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Molecular mechanisms of the effects of N-ethylmaleimide and 1,4-dithioerythritol on regulation of apoptosis in P19 cells under hypoxia

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ABSTRACT

Impairment of apoptosis regulation in P19 cells is correlated with generation of oxidative stress. Under hypoxia, changes in mitochondrial functions occur, which may exacerbate oxidative stress in the tumor cell.

The aim of the study was to evaluate the effects of N-ethylmaleimide and 1,4-dithioerythritol on implementation and regulation of apoptosis in P19 cells under hypoxia *in vitro*.

Materials and methods. P19 cells (mouse teratocarcinoma) cultured under hypoxia served as the material for the study. For redox status modulation, 5mM N-ethylmaleimide and 1,4-dithioerythritol in the final concentrations of 5 mM were used. The intracellular concentration of calcium ions, the transmembrane potential and the number of Annexin V, CD95 and CD120 positive cells were determined by flow cytometry. The levels of reduced, oxidized and protein-bound glutathione, protein SH groups, hydroxyl radical and protein carbonyl derivatives were measured by spectrophotometry.

Results. The alteration in the redox status of the glutathione system under hypoxia, accompanied by oxidative modification of proteins (glutathionylation and carbonylation), influences the metabolism in the tumor cell on the whole. Under the effects of 1,4-dithioerythritol, an SH group protector, this alteration promotes formation of additional mechanisms to escape apoptosis, whereas under the effects of N-ethylmaleimide, an SH group blocker, it, on the contrary, promotes apoptosis activation.

Conclusions. The changes in the redox homeostasis of the tumor cell and modulation of oxidative modification of proteins (glutathionylation and carbonylation) under hypoxia are one of the promising approaches to targeted regulation of cell death.

Key words: redox status, tumor growth, oxidative stress, glutathione system, apoptosis, hypoxia.

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Молекулярные механизмы влияния N-этилмалеимида и 1,4-дитиоэритритола на регуляцию апоптоза опухолевых клеток линии P19 при гипоксии

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РЕЗЮМЕ

Актуальность. Нарушение регуляции апоптоза в эпителиальных опухолевых клетках линии P19 сопряжено с формированием окислительного стресса. В условиях гипоксии происходит изменение функционирования митохондрий, что может выступать дополнительным фактором, усугубляющим окислительный стресс в опухолевой клетке.

Цель – оценить влияние N-этилмалеимида и 1,4-дитиоэритритола на реализацию и регуляцию апоптоза опухолевых клеток линии P19 при гипоксии *in vitro*.

Материалы и методы. Материалом для исследования служили культивированные в условиях гипоксии опухолевые клетки линии P19 (тератокарцинома мыши). Для модуляции редокс-статуса использовали N-этилмалеимид в концентрации 5 мМ и протектор SH-групп – 1,4-дитиоэритритол в конечной концентрации 5 мМ. Методом проточной цитофлуориметрии определяли внутриклеточное содержание ионов кальция, трансмембранный потенциал митохондрий, количество аннексин V-, CD95- и CD120-положительных клеток. Концентрацию восстановленного, окисленного и белково-связанного глутатиона, SH-групп протеинов, гидроксильного радикала и карбонильных производных белков измеряли методом спектрофотомерии.

Результаты. В условиях гипоксии изменение редокс-статуса системы глутатиона, сопровождающееся окислительной модификацией белков (глутатионилирование и карбонилирование), оказывает влияние на метаболизм опухолевой клетки в целом и, при применении протектора SH-групп белков – 1,4-дитиоэритритола, способствует формированию дополнительных механизмов ускользания от клеточной гибели, а в случае применения блокатора SH-групп протеинов – N-этилмалеимида – активации апоптоза.

Заключение. В условиях гипоксии изменение редокс-гомеостаза опухолевой клетки и модуляция окислительной модификации белков (глутатионилирование и карбонилирование) являются одним из перспективных подходов таргетной регуляции клеточной гибели.

Ключевые слова: редокс-статус, опухолевый рост, окислительный стресс, система глутатиона, апоптоз, гипоксия.

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INTRODUCTION

Steady growth of incidence and high mortality from cancer pose a challenge of finding molecular targets for the activation of tumor cell death to theoretical and practical medicine. A significant contribution to the change in the redox status of the cell is made by the functioning of mitochondria as the main source of reactive oxygen intermediate generation [1, 2]. It is known that the level of production of reactive oxygen intermediates directly depends on oxygen tension inside the cell and the activity of enzymes of the electron transport chain. Currently, reactive oxygen intermediates are considered as important signaling molecules that can cause oxidation of nucleic acids, lipids, and functional domains of proteins [3]. Oxidative modification of these macromolecules contributes to genome instability, uncontrolled proliferation, impairment of apoptosis regulation, activation of angiogenesis, and a change

in the direction of intracellular metabolic pathways of tumor cells [4–7]. Accumulation of oxidatively modified proteins in tumor cells is one of the aspects of proteasome activation and production of heat-shock proteins that are involved in the regulation of cell death [8–10]. The study of mechanisms that trigger tumor cell apoptosis under hypoxia is of particular interest, since the tumor cell acquires additional resistance not only to apoptotic death, but also to chemotherapy [11, 12].

From our point of view, glutathionylation and carbonylation of proteins are among promising molecular mechanisms for redox regulation of intracellular signaling and tumor cell death under hypoxia. Potential intracellular targets of reversible and irreversible modification of proteins may be key proteins that regulate the cell cycle, ion-transporting systems, and transcription factors [3, 13].

The aim of the study was to evaluate the effect of N-ethylmaleimide and 1,4-dithioerythritol on the implementation and regulation of apoptosis of P19 tumor cells under hypoxia *in vitro*.

MATERIALS AND METHODS

P19 tumor cells (C3H/He mouse teratocarcinoma) from the cell culture bank of the Institute of Cytology of the Russian Academy of Sciences (St.-Petersburg, Russia) served as the material for the study. The cells were cultured using the monolayer method in a CO_2 incubator (Sanyo, Japan) at a temperature of 37 °C in the 5% CO_2 atmosphere. Culture conditions were the following: αMEM culture medium (BioloT, Russia), 10% fetal bovine serum (BioloT, Russia), L-glutamine 0.3 mg/ml (BioloT, Russia) and gentamicin 100 μg/ml (Microgen, Russia). Cell viability was assessed using a 0.5% trypan blue solution (Serva, USA). A cell culture having no more than 5% of dead cells was used for the experiment.

For the purpose of additional production of reactive oxygen intermediates by tumor cells, hypoxia was simulated in a culture of P19 tumor cells in the Hypoxia Incubator Chamber (STEMCELL, Canada) using the following gas mixture: 5% O_2, 5% CO_2, 90% N_2. The creation of hypoxia conditions was monitored by measuring the concentration of dissolved oxygen using the Dissolved Oxygen Meter (HANNA HI 9146, Italy).

An SH group blocker – N-ethylmaleimide (NEM) (Sigma-Aldrich, USA) – at a concentration of 5 mmol [14] and an SH group protector – 1,4-dithioerythritol (DTE) (Sigma-Aldrich, USA) – at a concentration of 5 mmol [15] were used as redox status modulators.

After incubation, the tumor cells were washed from the culture medium and lysed by resuspension in phosphate-buffered saline (pH = 7.4) with the addition of 1% X-100 Triton (Sigma-Aldrich, USA). After that they were cooled down using ice, maintaining the standard cell concentration to determine the concentration of the hydroxyl radical and carbonyl derivatives of proteins. The content of the hydroxyl radical was determined through its ability [after preliminary opsonization of cells with zymosan (Sigma-Aldrich, USA)] to destroy the model substrate – 2-deoxy-D-ribose (Sigma-Aldrich, USA) – and form a reaction product with the maximum absorption at 532 nm [16]. The concentration of carbonyl derivatives of proteins was determined through their reaction with 2,4-dinitrophenylhydrazine, which product has the maximum absorption at a wavelength of 363 nm [17]. The results on the content of the hydroxyl radical and carbonyl derivatives of proteins were expressed in nmol/mg of protein.

To determine the content of reduced, oxidized, protein-bound glutathione and protein SH groups, the cell lysate was deproteinized using a 5% sulfosalicylic acid solution. The concentration of total, oxidized (GSSG) and reduced (GSH) glutathione was determined using the method proposed by M.E. Anderson modified by I. Rahman et al. [18]. The GSH/GSSG ratio was calculated as an indicator of the change in the cell redox status. The content of protein SH groups and protein-bound glutathione, after its preliminary release from binding with proteins using a 1% sodium borohydride solution, was determined based on the reaction with 5.5-dithiobis-(2-nitrobenzoic acid) [19]. The results on the content of fractions of glutathione, protein SH groups and protein-bound glutathione were expressed in nmol/mg of protein.

Protein content in the cells was determined using the Bradford method based on the interaction of amino acid residues of lysine and arginine with the Coomassie blue dye G-250 [20].

Extinction of results was determined using the SF-2000 spectrophotometer (OKB-Spektr, Russia).

Apoptotically modified cells were evaluated by flow cytometry using annexin-V-FITC and propidium iodide (PI) according to the manufacturer's instructions (eBioscience, USA). The number of Annexin V positive cells was counted with respect to the total number of studied cells and expressed in %.

The number of CD95 and CD120 positive cells was determined using a set of monoclonal antibodies to the corresponding antigens according to the manufacturer's protocol (R&D Systems, USA). The result was expressed in conditional units (cu).

The mitochondrial membrane potential ($\Delta\Psi m$) of the cells was evaluated with the help of the Flow Cytometry Mitochondrial Membrane Potential Detection Kit (BD, USA) for reduction of spectral luminescence using 5,5′,6,6′-tetrachloro-1,1′,3,3′-tetraethylbenzimidazolecarbocyanine iodide, which, upon depolarization

of the mitochondrial membrane, is unable to penetrate inside the organelles and form fluorescent aggregates. The number of cells with reduced fluorescence was expressed in %.

The concentration of calcium ions in cell cytoplasm was determined using the method based on their binding to the maximum fluorescence at 526 nm by the Fluo 3 AM lipophilic probe (Sigma-Aldrich, USA) [21]. The results were expressed in conditional units (cu) reflecting the level of probe luminescence per cell.

Flow cytometry results were detected using the FACSCanto II instrument (BD, USA) and FACSDiva Version 6.1.3 software.

Statistical processing of the obtained data was carried out using SPSS 17.0 software. The normality of distribution of quantitative indicators was checked using the Shapiro – Wilk criterion. The significance of differences was evaluated using the non-parametric Kruskal – Wallis and Mann – Whitney tests. The data were presented as a median (*Me*), upper and lower quartiles (Q_1–Q_3). The differences were considered statistically significant at $p < 0.05$.

RESULTS

The impairment of the regulation of apoptotic death of tumor cells against the background of a decrease in intracellular oxygen tension is associated with a change in their redox status. Excessive generation of reactive oxygen intermediates under hypoxia is associated with the loss of electrons from the mitochondrial respiratory chain due to the absence of the finite acceptor of electrons and a decrease in the activity of complex IV – cytochrome c oxidase. In this regard the main reason for the formation of oxidative stress inside the cell is the impairment of these organelles functioning [22]. The most toxic reactive oxygen intermediate for cell macromolecules is the hydroxyl radical. According to some authors, it is the $OH^{\bullet}$-radical that acts as the most powerful agent contributing to the oxidative modification of proteins [23, 24]. In the previous study on the effect of hypoxia on the metabolism of P19 tumor cells, we showed the formation of oxidative stress associated with a change in the state of the glutathione system and apoptosis activation, compared to tumor cells cultured at a normal oxygen concentration [25].

Under hypoxia, with the addition of the protein SH group protector (DTE) to the culture medium of P19 tumor cells, we obtained a decrease in the concentration of $OH^{\bullet}$-radical by 2.5 times ($p < 0.05$) as well as in the number of cells with reduced mitochondrial potential by 5.2 times ($p < 0.05$), compared with the results recorded in the cells under hypoxia (see the table). In addition, under hypoxia, the action of DTE was accompanied by a significant increase in the GSH content by 1.3 times ($p < 0.05$), in the concentration of free protein SH groups by 1.5 times ($p < 0.05$), and by a decrease in the content of protein-bound glutathione by 1.4 times ($p < 0.05$) against the background of a comparable concentration of carbonyl derivatives of proteins, GSSG and GSH/GSSG ratio, as opposed to cells cultured under hypoxia (see the table). When studying the implementation and regulation of apoptosis under conditions of hypoxia and addition of the protector of protein SH groups to the culture medium of P19 tumor cells, we obtained a significant decrease in the number of Annexin V positive cells by 2.0 times ($p < 0.05$) and in the intracellular content of Ca^{2+} ions by 1.1 times ($p < 0.05$) against the background of comparable values for CD95 and CD120 positive cells, as opposed to cells under hypoxia (see the table). So, 1,4-dithioerythritol contributed to the formation of additional resistance of P19 tumor cells to the mechanisms triggering apoptosis under conditions of hypoxia.

When modulating the redox status of P19 tumor cells using a protein SH group blocker (NEM) under hypoxia, we obtained a significant increase in the number of Annexin V positive cells by 9.1 times ($p < 0.05$), CD95 positive cells by 15.5 times ($p < 0.05$), CD120 positive cells by 2.9 times ($p < 0.05$), cells with a reduced mitochondrial potential by 8.8 times ($p < 0.05$) and the intracellular concentration of Ca^{2+} ions by 3.0 times ($p < 0.05$) against the background of a comparable value for the $OH^{\bullet}$-radical in comparison with the results recorded for the cells under hypoxia (see the table). The effect of NEM in P19 tumor cells under hypoxia was accompanied by a significant decrease in the concentration of GSH by 3.4 times ($p < 0.05$) and an increase in the concentration of GSSG by 1.6 times ($p < 0.05$), which led to a significant decrease in the GSH/GSSG ratio by 5.4 times ($p < 0.05$), compared with cells under hypoxia (see the table). In this case the effect of the blocker of protein SH groups in P19 tumor cells under hypoxia was accompanied by a significant increase in the concentration of protein carbonyl derivatives by 1.4 times ($p < 0.05$) against the background of comparable values for the content of free protein SH groups and protein-bound glutathione, compared with the findings obtained in tumor cells during hypoxia (see the table). The use of N-ethylmaleimide had an impact on the metabolism of tumor cells, including the functioning of mitochondria, a change in the content of Ca^{2+} ions and carbonylated proteins, which was accompanied by activation of apoptotic death under hypoxia.

The results of the study confirm the role of the cellular redox status and oxidative modification of proteins in ensuring the functioning of tumor cells, including their mitochondria, at a reduced oxygen tension.

Table

The effect of N-ethylmaleimide and 1,4-dithioerythritol on the implementation of apoptosis, parameters of the glutathione system, and the content of the hydroxyl radical and carbonyl derivatives of proteins in P19 tumor cells under hypoxia, *Me* (Q_1-Q_3)

Studied parameters	Conditions of culturing P19 cells		
	Hypoxia	Hypoxia + NEM	Hypoxia + DTE
Annexin V-FITC+, %	10.75 (4.50–10.90)	98.05 (97.70–98.40) #	5.50 (4.70–6.70)
CD95, u.	1.0 (0.9–1.1)	15.5 (12.8–15.7) #	0.9 (0.8–1.2)
CD120, u.	1.4 (1.3–1.5)	4.1(4.0–4.5) #	1.1 (1.0–1.2)
Cells with reduced ΔΨm, %	10.4 (10.4–10.6)	91.2 (90.8–92.0) #	2.0 (2.0–2.1) #
Hydroxyl radical concentration, nmol/mg of protein	27.21 (23.56–29.93)	29.94 (29.42–32.20)	10.70 (8.93–11.32) #
Intracellular concentration of Ca^{2+}, u.	10.24 (10.10–10.36)	30.23 (29.47–30.39) #	9.48 (9.43–9.49) #
GSH, nmol/mg protein	4.47 (4.40–4.58)	1.30 (0.67–1.88) #	6.00 (5.97–6.32) #
GSSG, nmol/mg protein	0.43 (0.39–0.45)	0.69 (0.50–0.72) #	0.72 (0.34–0.81)
GSH/GSSG	10.19 (9.88–11.35)	1.89 (0.93–4.22) #	8.37 (7.93–17.27)
Protein-bound glutathione, nmol/mg protein	2.08 (1.95–2.19)	2.96 (2.08–3.26)	1.48 (1.43–1.51) #
Protein SH groups, nmol/mg of protein	8.76 (7.83–10.55)	9.62 (9.40–10.28)	12.98 (12.75–13.36) #
Protein carbonyl derivatives, nmol/mg of protein	10.17 (8.92–10.39)	14.34 (14.27–17.63) #	7.54 (6.64–8.32)

Note. ROS – reactive oxygen species, GSH – reduced glutathione, GSSG – oxidized glutathione, NEM – N-ethylmaleimide, DTE – 1,4-dithioerythritol; # – significant differences ($p < 0.05$) compared with the hypoxia group

CONCLUSION

Changes in the redox status of the glutathione system as well as glutathionylation and carbonylation of proteins affect the metabolism of the tumor cell. The effect of 1,4-dithioerythritol promotes the formation of additional mechanisms to escape cell death, and the use of N-ethylmaleimide is accompanied by the activation of apoptosis of P19 tumor cells. Taking into account the leading role of the redox cell homeostasis in triggering apoptosis, our study confirms the need to study the mechanisms of cell death and development of resistance of tumor cells to antitumor drugs, the effects of which are based on a change in the cellular redox status.

REFERENCES

1. Sinha K., Das J., Pal P.B., Sil P.C. Oxidative stress: the mitochondria-dependent and mitochondria-independent pathways of apoptosis. *Archives of Toxicology.* 2013; 87 (7): 1157–1180. DOI: 10.1007/s00204-013-1034-4.
2. Redza-Dutordoir M., Averill-Bates D.A. Activation of apoptosis signalling pathways by reactive oxygen species. *Biochim Biophys Acta.* 2016; 1863(12):2977–2992. DOI: 10.1016/j.bbamcr.2016.09.012.
3. Moldogazieva N.T., Mokhosoev I.M., Feldman N.B., Lutsenko S.V. ROS and RNS signalling: adaptive redox switches through oxidative/nitrosative protein modifications. *Free Radical Research.* 2018; 52 (5): 507–543. DOI: 10.1080/10715762.2018.1457217.
4. Tew K.D., Manevich Y., Grek C., Xiong Y., Uys J., Townsend D.M. The role of glutathione S-transferase P in signaling pathways and S-glutathionylation in cancer. *Free Radical. Biology and Medicine.* 2011; 51 (2): 299–313. DOI: 10.1016/j.freeradbiomed.2011.04.013.
5. Stepovaya E.A., Ryazantseva N.V., Nosareva O.L., Zakirova E.V., Naumova A.I., Vesnina O.N., Orlov D.S., Shakhristova E.V., Ivanov V.V., Novitsky V.V. The role of oxidative protein modification in redox-dependent regulation of tumor cell apoptosis. *Molecular Medicine.* 2015; (4):60–64 (in Russ.).
6. Patwardhan R.S., Sharma D., Checker R., Thoh M., Sandur S.K. Spatio-temporal changes in glutathione and thioredoxin redox couples during ionizing radiation-induced oxidative stress regulate tumor radio-resistance. *Free Radical Research.* 2015; 49 (10): 1218–1232. DOI: 10.3109/10715762.2015.1056180.
7. Shakhristova E.V., Stepovaya E.A., Ryazantseva N.V., Nosareva O.L., Yakushina V.D., Ivanov V.V., Novitskii V.V. the role of glutathione system redox potential in apoptosis dysregulation in MCF-7 breast adenocarcinoma. *Bulletin of Experimental Biology and Medicine.* 2016; 160 (3): 364–367. DOI: 10.1007/s10517-016-3172-1.
8. Shashova E.E., Astakhova T.M., Plekhanova A.S., Bogomyagkova Y.V., Lyupina Y.V., Sumedi I.R., Slonimskaya E.M., Erokhov P.A., Abramova E.B., Rodoman G.V., Kuznetsov N.A., Kondakova I.V., Sharova N.P., Choinzonov E.L. Changes in proteasome chymotrypsin-like activity during the development of human mammary and thyroid carcinomas. *Bulletin of Experimental Biology and Medicine.* 2013; 156 (2): 242–244.
9. Kondakova I.V., Spirina L.V., Koval V.D., Shashova E.E., Choinzonov E.L., Ivanova E.V., Kolomiets L.A., Chernyshova A.L., Slonimskaya E.M., Usynin E.A., Afanasev S.G. Chymotrypsin-like activity and subunit composition of proteasomes in human cancers. *Molecular Biology.* 2014; 48 (3): 384–389.
10. Ryazantseva N.V., Stepovaya E.A., Nosareva O.L., Konovalova E.V., Orlov D.S., Naumova A.I., Didenko S.A., Vesnina O.N., Shakhristova E.V., Zima A.P., Novitskii V.V. Role of heat shock protein 27 in regulation of glutathione system and apoptosis of Jurkat tumor cells and blood lymphocytes. *Bulletin of Experimental Biology and Medicine.* 2015; 158 (3): 377–379. DOI: 10.1007/s10517-015-2766-3.
11. Marengo B., Nitti M., Furfaro A.L., Colla R., Ciucis C.D., Marinari U.M., Pronzato M.A., Traverso N., Domenicotti C. Redox Homeostasis and Cellular Antioxidant Systems: Crucial Players in Cancer Growth and Therapy. *Oxidative Med-*

icine and Cellular Longevity. 2016; 2016:6235641. DOI: 10.1155/2016/6235641.
12. Zhang T., Suo C., Zheng C., Zhang H. Hypoxia and metabolism in metastasis. *Advances in Experimental Medicine and Biology.* 2019; 1136: 87–95. DOI: 10.1007/978-3-030-12734-3_6.
13. Stepovaya E.A., Shakhristova E.V., Ryazantseva N.V., Nosareva O.L., Chil'chigashev R.I., Egorova M.Y. The thioredoxin system in regulating MCF-7 cell proliferation under Redox Status Modulation. *Siberian Journal of Oncology.* 2016; 15 (4): 50–55. DOI: 10.21294/1814-4861-2016-15-4-50-55 (in Russ.).
14. Sahaf B., Heydari K., Herzenberg L.A. Lymphocyte surface thiol levels. *Proc. Natl. Acad. Sci. USA.* 2003; 100 (7): 4001–4005.
15. Brunelli L., Crow J.P., Beckman J.S. The comparative toxicity of nitric oxide and peroxynitrite to *Escherichia coli. Archives of Biochemistry and Biophysics.* 1995; 316 (1): 327–334.
16. Thom S.R., Elbuken M.E. Oxygen-dependent antagonism of lipid peroxidation. *Free Radical Biology and Medicine.* 1991; 10 (6): 413–426.
17. Arutyunyan A.V., Dubinina E.E., Zybina N.N. Methods of assessment of free radical oxidation and antioxidative system of the organism. St. Petersburg: IKF "Foliant", 2000: 103 (in Russ.).
18. Kojima S., Nakayama K., Ishida H. Low dose gamma-rays activate immune functions via induction of glutathione and delay tumor growth. *Journal of Radiation Research.* 2004; 45 (1): 33–39. DOI: 10.1269/jrr.45.33.
19. Burchill B.R., Oliver J.M., Pearson C.B., Leinbach E.D., Berlin R.D. Microtubule dynamics and glutathione metabolism in phagocytizing human polymorphonuclear leukocytes. *Journal of Cell Biology.* 1978; 76 (2): 439–447. DOI: 10.1083/jcb.76.2.439.
20. Bradford M.M. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry.* 1976; 72: 248–254.
21. Merritt J.E., McCarthy S.A., Davies M.P., Moores K.E. Use of fluo-3 to measure cytosolic Ca^{2+} in platelets and neutrophils. Loading cells with the dye, calibration of traces, measurements in the presence of plasma, and buffering of cytosolic Ca^{2+}. *The Biochemical Journal.* 1990; 269 (2): 513–519. DOI: 10.1042/bj2690513.
22. Munro D., Treberg J.R. A radical shift in perspective: mitochondria as regulators of reactive oxygen species. *The Journal of Experimental Biology.* 2017; 220 (Pt. 7): 1170–1180. DOI: 10.1242/jeb.132142.
23. Zenkov N.K., Men'shhikova E.B., Tkachev V.O. Some principles and mechanisms of redox-regulation. *Oxygen and Antioxidants.* 2009; 1: 3–64 (in Russ.).
24. Men'shhikova E.B., Zenkov N.K., Lankin V.Z., Bondar' I.A., Trufakin V.A. Oxidative stress: pathological states and diseases. Novosibirsk: Sibirskoe Universitetskoe Izdatelstvo, 2017: 284 (in Russ.).
25. Orlov D.S., Ryazantseva N.V., Stepovaya E.A., Nosareva O.L., Shakhristova E.V., Ivanov V.V. Redox-dependent mechanisms of apoptosis dysregulation in tumor cells under hypoxia. *The Siberian Scientific Medical Journal.* 2017; 37 (1): 21–26 (in Russ.).

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