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State of free-radical processes in the heart cell mitochondria under melanoma B16/F10 growth against the background of chronic neurogenic pain as comorbid pathology

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Abstract

The aim hereof has been to investigate the dynamics of processes of free radical oxidation and antioxidant protection in the heart cell mitochondria in female mice of strain C57BL/6 at different stages of the B16/F10 melanoma growth under the comorbid pathology conditions, namely, chronic neurogenic pain.

Materials and methods

Our research work was conducted in female mice of strain C57BL/6 (n=168). The animals were randomly distributed in separate groups as follows: the group of intact mice (n=21); the reference group (RCNP) (n=21) to reproduce the model of chronic neurogenic pain (CNP); group M (n=63) with melanoma B16/F10 upon subcutaneous transplantation of the tumor; group CNP + M (n=63), where the B16/F10 melanoma was transplanted 3 weeks after CNP modeling. In the heart cell mitochondria samples, using ELISA tests, we have determined concentrations of superoxide dismutase 2 (SOD 2) (pg/mg protein), 8-hydroxy 2' deoxyguanosine (8-OHdG) (ng/mg protein); malone dialdehyde (MDA) (mcM/mg protein); the total SOD activity (units/mg protein), the Mn SOD activity (units/mg protein) and the Cu-Zn SOD activity (units/mg protein). The obtained statistics data have been processed with software Statistica 10.0.

Results

Under the CNP conditions, we have revealed in the female mice in the heart cell mitochondria that the SOD 2 level has decreased by 2,9 times, the total SOD activity has been diminished by 1,54 times ($p<0,05$), and the Cu-ZN SOD activity has been recorded to be 2,7 times lower, as compared with the respective data in the intact animals. 1 week after the melanoma growth stimulated by CNP, as against the reference values, an increase in the SOD 2 level by 3,2 times has been identified, and it has demonstrated high values upon expiration of 2 and 3 weeks of the tumor growth. Considering the same period of the tumor growth under the CHP conditions, we have observed an elevation of the activity of the total SOD and the Mn SOD by 1,7 and 2 times, respectively ($p<0,05$). On the contrary, 1 week after the stimulated tumor growth, the Cu-Zn SOD activity has been lowered reaching its undetectable values; upon expiration of 2 weeks it has been found to be at the level of the respective reference values, but after 3 weeks its decrease by 3 times has been recorded. The 8-OHdG concentration has been revealed to be increased after 1 week by 4 times and after 2 weeks by 6,6 times, respectively. The MDA level in week 1 and 2 has exceeded the reference levels on the average by 2 times. Upon expiration of 3 weeks of the stimulated tumor growth, both indicators have been found to reach the level of the reference values.

Conclusion

The accompanying chronic neurogenic pain has contributed to the functional re-setting of subcellular structures in the organs not affected by the tumor. In the heart cell mitochondria, which are considered as the most sensitive mechanisms in the cell regulation, we can observe surges in the prooxidant activity followed by further dynamics of its normalization. The initial suppression of the activity of the antioxidant system elements gives the way to a considerable surge and maintenance of a permanent or a variable high level of the activity that bears witness to the fact that stress or tension develops accompanied by depletion of energy resources of the heart under the CNP conditions.

Keywords

Mitochondria, Heart, Free radical oxidation, Antioxidant protection, Chronic neurogenic pain, Melanoma B16/F10, Female mice

Imprint

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Introduction

The heart always requires a large quantity of energy to be used primarily to maintain the cardiac muscle contractility and blood circulation. But unexpectedly the heart demonstrates its low-level capability of accumulating energy. Therefore, in order to keep up the required high-density energy flux by the heart, the ATP molecules should be synthesized rapidly, without any interruptions [1]. In cardiomyocytes, the mitochondria take up about one third of the entire volume of the cells and produce more that 95% of ATP that reflects a great demand of this cell type for energy. Therefore, it is not surprising that the mitochondrial dysfunction has much to do with development of cardiomyopathy and a higher risk of heart insufficiency [2]. Disorders in functioning of mitochondria may be attributed to the following factors: an imbalance in the mitochondrial dynamics [3], an aberrant mitochondrial lipid homeostasis [4], vitamin- and co-factor-related deficiency of metabolism and altered redox processes [5]. Many aspects of the mitochondrial dysfunction are also treated as cancer contributors [6].

At present, a break-through high-tech system is available that makes possible to apply a fresh digital approach to a phase-related analysis of pathological changes in the cardiovascular system performance. The above approach offers to non-invasively estimate the mitochondrial dysfunction in cardiomyocytes by delivering computed data on concentrations of metabolites like oxygen, lactate and phosphocreatine with the use of the original proprietary analytical software employed by hemodynamic analyzer CARDIOCODE (PC-assisted hemodynamic analyzer CARDIOCODE, designed and manufactured by STC Cardiocode, Certificate of Registration RU No. FSR 2011/12126, Taganrog, Russia). This high-tech system shows much promise for quantified assessment of functional loading and actual pathological processes including those in case of oncopathology. An extra option of the offered technology is establishing of laboratory biochemical relationships and parallels when determining the mitochondrial dysfunction of cardiomyocytes, especially with respect to levels of reactive oxygen species (ROS), in case of significant functional loading, under the normal conditions and pathology, including the tumor growth and accompanying comorbid states. In the framework of translational medicine, an application of experimental models can be a help in solving a variety of problems revealing dysfunctional factors [7, 8, 9].

An accumulation of ROS in cells is the primary cause of the damage and the dysfunction of mitochondria. In fact the mitochondria are the main cellular source of ROS. Electrons from coferments (NADH and FADH 2) are closely linked with oxidative phosphorylation to form ATP. However about 0,2-2% of the electrons escape the electron transport chain, and they are aberrantly accepted by O2 that results in formation of a superoxide anion radical. The electron escape can take place in every of the three complexes, i.e. in complex I, II and III. The superoxide is released from complex I and II into the matrix and from complex III into the intermembrane space and the matrix [2]. Long electron transportation pathways in mitochondrial associates in cardiomyocytes can be detected by photo-induced effects as chemiluminescence [10].

In the mitochondria, the ROS production is balanced by an effective detoxification protection. In the matrix layer, superoxide may be transformed by mitochondrial antioxidant manganese SOD – SOD2, and H2O2 can either freely move through the mitochondrial membranes or be further detoxified by other mitochondrial antioxidant ferments like glutathione peroxidase (GPx) [11]. At its lower levels, ROS may serve as a signal molecule for modification of the signal proteins [2]. But an excess of the ROS production is accompanied by a decline in antioxidant capacity and an elevation of oxidative stress. An excess of ROS leads to an interruption in energy generation, an increase in death of the cells and an irreversible oxidative damage of the mitochondrial DNA (mtDNA) and alterations in gene expression.

The aim of our study was to investigate indicators of free radical oxidation and antioxidant protection in the heart cell mitochondria in female mice of strain C57BL/6 under growing melanoma B16/F10 against the background of comorbid pathology, namely, chronic neurogenic pain.

Materials and methods

Our research work was conducted in female mice of strain C57BL/6 (n=168), at the age of 8 weeks, with an initial body mass of 21-22 g. The animals were randomly distributed in separate groups as follows: the group of intact mice (n=21); the reference group (RCNP) (n=21) to reproduce the model of chronic neurogenic pain (CNP); group M (n=63) with melanoma B16/F10 upon subcutaneous transplantation of the tumor cells; group CNP + M (n=63), where the

B16/F10 line melanoma was transplanted 3 weeks after the completion of CNP modeling.

The experimental animals have been delivered to us by the Federal State Medical & Biological Institution "Research Center of Biomedical Technologies" (Branch Andreevka, Moscow Region) at the Federal Medical & Biological Agency; mouse melanoma line B16/F10 have been supplied by the Russian National Medical Research Center of Oncology named after N.N.Blokhin, Ministry of Health, Russia. The tested animals have been kept under natural light conditions with a free access to water and food. Our research study has been conducted in accordance with the relevant regulations stated by EU Directive 86/609 EEC, the Declaration of Helsinki (DoH), the International Guiding Principles for Biomedical Research Involving Animals and Order No. 267 "Approval of the Rules of Laboratory Practice" dated June, 19, 2003 issued by the Ministry of Health of the Russian Federation; our study has been approved by the Commission on Bioethics at the Federal State Budgetary Institution "National Medical Research Center of Oncology", the Ministry of Health of the Russian Federation (Record No. 2 dated May, 31, 2018). All researchers have signed their written informed consent thereon.

Melanoma B16/F10 was transplanted in a standard manner under the skin of the right scapula in the volume of 0.5 ml of tumor cell suspension in a 1:10 dilution with saline solution. The chronic neurogenic pain (CNP) model was reproduced in animals by ligation of the sciatic nerves in both hind limbs under xyl-zoletil anesthesia [12]. For preanesthetic premedication, 10 minutes before anesthesia, used were xylazine (Xyl) intramuscularly at a dose of 0.05 ml/kg of body weight (according to the instructions), then, 10 minutes later, Zoletil-50 at a dose of 10 mg per 100 g of body weight. Manipulations with animals were carried out in the box in compliance with the generally accepted rules of asepsis and antisepsis.

Decapitation of the animals was performed with the use of the guillotine. Animals from groups M and CNP+M were decapitated after the B16/F10 melanoma transplantation in the following periods: 1 week of the melanoma growth, 2 weeks and 3 weeks of the melanoma growth. The CNP group mice were decapitated 3 weeks after the CNP model reproduction: their decapitation was performed in parallel with that of the intact mice. Mitochondria were separated by the method of Egorova M. V., Afanasiev S. A. [13] (with the use of refrigerants and differential centrifugation with high-performance refrigerated centrifuge Avanti J-E, BECMAN COULTER, USA). The tissues were washed with an ice-cold 0.9% KCl solution. To disrupt the intercellular bonds, the cell walls and the plasma membranes, mechanical treatment of tissues with grinding using scissors and homogenization in a glass homogenizer with a Teflon pestle (Potter-Elvehjem homogenizer) was utilized. Per gram of tissue, 10 ml of the isolation medium was added (0.22 M mannitol, 0.3 M sucrose, 1 mM EDTA, 2 mM TRIS-HCL, 10 mM HEPES, pH 7.4). The tissues were homogenized and centrifuged for the first time for 10 minutes at 1000 g at a temperature of 0-2 °C, the second and third centrifugation procedures were completed at 20000 g for 20 minutes at a temperature of 0-2°C. Between the centrifugation procedures, the mitochondria sediment was resuspended in the isolation medium. Mitochondria were further purified from lysosomes, peroxisomes, melanosomes, etc. using the Percoll 23% density gradient centrifugation. The suspension of the subcellular structures was layered on the Percoll gradient, centrifuged for 15 min at 21000 g, after which the separation into 3 phases was observed; the lower layer of mitochondria was left and resuspended with the isolation medium. The next mitochondria washing procedure was carried out by centrifugation for 10 minutes at 15000 g at a temperature of 0 - 2°C. The prepared mitochondrial samples (protein concentration 4-6 g/l) were stored at -80° C in the isolation medium before their analysis. In the mitochondrial samples, using ELISA tests, we have determined concentrations of superoxide dismutase 2 (SOD 2) (pg/mg protein) (ABfrontier, Netherlands), 8-hydroxy 2' deoxyguanosine (8-OHdG) (ng/mg protein) (Enzo Life Sciences, Switzerland); malone dialdehyde (MDA) (mcM/mg protein) (Cusabio Biotech, China);the total SOD activity (units/mg protein), the Mn SOD activity (units/mg protein) and the Cu-Zn SOD activity (units/mg protein) (Cayman Chemical, USA); the protein concentration in mg/ml was identified with biuret method (Olvex Diagnosticum, Russia) employing automatic analyzer ChemWell (Awareness Technology INC, USA). Coefficient values SOD 2 activity/ antigen have been computed.

The obtained statistics data have been processed with software Statistica 10.0.

The data were analyzed for the compliance of the features distribution with the normal distribution law

Table 1

Content of apoptosis factors in the mitochondria in the heart cells in female mice with standard versus stimulated by chronic neurogenic pain growth of melanoma B16/F10

Group	SOD 2 pg/mg protein	Total SOD activity, units/mg protein	Mn SOD activity, units/mg protein	SOD 2 activity/ antigen, units/mg protein	Cu-Zn SOD activity, units/ mg protein	8-OHdG ng/ mg protein	MDA) mcM/ mg protein
Intact	581,74±33,778	0,403±0,027	0,274±0,011	0,481±0,031	0,129±0,032	1,525±0,078	3,728±0,189
Reference CNP	196,68±16,889[1] p^1=0,0000	0,261±0,026[1] p^1=0,0025	0,213±0,021	1,126±0,134[1] p^1=0,0005	0,048±0,003[1] p^1=0,0283	1,63±0,082	2,909±0,254
Growth of melanoma B16/F10							
M week 1	121,72±10,179[1] p^1=0,0000	0,17±0,019[1] p^1=0,0000	0,166±0,019[1] p^1=0,0004	1,417±0,222[1] p^1=0,0013	0,009±0,0007[1] p^1=0,0030	1,323±0,118	3,175±0,292
M week 2	303,41±17,851[1,3] p^1=0,0000 p^3=0,0000	0,246±0,021[1,3] p^1=0,0006	0,188±0,022[1] p^1=0,004	0,633±0,097[3] p^3=0,0072	0,058±0,003[1,3] p^1=0,0108 p^3=0,0000	1,399±0,101	3,254±0,227
M week3	859,35±22,723[1,3] p^1=0,0000 p^3=0,0000	0,266±0,014[1] p^1=0,0007	0,211±0,021	0,249±0,029[1,3] p^1=0,0001 p^3=0,0026	0,055±0,006[1] p^1=0,0000	1,856±0,134	4,912±0,225[1,3] p^1=0,0017 p^3=0,0002
Growth of melanoma CNP + B16/F10							
CNP + M week1	627,8±22,462[2,3] p^2=0,0000	0,17±0,015	0,169±0,015	0,270±0,025[2] p^2=0,0000	0,001±0,0003[2] p^2=0,0000	6,499±0,266[2] p^2=0,0000	6,089±0,298[2] p^2=0,0000
CNP + M week2	853,76±19,793[2,3] p^2=0,0000 p^3=0,0000	0,236±0,025	0,170±0,015	0,198±0,015[2,3] p^2=0,0000	0,066±0,015[2,3] p^2=0,0021 p^3=0,0003	10,785±0,387[2,3] p^2=0,0000 p^3=0,0000	6,003±0,216[2] p^2=0,0000
CNP + M week3	710,12±30,587[2,3] p^2=0,0000 p^3=0,0018	0,446±0,033[2,3] p^2=0,0009 p^3=0,0003	0,429±0,026[2,3] p^2=0,0000 p^3=0,0000	0,617±0,056[2,3] p^2=0,0044 p^3=0,0000	0,016±0,008[3] p^3=0,0211	1,011±0,105[3] p^3=0,0000	3,767±0,248[3] p^3=0,0000

Notes: [1]statistically significant referred to the value in the intact animal group; [2]statistically significant referred to the value in the respective reference group RCNP; [3]statistically significant referred to the value obtained in the previous period of examination.

using the Shapiro-Wilk test (small sample size). The comparison of quantitative data in the above groups (independent samples) was performed by applying the Kruskal-Wallis test. The table data are presented in the M±m form, where M is the arithmetic mean, and m is the standard error of the mean; we have used the significance level p<0,05 (see Table 1 herein). The obtained statistics data have been processed in compliance with general applicable medical research recommendations.

Results

The results of our analysis of the obtained lipid metabolism and antixodidant protection indicators are given in Table 1 herein. It has been found that the only CHP effect recorded in the heart cell mitochondria, as compared with the data thereon in the intact animals, has been reflected in a statistically significant reduction in the SOD 2 level by 2,9 times, in the total SOD activity by 1,54 times (p<0,05) and in the Cu-Zn SOD activity by 2,7 times, respectively, while the coefficient value of SOD 2 activ./antig. has increased by 2,3 times.

The initial stage (week 1) of the standard growth of melanoma B16/F10 has produced a sharp decrease in the SOD 2 level in the heart cell mitochondria by 4,8 times, then later, since week 2, we have observed a gradual increase therein, when the difference against that found in the intact animals has been recorded to be not so substantial: it has decreased by 1,9 times (p<0,05) only, and upon expiration of 3 weeks the value in question has exceeded the intact animal level by 1,5 times (p<0,05). The total SOD activity within the entire period of the standard melanoma growth have been recorded to be below the intact group values by 1,8 times on average. The Mn SOD activity in week 1 and 2 in the context of the standard growth of the tumor has declined by 1,5 times (p<0,05) and by 1,4 times (p<0,05), respectively, and the above indicator has been found to be at the same level that is the case with the intact animals. As to the Cu-Zn SOD activity, it has been recorded that at all stages of the standard growth of melanoma its indictors are below the respective intact animal values: after week 1 it has been reduced by 12,9 times, and after week 2 and 3 the value has been recorded to be 2,2 times lower on average. At the initial stage, the coefficient value SOD 2 activity/ antigen has been found to be 3,1 times higher than the respective value in the intact animals; but beginning with week 2 it has become lower, and by week 3 it has been recorded

to be 1,9 times lower. The standard growth of melanoma has produced no effect on the 8-OHdG amount in the heart mitochondria at all stages of our observation. The MDA level has remained stable in week one and two in the period of the standard melanoma growth, and it has demonstrated its tendency to increase by 1,32 times ($p<0,05$) as against the intact animal value.

With the growth of melanoma under the CNP conditions, another dynamics of the studied biochemical indicators in the heart cell mitochondria has been revealed. So, after week 1 of the tumor growth accompanied by CNP, the SOD 2 level has increased by 3,2 times, as against the values in the reference group, and remained high after 2 and 3 weeks exceeding the reference values by 4,3 and 3,6 times, respectively. Some changes in the total SOD activity have been recorded after 3 weeks of the melanoma growth against the CNP background: this indicator has demonstrated an increase by 1,7 times ($p<0,05$). A similar dynamics has been detected in case of the Mn SOD activity: we have observed an increase therein by 2 times after 3 weeks of the melanoma growth accompanied by CNP. The Cu-Zn activity measured after 1 week against the CNP background drops reaching practically undetectable values, then, 2 weeks later, it is recorded to be within the reference value range followed by its decline by 3 times upon expiration of 3 weeks. As to the values of coefficient SOD 2 activity/ antigen, throughout the entire period of the tumor growth against the CNP background, we have recorded low values thereof: after weeks 1-2 we have them recorded by 4,6 times and after week 3 by 1,8 times lower on average ($p<0,05$). The initial and logarithmic progression stages of the tumor growth against the CNP background (1 – 2 weeks) have been accompanied by an accumulation of products of free radical oxidation (FRO) in the heart cell mitochondria. So, after week 1, the 8-OHdG level has become 4 times higher, and upon expiration of week 2 it has been recorded to be 6,6 times higher. The MDA level measured after 1 week and 2 weeks has exceeded the reference values by 2 times on average. Upon expiration of 3 weeks of the tumor growth with CNP, both indicators have been found to be within the reference value range. The complete set of the presented data on the lipid metabolism and antixodidant protection system under the melanoma growth conditions against the CNP background in female mice demonstrates an aggravation of the dysfunction of mitochondria in the presence of comorbid pathology.

Discussion and conclusions

We have used in our research work the CHP model as comorbid pathology with the growing inoculated melanoma B16/F10 in mice [12]. The progression of melanoma against the CNP background in the examined mice cohort has been characterized by rapid formation of the tumor, the double-focused growth of the latter, early metastazing and a decline in the animal life duration. In the female mice, under the growing tumor accompanied by CNP, metastases have been detected not only at their usual locations like the liver or the lungs, but also at their unusual destinations, namely, in the spleen and uterus. As a consequence, we may conclude that CNP as comorbid pathology used in our experimental study can cancel the genetically determined inhibition of a malignant tumor [12, 14].

In the framework of this experimental study, we have revealed that there is a high level of a secondary product of lipid peroxidation (LPO): it is MDA found in the heart cell mitochondria under the melanoma growth stimulated by CNP, in week one and two of the tumor formation, followed by a decrease in the level of this indicator after three weeks. It is known that MDA belongs to highly reactive molecules and is capable of damaging DNA due to production of exocyclic DNA adducts: most of them demonstrate a strong mutagenic effect. MDA is considered to be the highly mutagenic product formed in lipid peroxidation [15].

The capability of MDA to damage DNA has been evidenced in our experiment by an increase in the level of a biomarker of oxidative stress: it is biomarker 8-OHdG, which indicates DNA damage. Therewith, we have recorded a similar tendency of changes in the 8-OHdG and MDA contents in the heart cell mitochondria under the melanoma B16/F10 growth stimulated by CNP. So, we have established that there is an increase in the 8-OHdG content in the first two weeks of the tumor growth and a decrease therein after three weeks. It follows that the obtained evidence data on the LPO product content in the heart cell mitochondria under the melanoma growth stimulated by CNP can point to an immediate damaging effect produced by MDA on DNA.

A rise in the 8-OHdG level at the initial stages and a decline therein at the later stage of the stimulated tumor growth are in good agreement with the data obtained previously by other researchers, who note an increase in the 8-OHdG level at early stages of the malignant process and a decrease therein at its later stag-

es [16]. Moreover the authors of the research reports show that there is an increase in 8-OHdG available in oncology patients, who suffer from chronic diseases. A higher rate of production of reactive species of oxygen (ROS) leads to many modifications of the DNA nucleotide bases. 8-hydroxy 2' deoxyguanosine (8-OHdG) is a product of oxidation of the guanine base, which shows the least oxidation potential as compared with other bases. Consequently, the guanine residues are more liable to attacks by free radicals. 8-OHdG attracts a great attention by researchers and is usually involved as a biomarker of oxidative stress indicating DNA damage. This damage of DNA (the 8-OHdG residues) induces a tranversion mutation by pairing with adenine or cytosine in the process of replication. The above type of mutation has been considered to be the major somatic mutation which appears in case of human cancer. As a consequence, the presence of 8-OHdG in the cells bears witness to the mutagenesis ability and increases potential risks of cancerogenesis.

To defend attacks produced by the free radical oxidation system, there is an antioxidant protection system in operation, and, according to our evidence data, within the first two weeks of the melanoma growth stimulated by CNP, weakening of the mitochondria protection takes place due to lowering of the Mn SOD activity and the total SOD activity, but by week three observed has been an activation of the ferments. When comparing the obtained data on the operation of the activators and inhibitors involved in oxidative stress, it can be seen that under weakening of the protection function of the antioxidant ferments we observe an immediate accumulation of the LPO products, while under growing of the antioxidant activity, on the contrary, we can identify a reduction in the LPO product concentration.

Previously, in our research works [17] we have demonstrated that the state of the antioxidant protection system (APS) in humans depends on the volume of the tumor node. Since CNP affects the melanoma progression that is manifested, among the other things, by a double-focused growth of the tumor [12], we cannot exclude the fact of making an effect by the tumor volume on the detected changes in FRO-APS in the heart cell mitochondria. In our experimental study, we have recorded a permanently stable level of SOD 2 within the entire period of the melanoma growth stimulated by CNP, and in this case the SOD 2 activity has been found to be not in conformity with the amount of this ferment in question. It is probable that within the first two weeks of the stimulated melanoma growth the SOD 2 active centers in the heart cell mitochondria have been disabled/closed that has reflected in the value of coefficient SOD activ./antigen. We cannot state that under this variant of the tumor growth, disabling of the above active centers in week 1 and 2 has been performed by neutralizing of the reactive oxygen species, because we have recorded an increase in MDA and 8-OHdG content. It is possible that in this case closing of the SOD 2 active centers is linked with some conformative changes in the protein molecule of the ferment.

Therefore, to generalize the evidence data obtained from our research, we can arrive at conclusions as follows: 1. The revealed changes in the FRO-APS system in the heart cell mitochondria in female mice under the standard and CNP stimulated melanoma growth conditions point to a pronounced systemic character of the damaging effect of the tumor disease on the organism with comorbid pathology. 2. Concomitant chronic neurogenic pain is a contributor to a substantial re-setting of the oxygen-dependant systems in the cell mitochondria of the heart not affected by the tumor that develops stress and promotes further depletion of the heart energy resources under the growing tumor accompanied by CNP.

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.

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References

1. Nguyen BY, et al. Mitochondrial function in the heart: the insight into mechanisms and therapeutic potentials. British journal of pharmacology. 2019; 176(22): 4302–4318. DOI: 10.1111/bph.14431.

2. Murphy E, et al. Mitochondrial function, biology, and role in disease. Circ Res. 2016; 118: 1960–1991. DOI: 10.1161/RES.0000000000000104.
3. Janer A., et al. SLC25A46 is required for mitochondrial lipid homeostasis and cristae maintenance and is responsible for leigh syndrome. EMBO Mol. Med. 2016; 8: 1019–1038. DOI: 10.15252/emmm.201506159.
4. Wortmann S. B., et al. Mutations in the phospholipid remodeling gene SERAC1 impair mitochondrial function and intracellular cholesterol trafficking and cause dystonia and deafness. Nat. Genet. 2012; 44: 797–802. DOI: 10.1038/ng.2325.
5. Khan N. A., et al. Effective treatment of mitochondrial myopathy by nicotinamide riboside, a vitamin B3. EMBO Mol. Med. 2014; 6: 721–731. DOI: 10.1002/emmm.201403943.
6. Vyas S., et al. Mitochondria and cancer. Cell. 2016; 166: 555–566. DOI: 10.1016/j.cell.2016.07.002.
7. Kit O.I., Franciyanc E.M., Kaplieva I.V., Trepitaki L.K., Evstratova O.F. A method for producing liver metastases in the experiment. Byulleten' eksperimental'noj biologii i mediciny. 2014. 157. №6. S. 745-747. [in Russian]
8. Kit OI, et al. Neoangiogenesis and fibrinolityc system biomarkers expression in the dinamics of experimental kidney ischemia in rats/ Eksperimental'naya i klinicheskaya urologiya. 2015;1:20-3. [in Russian].
9. Zhukova GI, et al. Effekty kombinirovannogo vozdejstviya nizkointensivnogo elektromagnitnogo izlucheniya millimitrovogo diapazona i kompleksov nezamenimyh aminokislot u krys-opuholenositelej starcheskogo vozrasta. Yuzhno-rossijskij onkologicheskij zhurnal. 2020;1(4):38-46 [in Russian]
10. Kit OI, et al. Processy samoorganizacii mitohondrij pri roste eksperimental'nyh opuholej v uslovi-yah hronicheskoj nejrogennoj boli. Izvestiya vysshih uchebnyh zavedenij. Severo-Kavkazskij region. Seriya: Estestvennye nauki. 2019; 2 (202):97-105. [in Russian]
11. Bhattacharyya A, et al. Oxidative stress: an essential factor in the pathogenesis of gastrointestinal mucosal diseases. Physiol Rev. 2014; 94: 329–354. DOI: 10.1152 / Physrev.00040.2012.
12. Kit OI, et al. Vliyanie hronicheskoj nejropaticheskoj boli na techenie zlokachestvennogo processa melanomy V16/F10 u samcov myshej. Izvestiya vysshih uchebnyh zavedenij. Severo-Kavkazskij region. Seriya: Estestvennye nauki. 2019; (201):106-111. [in Russian]
13. Egorova MV, Afanasiev SA. Isolation of mitochondria from cells and tissues of animals and human: Modern methodical approaches. Sibirskiy meditsinskiy zhurnal – Sibirian Medical Journal. 2011; 26(1-1): 22-28 [in Russian]
14. Franciyanc EM, et al. Vliyanie nokauta po genu urokinazy na rost melanomy v eksperimente. Sibirskij nauchnyj medicinskij zhurnal. 2019;39(4):62-70. [in Russian]
15. Georgia-Persephoni Voulgaridou, et al. DNA damage induced by endogenous aldehydes: Current state of knowledg. Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis. 2011; 711(1–2, 3): 13-27. DOI: 10.1016/j.mrfmmm.2011.03.006.
16. Essam Eldin Mohamed Nour Eldin, et al. 8-Hydroxy-2'-deoxyguanosine as a Discriminatory Biomarker for Early Detection of Breast Cancer. Clinical Breast Cancer. 2019; 19(2): e385-e393. DOI: 10.1016/j.clbc.2018.12.013.
17. Rozenko LY, et al. Vliyaet li ob"em opuholi na sostoyanie antioksidantnoj zashchity organizma. Voprosy onkologii. 1999; 45(5):538-41. [in Russian]