: 10.1007/s10517-014-2666-y.
13. Shashova E.E., Kolegova E.S., Zav'yalov A.A., Slonimskaya E.M., Kondakova I.V. Changes in the Activity of Proteasomes and Calpains in Metastases of Human Lung Cancer and Breast Cancer. *Bull. Exp. Biol. Med.* 2017; 163 (4): 486–489. DOI: 10.1007/s10517-017-3834-7.
14. Zhang S., Deen S., Storr S.J., Chondrou P.S., Nicholls H., Yao A., Rungsakaolert P., Martin S.G. Calpain system protein expression and activity in ovarian cancer. *J. Cancer Res. Clin. Oncol.* 2019; 145 (2): 345–361. DOI: 10.1007/s00432-018-2794-2.
15. Livak K.J., Schmittgen T.D. Analysis of Relative Gene Expression Data Using Real-Time Quantitative PCR and the $2^{-\Delta\Delta CT}$ Method. *Methods.* 2001; 25 (4): 402–408. DOI: 10.1006/meth.2001.1262.
16. Ma D., Fang J., Liu Y., Song J-J., Wang Y-Q., Xia J., Cheng B., Wang Z. High level of calpain1 promotes cancer cell invasion and migration in oral squamous cell carcinoma. *Oncology Letters.* 2017; 13 (6): 4017–4026. DOI: 10.3892/ol.2017.5970.
17. Sletov A.A., Mozheiko R.A. Features of differential diagnostics of cancer of the mucous membrane of the oral cavity with the use of specific markers of tumor progression. *Science Almanac.* 2017; 28 (2–3):389–397 (in Russ.). DOI: 10.17117/na.2017.02.03.389.
18. Koval' V.D., Spirina L.V., Kondakova I.V., Kolomiets L.A., Shpileva O.V. Proteasome and calpain activities in endometrial hyperplasia and endometrial cancer. *Molecular Medicine.* 2012; (4):45–48 (in Russ.).
19. Shashova E.E., Doroshenko A.V., Bondar L.N., Slonimskaya E.M., Kondakova I.V. Proteasomal and calpain proteolysis systems in different molecular subtypes of breast cancer. *Siberian Journal of Oncology.* 2017; 16 (3): 33–39 (in Russ.). DOI: 10.21294/1814-4861-2017-16-3-33-39.

Authors contribution

Sidenko E.A. – carrying out of laboratory research, determination of the calpain gene expression, drafting of the article. G.V. Kakurina – determination of the calpain gene expression, analysis and interpretation of data. Cheremisina O.V. – collection of biopsy material from patients with laryngeal and laryngopharyngeal cancer and precancerous diseases of the upper respiratory tract. Spirina L.V. – carrying out of laboratory research, determination of the calpain activity. Shashova E.E. – statistical processing of data. Korshunov D.A. – preparation

of the illustrative material, drafting of the article. Kondakova I.V. – conception and design, substantiation of the manuscript, critical revision for important intellectual content, final approval of the manuscript for publication.

Authors information

Sidenko Evgeniya A., Post-Graduate Student, Laboratory of Tumor Biochemistry, Cancer Research Institute, Tomsk NRMC RAS, Tomsk, Russian Federation. ORCID 0000-0001-5838-9459.

Kakurina Gelena V., Cand. Sci. (Med.), Senior Researcher, Laboratory of Tumor Biochemistry, Cancer Research Institute, Tomsk NRMC RAS, Tomsk, Russian Federation. ORCID 0000-0002-4506-9429.

Cheremisina Olga V., Dr. Sci. (Med.), Professor, Head of the Endoscopy Department, Cancer Research Institute, Tomsk NRMC RAS, Tomsk, Russian Federation. ORCID 0000-0001-7234-4708.

Spirina Liudmila V., Dr. Sci. (Med.), Leading Researcher, Laboratory of Tumor Biochemistry, Cancer Research Institute, Tomsk NRMC RAS, Tomsk, Russian Federation. ORCID 0000-0002-5269-736X.

Shashova Elena E., Dr. Sci. (Med.), Senior Researcher, Laboratory of Tumor Biochemistry, Cancer Research Institute, Tomsk NRMC RAS, Tomsk, Russian Federation. ORCID 0000-0002-7752-9346.

Korshunov Dmitry A., Cand. Sci. (Med.), Researcher, Laboratory of Tumor Biochemistry, Cancer Research Institute, Tomsk NRMC RAS, Tomsk, Russian Federation. ORCID 0000-0002-1058-3882.

Kondakova Irina V., Dr. Sci. (Med.), Professor, Head of the Laboratory of Tumor Biochemistry, Cancer Research Institute, Tomsk NRMC RAS, Tomsk, Russian Federation. ORCID 0000-0002-0947-8778.

(✉) **Sidenko Evgeniya A.,** e-mail: sidenkoevgeniyaaleksandrovna@gmail.com.

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