Dear Colleagues!

We are pleased to inform you that the All-Russian Scientific & Practical Conference on fundamental oncology with international participation, titled FUNDAMENTAL RESEARCH IN ONCOLOGY 2023, has been held.

About 200 experts took an active part in the work thereof, among which were full academicians and corresponding members of the Russian Academy of Sciences, professors, doctors and candidates of science. It is such a large cohort of highly qualified experts from Moscow, Tomsk, Krasnoyarsk, Novosibirsk, Rostov-on-Don, Saratov, Simferopol, Minsk, Osaka and London, representing various scientific centers and cities, who joined their efforts to demonstrate the living vitality of the fundamental science and answer the main question about the relevance, timeliness and demand for fundamental research in modern oncology. You can find Conference papers published herein, in November issue 29 of the journal Cardiometry.
**Introduction.** Drug-induced nephrotoxicity is a common side effect in chemotherapy treatment for cancer patients. Every year the relevance of this problem increases due to the widespread use of various chemotherapy (CT) protocols, including nephrotoxic drugs, in particular cisplatin. Standard measures of kidney function include glomerular filtration rate (GFR), serum creatinine (sCr) and blood urea nitrogen concentrations. However, their changes are revealed after a rather long period after nephrotoxic exposure only; in addition, they deviate significantly from the norm only with a significant degree of kidney damage. Therefore, the search for new markers of nephrotoxicity for early and reliable diagnostics of renal dysfunction is an extremely urgent task. One such promising marker is KIM-1.

**Aims:** To investigate urinary KIM-1 (uKIM-1) as a marker for prognosis and monitoring of cisplatin-induced nephrotoxicity in cancer patients.

**Materials and methods.** The study included 19 patients with tumors of various locations (15 men and 4 women), who received chemotherapy at the Moscow Research Oncology Institute named after P.A. Herzen in 2021-2022. 16 of them received chemotherapy containing cisplatin, 3 patients were medicated with oxaliplatin-containing drugs. Morning urine samples to determine the level of uKIM-1 were taken during three cycles of chemotherapy as follows: on the day of the beginning of each cycle, before the administration of cytostatics, and one day after administration of the latter. The content of uKIM-1 (ng/ml) was determined by enzyme immunoassay using the EnzoLife Sciences KIM-1 ELISA test system (USA). The uKIM-1 concentration was normalized to the urinary creatinine (uCr) concentration.

**Results.** Before treatment, the laboratory test parameters of the functional state of the kidneys (sCr and GFR) in the patients were within the range of the reference values. The median uKIM-1/Cr value in the patients at the beginning of treatment was 2.1 (1.4; 3.2) ng/mguCr, which was almost 3 times higher than the median value in healthy individuals (0.8 ng/mguCr). The uKIM-1/Cr levels increased with each chemotherapy course; by the beginning of the 3rd course, it exceeded the upper limit of the norm in 12 patients (63.2%), and the median increased to 4.9 (1.9; 6.5) ng/mguCr.

In most observations, an increase in uKIM-1/Cr was recorded already within 24 hours after the administration of cytostatics. At the same time, the average level of sCr, GFR and urea remained within the reference values throughout those 3 courses of chemotherapy (CT). An increase in the level of uKIM-1/Cr (by 0.6 ng/mguCr or more) after the first administration of cytostatics was detected in 13 patients (68.4%), after the second administration - in 11 patients (57.9%), and after the third administration - in 13 patients (68.4%).

The probability of an increase in uKIM-1/Cr one day after the administration of cytostatics in the test group became greater with an increase in the value of the indicator immediately before the next course of treatment. Thus, in cases where uKIM-1/Cr did not exceed 3.0 ng/mguCr before the administration of cytostatics, no episodes of a significant rise of the indicator after the administration of cytostatics were observed. In those cases, where uKIM-1/Cr before the introduction of cytostatics varied in the range from 3.1 to 6.0 ng/mguCr, one such episode was recorded (5.6% of the cases). In the observation group, when the level of uKIM-1/Cr before the start of the next
course exceeded 6.0 ng/mg uCr, a significant rise of the indicator was detected in 41.7% of the cases.

Conclusion. The results obtained by us bear witness to the significance of uKIM-1/Cr as a potential risk factor for the development of AKI. An initially elevated uKIM-1/Cr level or its increase at the beginning of the next course of chemotherapy may be the basis for taking enhanced preventive measures or changing the antitumor treatment regimen to a less nephrotoxic one. The development of reliable objective criteria for kidney damage using uKIM-1/Cr and the optimal timing of their analysis require their further research.

Keywords: KIM-1, Nephrotoxicity, Cisplatin, Chemotherapy

TUMOR GROWTH ADHESION

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Background. The concept that the key mechanism of the tumor process is the disruption of adhesive interactions is based on the participation of local, general and central mechanisms.

Methods. Immunofluorescent, immunohistochemical, immunoenzyme, morphological, biochemical research methods, as well as statistical analysis were used in the research.

Result. The local features of adhesion dysregulation include insufficient expression of histospecific adhesion molecules resulting from a genetic mutation, which damages an important mechanism of antitumor tissue defense, disrupting the processes of proliferation and differentiation. Deficiency of histononspecific homotypic adhesion molecules, which arises much later, escalates the disorders. This leads to general contact “breakdowns”: firstly, to a decrease in the expression of ligands of the β2 family of leukocyte integrins (LFA-1, Mac-1) on the surface of immune effectors, and secondly, to increased expression on tumor cells of molecules of adhesion to the substrate, late activation antigens VLA (very late activation) of the β1-integrin family. The first event limits the interaction of molecules of the ICAM family with their counterreceptors from the β2-integrin family, reducing the elimination of target cells by immune effectors, which contributes to shielding the tumor from immune surveillance. The second “breakdown” promotes tumor invasion and the formation of blood vessels - heterotypic adhesion with other cells and tissues, which additionally stimulates the processes of cell proliferation and tumor growth. Thus, adhesion molecules can be compared to a phoenix bird: disappearing at the beginning of the process (between “native” cells), they appear again, but in a different capacity (strengthening adhesion to “foreign” cells), elevating the totalitarian behavior of the tumor. It should be taken into account that tumor cells, due to adhesion dysregulation, lose their differentiation, losing their maturity, and are “isolated from society”, being unable to carry out their specific, “adult” functions. Therefore, tumor growth can be considered as rapid aging of organ cells.

Conclusion. Features of local adhesion dysregulation, which provides the basic properties of the tumor: loss of tissue control of proliferation, anaplasia, invasion, metastasis, deficiency of immune surveillance, can be controlled by central mechanisms involving the dopaminergic system, which is able, using immunoadhesive interactions, to regulate the active phase of immune reactions against tumors, interfering with the process and thus interrupting the development of a malignant neoplasm initiated by a local mutation in a specific tissue. The concept reveals the stress character of cancer etiology and creates prospects for new methods of diagnosis, prevention and treatment of tumors, which could be another step towards solving the problem of malignant neoplasms.

Keywords: Homotypic, Heterotypic adhesion, β1 and β2 integrins, Cadherines, Dopamine, Malignant neoplasms, Aging, Mutations.
PHENOTYPIC COMPOSITION OF TUMOR CELLS AND THEIR ROLE IN THE PROCESS OF METASTASIS WHEN DEVELOPING A PDX MODEL OF BREAST CANCER

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Introduction. To study the mechanisms of metastasis, one of the effective tools is mouse models using primary patient tumors (Patient-Derived Xenograft, PDX). The phenomenon of changes in the cell phenotype in response to the dynamic conditions of the tumor microenvironment is one of the key processes forming the basis for metastasis.

The aim hereof is to investigate the phenotypic composition of tumor cells during the process of metastasis using an example of the PDX models of breast cancer.

Materials and methods. Balb/c nude mice (♀, 8 weeks) were used in our experiment. The first PDX model was developed using a suspension of primary tumor cells obtained from the surgical material of 14 patients with breast cancer; the suspension was injected with DMEM and Matrigel in a volume of 100 μl. In the second model, a fragment of the primary tumor from 16 patients with breast cancer was sutured into the breast area; after the formation of the vascular network, it was partially removed. Tumor cell phenotypes were identified by flow cytometry (NovoCyte 3000 ACEA Biosciences, Agilent, USA) using antibodies to CD45, EpCam, CK7/8, CD44, CD24 and N-cadherin.

Results. In 7 of 40 mice, voluminous formations sufficient for phenotyping were obtained. The most noticeable group consisted of cells with the CD45- mEpCam- CK7/8- CD24+ N-cadh- phenotype, their cellularity ranged from 4.5 to 85.3%. In 5 of 7 xenografts, an increase in the share of the cells, compared to the primary tumor, averaged 96.5%. Metastasis occurred in two mice. In the metastases, an increase in the number of cells with the CD45- mEpCam-CK7/8-CD24+N-cadh-icEpCAM-panCK-CD133-ALDH1-Ki67- and CD45- mEpCam-CK7/8-CD24+N-cadh-icEpCAM-panCK-CD133-ALDH1-Ki67+ phenotypes by 4 – 6.8 times was noted, compared to the primary tumor.

Conclusion. The phenotypic composition of the primary tumor, the xenografts and the metastases is identical, but some differences are observed at the quantitative level of the tumor cell populations.

Keywords: Patient-Derived Xenograft PDX, Breast cancer, Model, Balb/c nude mice

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MOLECULAR PROFILE OF METASTATIC COLON CANCER

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Introduction. Metastatic colon cancer (mCC) is a heterogeneous disease, which significantly complicates its prognosis and the choice of an effective treatment strategy for patients. The use of mCC mo-
molecular profiling data will allow identifying an optimal approach to justify the choice of therapy and increase the patient's survival rates.

**Aim.** Our aim was to assess the molecular subtypes of the primary CC tumors and liver metastases based on the transcriptomic analysis.

**Materials and methods.** The study included samples of tumor and adjacent normal tissue of the colon, as well as metastatic nodules to the liver, obtained from two patients before the stage of their antitumor treatment. Using the PureLink RNA Mini Kit (Invitrogen, USA), RNA was isolated from biopsy samples. Sequencing libraries were prepared using the KAPA RNA HyperPrep Kit with RiboErase (HMR) (KAPA, South Africa). Whole-transcriptome sequencing was performed with the NextSeq500 system (Illumina, USA).

**Results.** Our analysis of the studied transcripts of colon tumor tissue using the Phantasus web tool showed the top 20 overexpressed genes: WNT5A, FENDRR, DES, SELENOM, APLN, DMBT1, SNHG3, TSPAN11, MUC1, PCSK1, C1QB, MFSD4A, LEFTY1, NOS2, COL9A1, PDPN, FZD8, TNFRSF11A and CDCA4. The signaling cascades most enriched in the colon tumor tissue were identified: c-Myc, RB/E2F, mTOR, Hedgehog, and oxidative phosphorylation pathways were recognized. Thus, the studied samples of the colon tumor tissue are potentially associated with the canonical molecular subtype of CC (CMS2), which, according to the reference literature, is characterized by activation of the Wnt and c-MYC signaling cascades, a microsatellite-related stable phenotype (MSS), as well as high chromosomal instability (CIN). In the metastatic liver nodules, overexpression of the top 20 genes was detected: ALB, APOA1, FGG, ITIH3, CYP2A6, GC, SERPINC1, CRP, ADH1A, CYP4A11, C8B, ORM2, F11, ACSM5, SERPIND1, HP, FGA, TF, AMBP, P.L.G. A detailed analysis of the signaling cascades showed a high enrichment in those samples of metabolic pathways with xenobiotics and steroids, as well as the cascades of the complement system and the intracellular pathways regulating the coagulation process. Thus, functional annotation of the transcripts of metastatic nodules in the liver allows us to assign them to the molecular subtype CMS3, which is associated with dysregulation of metabolic processes, a heterogeneous MSI phenotype, a low level of CIN and a high occurrence rate of KRAS gene mutations.

**Conclusions.** Fundamental differences have been established in the molecular genetic profile of colon carcinomas and the associated liver metastases. Our analysis of the transcriptome of the colon tumor tissue allows us to classify them as the molecular subtype CMS2; the transcripts of the metastatic nodules in the liver potentially correspond to the CMS3 variant.

**Keywords:** Metastatic colon cancer (mCC), Whole transcriptome sequencing, Consensus molecular subtyping (CMS)

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**MEDICINAL PLANTS CONTAINING ALKALOIDS: PROSPECTS FOR THEIR USE IN ONCOLOGY**

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The aim of our study was to search and identify new promising plant species containing alkaloids in order to develop new effective herbal medicines for antitumor therapy.

**Materials and methods.** At the All-Russian Scientific Research Institute of Medicinal and Aromatic Plants (ARSRIMAP) with participation of the National Medical Research Center of Oncology named after N.N. Blokhin (The N.N. Blokhin National Medical Research Center of Oncology, Moscow) we conducted screening studies (in vitro) of the antitumor properties of more than 30 plant species containing alkaloids. Those plants, which showed the greatest antitumor activity, were properly identified.
**Results.** Studies have been carried out on extracts and individual compounds from a number of plants collected by the ARSRIMAP specialists in their field expeditions and obtained from the Institute’s own bio-collections. The biological diversity of alkaloid-bearing plants is wide and includes representatives of a number of families of higher plants (Amaryllidaceae, Fabaceae, Apiaceae, Papaveraceae, Nymphaceae, Berberidaceae, etc.). Among the most active, the following plants and alkaloids are noted: species of the genus aconite (A. Karakolsky - *Aconitum karakolicum* Rapaics, A. Baikalsky - *A. baikalense* Turcz. ex Rapaics, family Ranunculaceae), alkaloid of the hemlock spotted (*Conium maculatum* L., family Apiaceae) - conine, alkaloids of the bulbs of the magnificent crocus (*Colchicum speciosum* Steven, family Melanthiaceae) - colchicine and colchamine, alkaloids from the roots of shrubby sophora (*Sophora flavescens* Aiton, family Fabaceae) - matrine and oxymatrine, the alkaloid galantamine from the bulbs of the Siberian sqill (*Scilla siberica* Haw., family Hyacinthaceae) and the Voronov's snowdrop (*Galanthus woronowii* Losinsk. family Amaryllidaceae).

The ARSRIMAP researchers have shown the prospects for use in oncology of the alkaloid nuflein, isolated from the rhizomes with roots of yellow water-lily (*Nuphar lutea* (L.) Sm., family Nymphaeaceae), which shows a 200 times greater activity against human cervical cancer cells than the drug cisplatin.

In our experiments using xenografts of stomach cancer and human hepatocellular carcinoma transplanted into immunodeficient animals, the alkaloid sanguinarine, isolated from the herb of the plants of the genus *Macleaya* (*Macleaya R.Br.*) of the poppy family (Papaveraceae), caused a significant (3-5 times) inhibition of the tumor growth [1]. The safety of the substance used in therapeutic doses and the multiplicity of the mechanisms responsible for implementing its antitumor effect were also demonstrated. It can be assumed that the antitumor effect of the *Macleaya* alkaloids may be enhanced due to systemic effects based on their immunostimulating properties. The alkaloid sanguinarine has also been found to induce apoptosis of tumor cells through the release of nitric oxide (NO) and superoxide radicals in prostate cancer cells, in particular, apoptosis of lymphoma cells in the primary effusion, as well as dose-dependent apoptosis of A431 and NHEKs carcinoma cells [2]. Sanguinarine and chelerythrine are the active substances of the drug “Sanguinarine”, developed by ARSRIMAP.

The antitumor drug “Vincristine”, developed by ARSRIMAP together with the National Medical Research Center of Oncology named after. N.N. Blokhin on the basis of the alkaloid from the herb *Catharanthus roseus* (L.) G. Don, family Apocynaceae, has found its international recognition and is included in the Russian National Registry of Vital Essential Medicines.

The alkaloid matrine, one of the main alkaloids isolated from the rhizomes of *Sophora flavensis*, suppresses significantly the tumor growth and induces apoptosis in vivo and in vitro of the murine hepatocarcinoma H 22 cells.

The experience in using alkaloid-containing plants in medicine by experts of the Phytotherapeutic Society (Moscow) and the St. Petersburg Society of Therapists named after. S.P. Botkin (section of herbal medicine) allows us to recommend the above plants with their antitumor and antiviral activity as an accompanying agent, significant for practical herbal medicine in oncology.

**Conclusion.** We have succeeded in demonstration of the prospects and the needs to expand investigations in the search for new plant species of the world flora containing alkaloids and other classes of biologically active natural compounds for the development of effective herbal medicines, which can be used in complex antitumor therapy both as direct inhibitors of the tumor growth and effective modulators of the immune system to promote the activation of the systemic and cellular mechanisms of the antitumor resistance by the body.

**Keywords:** Alkaloids, Plant bio-collection, Apoptosis, Herbal medicine

The above study was carried out within the framework of the State Assignment (Research work No. FGUU-2022-0014) using bio-objects of the Unique Scientific System “Biocollections of the Federal State Budgetary Institution ARSRIMAP”.

**References**

THREE-DIMENSIONAL IN VITRO MODELS FOR STUDYING THE CHEMOSENSITIVITY OF BREAST CANCER CELLS


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Introduction: Scientists agree that three-dimensional tumor cell cultures reflect the in vitro biological features of malignant tumors to a greater extent than it is the case with the conventional monolayer cultures. In particular, the results of assessing the effectiveness of anticancer drugs obtained in three-dimensional cell models are considered as more accurate and consistent with the tumor response in vivo, where sensitivity is usually lower than that found in the in vitro experiments. Some of the most developed cellular models are structures obtained using three-dimensional bioprinting, which include not only malignant cells, but also biogels to imitate the chemical and mechanical properties of the extracellular matrix of the tumor.

Our aim is to create an in vitro model of breast cancer (BC) using the bioprinting method and study the possibilities of its use to evaluate the effectiveness of antitumor chemotherapy.

Material and methods: Immortalized breast cancer cell cultures BT20, BT474 and MDA-MB-453 were grown under standard conditions as a monolayer in DMEM supplemented with a 10% FBS. To create a three-dimensional culture, the cells were removed from the plastics using a trypsin-versene solution and mixed with GelMA bioink (Cellink, USA) in a ratio 1:10. The final cell concentration in all cases was 2.5 × 10^6 cells per ml. Next, three-dimensional structures were printed with the use of a BIO X bioprinter (Cellink, USA) and cured with light with a wavelength of 405 nm for 20 seconds from a distance of 5 cm. To study the morphology of the cells, square constructs with a side length of 1 cm were printed with orthogonal filling (fill pitch 0.5 cm), consisting of 3 layers.

Next, the constructs were cultured under standard conditions for 1 week, with recording changes in cell morphology using an inverted microscope. To study the response made by the breast cancer cells in the constructs to chemotherapeutic drugs, bioink with cells was deposited into 96-well plates in the form of drops with a volume of 50 μl. Next, a solution of 5-fluorouracil to the culture medium was added into the wells in a set of two-fold dilutions from 10^-1 to 10^-4 mg/ml. After 72 h of cultivation, the number of living cells was determined using the MTT assay. In parallel, a similar experiment was carried out with monolayer cultures of the same breast cancer cell lines.

Results: The breast cancer culture cells showed high proliferative activity, forming colonies of 4-8 cells throughout the thickness of the biogel already on day 3 of the cultivation. After a week of the cultivation, the colony size reached 30-50 cells in all studied cultures. As a result from the study, it was found that the sensitivity of malignant cell cultures to 5-fluorouracil as part of a bioprinted construct has been reduced compared to the monolayer culture. The greatest difference, in that case, was achieved for the BT20 line, for which the IC50 in the monolayer culture was 35 ± 12 μg/ml, while in the three-dimensional model it was recorded to be 64 ± 15 μg/ml. For the BT474 line, those values were 110±19 μg/ml and 131±25 μg/ml, respectively. The MDA-MB-453 line showed the smallest difference between the experimental variants: IC50 in the monolayer culture was 81 ± 9 μg/ml, while in the construct it was 97 ± 10 μg/ml. The difference between the experimental variant with the monolayer and the bioprinted construct variant in all cultures was significant (p<0.01).
**Introduction.** The development of new therapeutic strategies for the treatment of various diseases associated with impaired protein kinase activity is one of the most urgent tasks in modern medicine. The search for new protein kinase inhibitors is an effective approach in the struggle against cancer. However, the existing protein kinase inhibitors may have limitations in their effectiveness or may produce undesired side effects. Therefore, the development of new methods for searching for new inhibitors, which should be more effective and safe, is a topical issue. One of the promising areas is the use of molecular modeling to search for new protein kinase inhibitors.

**Materials and methods.** In our work, automated molecular docking methods were applied in combination with the use of convolutional neural networks of deep learning and molecular dynamics to analyze the general properties of protein kinases and select the optimal method for searching for inhibitors. Molecular docking of a large array of molecules with known biological activity was performed, as well as a quantitative analysis of the relationship between their structure and the ability to inhibit protein kinases was completed [1]. The analysis was performed for the protein kinases MAPK (240 substances), MEK (142 substances), B-Raf (325 substances), PI3K (325 substances), JAK (550 substances), as well as the receptor kinase EGFR (185 substances). Molecular docking was completed using the AutodockGPU software [2, 3], taking into account the mobility of the amino acids of the binding site.

**Results.** For each substance, 100 conformations of the ligand-enzyme complex were obtained. The docking results were analyzed based on the average and minimum binding energies. Molecular docking was also performed using the Gnina software [4]. In that case, the selection of the best conformations and prediction of the inhibition constant were provided using a convolutional neural network included in the Gnina software. For the obtained conformations, the ligand-enzyme complex was simulated by the molecular dynamics method in the Gromacs [5, 6] and Bioeureka [7] softwares. Molecular dynamics simulations made it possible to clarify the characteristics of the binding of ligands to the catalytic site (duration and energy of binding).

**Conclusions.** The results obtained make it possible to construct a high-performance method for a detailed study of the quantitative relationship between the structure and the biological activity for the targeted search for new protein kinase inhibitors.

**Keywords:** Protein kinase inhibitors, Automated molecular docking, Molecular dynamics, Simulation, Convolutional neural networks of deep learning

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SONODYNAMIC THERAPY (SDT) AND GENE THERAPY

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One in Four Deaths in Japan Caused by Cancer.

Cancer is the most common cause of death for Japanese people who are 40 and over, while suicide is the leading cause among young people.

In 2020, 378,356 people died of cancer in Japan, according to a Ministry of Health, Labor, and Welfare report. This accounts for 27.6% of all deaths that year and equates to one in four people dying of cancer. The death rate for cancer—the number of deaths per 100,000 people—has been rising steadily and in 2020 reached 307.0. For men, the type of cancer with the highest death rate at 88.8 was lung cancer (53,244 deaths), followed by stomach cancer (27,769), and colorectal cancer (27,715). For women, the most common type with a death rate of 38.0 was colorectal cancer (24,069) and then lung cancer (22,337).

Cancer is also a leading cause of death worldwide, accounting for nearly 10 million deaths in 2020, or nearly one in six deaths. The most common cancers are breast, lung, colon and rectum and prostate cancers.

Saisei has been collaborating with 6 universities in Japan and actually, 4 out of 6 universities are not medical universities. This is because we need the departments of Technology, Science, Veterinary Medicine and Pharmacy in order to develop new cancer treatments.

The key concept I’d like to discuss is the destruction of local cancer tissue in combination with natural immunotherapy. It is important that we destroy local cancer tissue with minimal side effects and kill cancer cells selectively.

Our goal is to help fight cancer cells with non-toxic medical treatments, with little to no side effects shown for most of our cancer patients.

This time, I’d like to introduce two of our treatments which are Sonodynamic therapy and Liposomal P 53 and PTEN gene therapy.

Sonodynamic therapy (SDT) is a new cancer modality which has huge potential in treating cancer. Photodynamic therapy (PDT) has a limited success due to limited penetration depth (usually 2-5 mm).
Clinically, PDT has a very low success rate for liver and bone tumours and no success in the treatment of brain tumours.

SDT has been considered as a local treatment to destroy local cancer tissue, but SDT can be taken in a hot bath tub as a whole-body treatment. It can target primary tumours, both known and unknown, metastatic tumours, both known and unknown, circulating tumour cells (CTCs) in the blood and disseminated tumour cells (DTCs) in the bone marrow.

Acquired gene mutations are the most common cause of cancer. They occur from damage to genes during a person’s life and they’re not passed from parent to child.

Cancer gene therapy is a type of treatments which uses normal P53 and PTEN genes to destroy cancer cells at Saisei.

New liposomal P53 and P TEN gene therapy were developed by Saisei, collaborating with 2 big laboratories in Japan. We don’t use inactivated viruses as a vector instead we use liposomes to reduce side effects of the gene therapy and to use much higher doses of normal genes. So, gene therapy attempts to introduce genetic materials (DNA or RNA) into living cancer cells to cause apoptosis.

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FEATURES OF BIOCHEMICAL HOMEOSTASIS IN RATS WITH ANTI-TUMOR EFFECT PRODUCED BY IRON NANOPARTICLES

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Introduction. Overloading a tumor with iron (Fe) is a promising strategy for cancer therapy. A number of papers have demonstrated a self-dependent anti-tumor effect of Fe nanoparticles (NPs) in vivo. However, an excessive amount of this trace metal can produce a negative effect on the organism.

The aim of our study was to evaluate the metabolic changes in the rats with lymphosarcoma when exposed to FeNPs with anti-tumor activity.

Materials and methods. The tumor growth in outbred male rats was induced by a subcutaneous injection of 0.3 ml (2~106 cells) of the cell suspension of Pliss lymphosarcoma (Pliss LS). The animals were randomly divided into groups as follows: LS (n=23) and LS+Fe NPs (with treatment, n=41). The rats of the LS+FeNPs group received 8 intratumoral (n=18) or intraperitoneal (n=23) injections of the Fe NPs (20–40 nm) suspension at a total dose of 10 mg/kg body weight. 5–7 days after the 14-day treatment, a share of the animals were examined using the clinical chemistry tests of the blood plasma, and the healthy animals served as the reference group (n=22).

Results. FeNPs, with both types of the administration, showed their anti-tumor effects in 48.7% of the cases; the complete tumor regression was recorded in 17 rats; an inhibition of the tumor growth was found in 2 rats, and a partial tumor regression (by 45%) was revealed in 1 rat; the other animals (51.3%) showed the tumor growth.

In the LS rats, their weight loss by an average of 23% was combined with a decreased values of total protein (by 36%, p=0.000), albumin (by 22%, p=0.000) and urea nitrogen (by 8.3%, p=0.043) vs. the reference group. Also we observed a decrease in the activity of alanine transaminase (ALT) by 29% (p=0.009). In the LS rats, we revealed an increase in the level of triglycerides (TRIGL) in blood plasma by 5.9 times in parallel with a reduction of the level of high-density lipoproteins (HDL) (by 39%, p=0.000) vs. the reference group.

In the rats with the LS growth, after their FeNPs treatment, the loss of their body weight was an average 18.5% of the initial mass. In the rats, receiving NPs, recorded were a decrease in the values of total protein (by 28.0 %, p=0.000) and the largest reduction in albumin (by 37.6%, p=0.000) vs. the reference group; the ALT activity decreased by 26.0% (p=0.005). Also in the rats without anti-tumor effect, the decreased HDL levels (by 42.2%, p=0.000) and the mobilization of triglycerides (3.4 times higher than that in the healthy rats) were found.
In the rats with the tumor regression, after their exposure to FeNPs, compared with the rats with the tumor growth, signs of restoration of the balance of metabolism were observed. The animals showed an increase in their body weight by 8.3%, and the decrease in total protein in them was less pronounced than in the rats with the LS growth (by 8.7% vs. the reference group, p = 0.013). At the same time, in those rats, the albumin level remained low (by 29.0% compared to the healthy rats), and that was also the case with creatinine (by 29.3%, p=0.000) and urea nitrogen (by 38.6%, p= 0.000). In the LS+FeNPs group, the values of lipid metabolism parameters were not significantly different from those recorded in the reference group.

**Conclusion.** Thus, in the LS rats without the above treatment, signs of metabolic disorders characteristic of the tumor progression were observed. The direction and severity of the changes in metabolism in response to the action of FeNPs depended on the presence of an anti-tumor effect. The effect produced by NPs on the rats with the growing tumors did not significantly affect their metabolic status, since it did not differ from the rats without NPs treatment. The reorganization of metabolism in the rats with the tumor regression after exposure to NPs was associated with a change not in lipid, but in protein metabolism.

**Keywords:** Rats, Lymphosarcoma, Metallic iron nanoparticles, Anti-tumor effect, Biochemical homeostasis

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**COMPARATIVE ASSESSMENT OF CYTOTOXICITY AND ACCUMULATION OF LITHIUM AND BORON IN SKIN MELANOMA CELLS IN VITRO**

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**Introduction.** Boron neutron capture therapy (BNCT) is a binary radiation therapy modality based on the high capability of the non-radioactive isotope ¹⁰B to absorb thermal neutrons. The use of alternative isotope ⁶Li for lithium neutron capture therapy (LiNCT) may be a promising area in the treatment of oncological diseases, but at present the data on the possibilities of lithium accumulation in tumor cells are limited to some sporadic studies.

The aim hereof is a comparative assessment of cytotoxicity of various boron and lithium compounds, as well as an analysis of the accumulation of boron and lithium in vitro to determine the possibility of using lithium salts in neutron capture therapy.

**Materials and methods.** Our study used some cell cultures of human skin melanoma SK-Mel-28, murine skin melanoma B16 and human dermal fibroblasts BJ-5ta. In our research, we utilized boron compounds approved for clinical use for BNCT (boron phenylalanine and boron captate), as well as lithium salts most often applied in clinical practice (lithium carbonate, lithium citrate and lithium chloride). The cytotoxicity of the drugs was determined using the MTT assay. The concentration of boron and lithium in the cell precipitate was measured by inductively coupled plasma atomic emission spectrometry (ICP AES). Statistical processing of the results was carried out by applying Statistica 10.0 with the use of the Mann–Whitney U test at a statistical significance level of 95% (p < 0.05).

**Results.** It has been found that the incubation of BJ-5ta dermal fibroblasts with boron phenylalanine and boron captate at a boron concentration of 80 μg/ml in a culture medium for 24 hours does not produce a toxic effect on the cells. The cell survival significantly decreased in the experimental groups as against the reference group at boron concentrations ≥ 160 μg/ml. When incubated with boron captate, the survival rate of the B16 cells decreased statistically significantly at boron concentrations of 160 μg/ml and higher. The cytotoxic effect by boron phenylalanine was observed...
at boron concentrations ≥ 320 μg/ml. Cytotoxicity of boron compounds during the incubation with the SK-Mel-28 cells was noted at medium-related boron concentrations ≥ 640 μg/ml. When the BJ-5ta cells were incubated with three test lithium salts at a lithium concentration of 80 μg/ml (necessary, according to theoretical calculations, for successful neutron capture therapy), no toxic effect was detected. A lithium concentration of 160 μg/ml under the incubation of the BJ-5ta cells with every of the three lithium salts was found to be toxic. Lithium carbonate had no toxic effect on the SK-Mel-28 and B16 melanoma cells in the lithium concentration range up to 160 μg/ml. Lithium citrate also did not make a toxic effect on the B16 cells at a lithium concentration of 160 μg/ml, but however, for the SK-Mel-28 culture, a concentration of 160 μg/ml or greater statistically significantly reduced the percentage of the cell survival compared to the reference group. The survival of the B16 cells when exposed to lithium chloride at lithium concentrations up to 320 μg/ml did not differ significantly from the reference, but for the SK-Mel-28 cells the cytotoxic effect was revealed at lithium concentrations ≥ 160 μg/ml.

The maximum boron concentration was determined, when the SK-Mel-28 and B16 cells were incubated with boron phenylalanine, and it was 0.29 μg/106 cells; Moreover, the highest concentration of lithium was detected during incubation of the B16 culture with lithium carbonate and amounted to 0.79 μg/106 cells.

**Conclusion.** Lithium salts, as well as boron compounds, do not significantly reduce the survival of tumor cells and normal fibroblasts at concentrations of 40-80 μg/ml required for neutron capture therapy. Lithium carbonate is most effectively accumulated by tumor cells, compared to other lithium salts and boron compounds, and can be used as a drug for delivering lithium to a tumor for NCT.

**Keywords:** Neutron capture therapy, Boron-containing compounds, Lithium salts, Skin melanoma

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**STRUCTURAL AND FUNCTIONAL DIFFERENCES IN CARDIAC AND BRAIN MITOCHONDRIA IN ANIMALS WITH DIFFERENT TOLERANCE TO OXYGEN DEFICIENCY**

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**Introduction.** Depending on the individual resistance to hypoxia, many cell-adaptive reactions are formed, but the mechanisms of their occurrence are still not clear. Hypoxia is associated with many biological processes, including pathogenic microbial infection, cancer, acute and chronic diseases, and more other. Hypoxia-inducible factors (HIFs) regulate the expression of a number of genes involved in cellular metabolism, cell growth/death, cell proliferation, glycolysis, immune response, tumorigenesis, and metastasis. The brain and the heart are the organs most sensitive to oxygen deficiency, and their mitochondria, as the primary consumers of cellular oxygen, help tune cellular and organismal responses to hypoxia through structural or functional modifications. To date, there have been no comparative studies of the ultrastructural features of brain and cardiac mitochondria in animals demonstrating different tolerance types to oxygen deficiency: low-resistance (LR) and high resistance (HR) to hypoxia, which influence the general resistance of the body to oxygen deficiency.

**Materials and methods.** The ultrastructure of mitochondria was studied with the JEM 100-B microscope.
(Japan); subsequent data processing was carried out using the Image J software.

**Results.** Based on their localization in the cell, cardiomyocyte mitochondria are usually divided into three mitochondria subpopulations as follows: interfibrillar (IF), subsarcolemmal (SS) and perinuclear (PN) mitochondria. Our research work has established that the LR and HR animals have initial differences in the structure of all three types of cardiac mitochondria, which possibly shape the nature of the resistance of these animals to hypoxia:

1. Normally, the IF mitochondria of both studied phenotypes in the animals had a shape characteristic of that subpopulation and were located clearly along the rows of myofibrils, however, the IF mitochondria of the LR group had a lighter matrix than mitochondria of the HR group. In addition, while the IF mitochondria in the LR animals, as a rule, are located in one row between the myofibrils, in the HR animals they are often localized in 2 or more rows.

2. The SS mitochondria in the LR animals were characterized by the location of one organelle each in the invaginations of the sarcolemmal membrane, which gave the membrane a convoluted shape, while the same mitochondria in the HR group were located in small clusters under the sarcolemmal membrane.

3. The average density of the PN organelles, as well as the average number of small mitochondria, was found to be significantly higher in the HR animals.

Our investigation of the ultrastructure of the prefrontal cortex (PFC) showed that mitochondria both in the LR and HR rats have a round or an elongated shape with a diameter of 0.6-1 μm, without abnormalities in the location of the cristae and without significant damages to the external and internal membranes. At the same time, the ultrastructure of mitochondria in the PFC of the two studied types of animals differs significantly. The morphometric analysis shows that:

1. the number of mitochondria with an electron light matrix in the PFC of the LR animals is 3 times greater than that in the HR animals;
2. the number of small mitochondria measuring 0.14-0.25 microns in the LR animals is almost 3 times less than that found in the HR animals.

All the described changes are characteristic of adaptation to hypoxia.

**Conclusions.** Thus, the data obtained indicate the existence of the basic ultrastructural differences between mitochondria in the heart and the PFC of two animal phenotypes. A reduced matrix density, less dense packing of cristae and a smaller total number and the number of small-sized mitochondria in the PFC and in the IF and PN type of the cardiac mitochondria, as well as the nature of the location of mitochondria in the subsarcolemmal zone in the LR animals, compared to the HR animals, apparently determine the decreased resistance to hypoxia in the LR rats.

**Keywords:** Hypoxia, Interfibrillar, Subsarcolemmal, Perinuclear mitochondria, Ultrastructure

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A MODERN APPROACH TO DISEASE MODELING FOR THE DEVELOPMENT OF ONCOLOGY DRUGS


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**Introduction.** Oncological diseases are characterized by the complexity of pathogenesis and demonstrate a number of specific signs, such as uncontrolled proliferation, stimulation of angiogenesis, activation of invasion and metastasis. Molecular genetic testing to examine genetic abnormalities in tumor cells has been increasingly using in oncology. With an accumulation of the data on the repertoire of mutations
or other events in the patient’s tumors, new goals for the development of more targeted and specific anti-tumor therapies appear. For example, membrane proteins EGFR, HER-2, PSMA and C-met are identified as such targets. Despite the large volume of research conducted, no more than 11% of the total drug candidates are approved by regulatory authorities for clinical use. This is often due to their lack of effectiveness and the incomparability of the results obtained in preclinical trials in animal models with actual results in patients. Therefore, the development of experimental approaches for modeling tumor processes in laboratory animals is an urgent problem.

The aim of our research work is to develop some relevant in-vivo models for studying and assessing the effectiveness of targeted drugs.

**Materials and methods.** Tumor cells from breast cancer lines BT-474 (ATCC®) were used in the present research work; 22Rv1, LnCap and PC-3 prostate cancer cells (ATCC®); bladder cancer cells EJ (D.I. Ivanovsky Research Institute of Virology®) and 5637 (ATCC®); human colorectal cancer cells HT29 (D.I. Ivanovsky Research Institute of Virology®) and HCT116 (ECACC®). Immunodeficient Balb/c nude mice were used as test systems. Tumor cells were inoculated into animals subcutaneously or orthotopically (BT-474 cells were transplanted into the area of fat deposits near the mammary glands, 22Rv1, LnCap and PC-3 were inoculated into the inguinal fold area, and EJ and 5637 were transplanted intravesically).

**Results.** It was shown in-vitro and in-vivo, by immunocytochemical and immunohistochemical methods, respectively, that the BT-474 breast cancer tumor cells have a pronounced ability to express the membrane receptor of the epidermal growth factor receptor family (EGFR/ErB) - HER-2; prostate cancer cells 22Rv1 and LnCap – the receptor for prostate-specific membrane antigen PSMA; bladder cancer cells EJ and 5637 – epithelial growth factor receptor EGFR; colorectal cancer cells HT29 and HCT116 – C-Met receptor associated with the epithelial-mesenchymal transition gene Met. The PC-3 prostate cancer cells are characterized by weak, conditionally “negative”, expression of the PSMA receptor.

**Conclusion.** Thus, the models (the ectopic and the orthotopic ones) of human tumors of various histogenesis have been developed, produced by inoculation of tumor cells with high expression of target proteins, that can be effectively used for preclinical study of targeted drugs with targeted drug delivery to tumor cells.

**Keywords:** In-vivo, BT-474, 22Rv1, LnCap, PC-3, EJ, 5637, HCT116

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**CHANGES IN THE ENERGY ACTIVITY OF BLOOD LYMPHOCYTES AND MORPHOMETRIC PARAMETERS OF THE BRAIN IN A MODEL OF BRAIN DAMAGE IN RATS**

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**Introduction.** It is known that Parkinson’s disease (PD) being a neurodegenerative disease is accompanied by increased levels of reactive oxygen species, oxidative stress and impaired mitochondrial activity. It has been shown that the development of malignant tumors can be accompanied by disorders that mimic PD. In this regard, shifts in cellular metabolism associated with the development of true PD are of interest.
The aim of our research work was to determine the activity of the mitochondrial and glycolitic marker enzymes succinate dehydrogenase (SDH) and lactate dehydrogenase (LDH) in blood lymphocytes and the level of oxidative stress in the body in rats in an experimental model of Parkinson’s syndrome induced by the introduction of the neurotoxin 6-hydroxydopamine (6-OHDA). In addition, our aim was to investigate the effect made by the mitoKATP binding channel activator Uridine on animals with PD.

**Materials and methods.** The study was carried out in male Wistar rats (n=27), weighing 200-250 g, kept by the facility at the Institute of Theoretical and Experimental Biophysics at the Russian Academy of Sciences. Pathology modeling was carried out by bilateral stereotaxic injection of neurotoxin (6-OHDA) into the substantia nigra. To activate the mitochondrial ATP-dependent potassium channel (mito-KATP), an intraperitoneal administration of Uridine at a dose of 30 mg/kg body weight was used for 22 days. Determination of the activity of SDH and LDH was performed by the developed Cytobiochemical (CBC) method under the reduction of Nitroblue tetrazolium in immobilized blood lymphocytes. Lipid peroxidation products were assessed by the amount of malondialdehyde (MDA). Histological cryotomic sections of the striatum were stained with hematoxylin and eosin.

**Results.** When the neurotoxin 6-OHDA was injected into the substantia nigra, the animals experienced a 70% impairment of the motor function in the rotarod test. When determining the content of lipid peroxidation products in mitochondria of the cerebral cortex and in the blood serum in the animal group with the neurotoxin, the level of MDA in mitochondria of the rat brain was 18% higher, and in the blood serum it was increased by 37% as against the reference group. The administration of Uridine to sick animals reduced that indicator to the reference level. The introduction of the mito-KATP inhibitor - 5-HD removed the positive effect of Uridine in blood serum and led to an increase in the content of the MDA products by 45%. The CBC measurement of the SDH activity in lymphocytes in the PD rats did not show significant differences, however, the administration of Uridine to sick animals led to an increase in the SDH activity by 80% as compared to the reference group. The measurement of the LDH activity in the rats with PD reliably showed an increase in that activity type by 35% of the reference group value, and treatment with Uridine reduced that activation to the level of the reference values. The introduction of 5-HD removed the effect of Uridine both in mitochondria and cytosol of lymphocytes.

**Conclusions.** Thus, in a model system of Parkinson’s syndrome in rats, induced by the administration of 6-OHDA, considering blood lymphocytes, the predominance of glycolysis over oxidative phosphorylation of mitochondria was revealed. The administration of Uridine led to an increase in mitochondrial respiration by 80% and a decrease in glycolysis. An increase in lipid peroxidation was found in the blood serum of sick animals compared to the reference values that was also removed by Uridine. In sick animals, an increase in the number of dead neurons in the striatum and their restoration in rats with Uridine was also detected. An inhibitory analysis showed that the therapeutic effect of Uridine is associated with the activation of the mitoK-ATP channel.

**Keywords:** Mitochondria, Blood lymphocytes, Succinate dehydrogenase, Lactate dehydrogenase, Lipid peroxidation, Parkinson’s disease

*The above research work was supported by the Russian Science Foundation grant No. 23-25-00441.*
**Introduction.** Morphological studies represent the most important part of evidence-based medicine, and in oncology they are an essential attribute in the diagnostics of malignant neoplasms. In experimental oncology, current models of the tumor growth are developed to be as close as possible to the actual biological life conditions. There is a need to study the pathogenesis of tumors in various variants of their orthotopic growth, the formation of bi-model systems with a combination of the malignant growth and comorbid conditions (chronic neurogenic pain, diabetes mellitus, hypothyroidism, obesity), the use of subcellular substrates for the induction of carcinogenesis and biotherapy. Among the important methods for studying the pathogenesis of tumors, morphology is on a par with advanced molecular genetic and biochemical methods, as well as radioimmunoassay techniques.

The aim of our research work is to study, identify and discover some structural transformations in tumors and organs in experimental animals, when modeling the processes of malignant transformation under the conditions of comorbid pathology and mitochondrial therapy.

**Materials and methods.** This work is based on morphological data from our examinations of the tumor tissue, the heart, the liver and other visceral organs in tumor-bearing animals under conditions of chronic neurogenic pain (CNP), diabetes, hypothyroidism, and biotherapy. The prepared tissue sections were fixed with formaldehyde, followed by staining with hematoxylin-eosin. We used the methods of light, dark field and polarization microscopy techniques with the Leica DM LS2 microscope having a magnification of x10, x40, x100, by capturing photo and video images with a digital camera OLYMPUS Camedia C-5050 (Germany) and device OLYMPUS S-3040 ADU (Japan) to supply the obtained image data to a computer.

**Results.** When creating an experimental model of synchronous primary multiple polyneoplasia by simultaneous transplantation of a suspension of the melanoma B16/F10 and sarcoma 45 cells in the regional proximity, an accelerated production and a rapid growth of the tumors were observed, and melanoma metastasized in addition to typical sites in sarcoma. The morphological picture of the metastasis was characterized by the appearance of signs of cellular mimicry on the part of melanoma (such as melanosarcoma), enhancing the growth of sarcoma 45. The structural features of malignant transformation under the CNP conditions, as well as against the background of such comorbid conditions as diabetes, hypothyroidism, included a powerful stimulation of the tumor growth, generalization and spreading it to the liver, the kidneys, the ovaries, and the lungs. The morphological examination of the myocardium under CNP and the tumor growth revealed deep dystrophic and necrotic changes characteristic of the development of extensive infarctions. However, experimental mitochondrial biotherapy in more than 70% of the cases helped prevent the development of structural and functional disorders of the heart muscle in the tumor-bearing animals with CNP, and it was also accompanied by suppression of the growth and metastasis of the tumors that was morphologically confirmed.

**Conclusion.** Visualization of the microstructure of the experimental tumors under the conditions of modeling polyneoplasia, the bi-model systems, including the tumor growth against the background of the comorbidities, the malignized and non-malignized organs in the tumor-bearing organism, has revealed the nature and the mechanisms of the cellular relationships and interactions and documented the effectiveness of mitochondrial biotherapy in suppressing the growth of the malignant tumors with preventing disorders in the performance of the vital organs.

**Keywords:** Morphological studies, Malignant tumors, Comorbid conditions, Mitochondrial therapy
Introduction. It is well known that extraordinary stress or chronic exposure to stressors is characterized by adverse immunological consequences due to stress-induced immunosuppression. It has been established that stress causes an increased incidence of malignant tumors and also worsens the prognosis in patients with already existing tumors. Among the mechanisms of carcinogenesis under stress, there is an increase in the level of cortisol, which has a significant inhibitory effect on the immune system and the secretion of pro-inflammatory interleukins. Post-traumatic stress disorder (PTSD) involves inactivation of the hypothalamic-pituitary-adrenal (HPA) axis and decreased secretion of cortisol in humans, or corticosterone in rats and mice. However, the question of whether PTSD is a risk factor for the development of cancer or tumor growth remains practically undeveloped.

The aim of the study is to use predator stress to create an experimental model of PTSD in order to investigate the characteristics of the growth and development of transplantable melanoma in sexually mature male C57Black/6 mice.

Materials and methods. Mature male C57Black/6 mice (n=40) were randomly divided into 2 groups as follows: the reference group (n=20), covering the mice without stress, implanted with a suspension of B16 melanoma cells (1.44x106 cells per mouse in the area of the left scapula) and the experimental group (n=20), where PTSD was modeled by inducing predator stress in the mice (with the use of cat urine odor for 10 min, within 10 days, later under the normal vivarium conditions for 14 days), after which the experimental animals were implanted with B16 melanoma cells. After 10 days, all animals were tested in the elevated plus maze (ECM) and then subjected to decapitation under anesthesia; the tumor was excised, its size was measured, and then histological sections were made, which were stained with hematoxylin and eosin. The level of corticosterone and interleukins in blood was determined using the ELISA method: IL-2, IL-6, IL-4, IL-10. Statistical methods were applied to process the data. All experimental procedures were carried out in accordance with Directive 2010/63/EU of the European Parliament and of the Council of 22 September 2010 on the protection of animals used for scientific purposes.

Results. The development of PTSD was judged by the conventional signs of the behavior for the model in question, manifested during testing in the ECM, as well as by a decrease in corticosterone levels. Compared to the reference group animals, the concentration of pro-inflammatory IL-6 and IL-2 was 40% higher, and the level of anti-inflammatory IL-10 and IL-4 was lower by 14% and 30%, respectively. The tumor volume and its thickness in the stressed mice were 49% smaller, the number of cells in the tumor was reduced by 18%, and the mitotic index was reduced by 69%.

Conclusion. When modeling PTSD, the factors responsible for inhibiting the growth of B16 melanoma are an increase in the concentration of pro-inflammatory interleukins and a decrease in the secretion of anti-inflammatory cytokines.

Keywords: C57Black/6 mice, B16 melanoma, Post-traumatic stress disorder, Corticosterone, Anti-inflammatory and pro-inflammatory interleukins.
**Introduction.** The process of transferring the technology for obtaining a drug from the laboratory to production and, then, to the clinical practice is always a very complicated and time-consuming way, requiring many adaptation solutions. Regarding biomedical cell products (BMCP), this is even a more complex process, requiring large-scale expansion of procedures to obtain clinically significant numbers of cells, adaptation to reagents and equipment, and establishment of safety parameters for reagents, equipment and the final product. Nevertheless, cell therapy has become an integral part of treatment regimens for malignant neoplasms. Today, thousands of studies of BMCP are being conducted around the world, both in mono-regimen and in various regimen combinations.

The quality of the final product at output, regardless of the production base (a research center or a pharmaceutical company), must be impeccable. In the process of developing an innovative medical drug, a cellular product, its composition, quantitative and qualitative indicators and their reference values are to be determined. Although currently in the Russian Federation there is no regulatory document defining the requirements and methods for establishing the quality of BMCP, research centers developing products for cell therapy, in the process of work, shall specify in each individual case the required quality indicators to be achieved by the product in question.

A mandatory test for BMCP is an assessment of sterility, which can be carried out in accordance with GPM 1.2.4.0003.15, and microbiological purity in accordance with GPM 1.2.4.0002.15.

**Materials and methods.** A very important indicator is the viability of cells in the final population. An acceptable indicator is considered to be at least 70% of living cells. For evaluation, trypan blue assay method and/or flow cytometry with 7-AAD (7-aminoactinomycin D) staining are used. In our studies, the survival rate of freshly prepared cells varied from 80 to 95%. If the cells were cryopreserved, then immediately after thawing the cell survival rate ranged from 70 to 90%. Very often, after thawing, the cells die within 24 hours, and the viability can decrease by up to 50%. In most cases, the cells are prepared for infusion as soon as possible after thawing to ensure their proper viability.

Since the NK cells are, in most cases, grown with feeder cells, an assessment of the presence of the residual feeder cells should be mandatory. The genetically modified K562-mIL15-mIL21-41BBL cells were used by us as a feeder line. For the feeder cells, specific surface markers were identified with flow cytometry. In our case, it has been shown that the specific markers of the feeder cells have not been found in the target population of the NK cells.

**Results.** In our case, the NK cells were identified by using the phenotype CD3-/CD16+/CD56+. Upon completion of the cultivation, the percentage of the NK cells in the produced test cell population amounted to 85-95%.

For the purpose of clinical use, the most significant event/effect of therapy is the patient’s response to therapy and, accordingly, tumor regression. The main biological function of the NK cells is to protect the body from foreign and pathological cells. Therefore, assessing an effectiveness of the final product in this case is identifying the biological function of the NK cells: this is cytotoxicity.

The cytotoxic activity of the above cells can be measured either indirectly by changes in the degranulation marker (CD107a) with flow cytometry or in a direct cytotoxic test with fluorescence imaging, when testing effectiveness against tumor cell lines. When the NK cells and the K562 cells were cocultured, the degranulation marker on effector cells increased on average 4 times compared to the intact cells. In a direct cytotoxic test against tumor cell lines (K562, melanoma, neuroblastoma), a 100% target death was observed after 48-72 hours of cocultivation.
Conclusion. Since at present there are no applicable guidelines or regulations available, each manufacturer has to decide what quality control methods are to be used, considering their feasibility, applicability and cost-effectiveness. So, at present, the proper selection and application of methods for assessing the quality of BMCP is an urgent problem to be solved.

Keywords: NK cells, BMCP, Quality control, Cytotoxicity test

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DENDRITIC CELLS AND THEIR ROLE IN CANCER IMMUNOTHERAPY

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Introduction. Cytotoxic CD8+ T lymphocytes (CD8+ CTLs) are key effector cells, which recognize and kill tumor cells, and are therefore preferred targets for improved cancer immunotherapy.

Aim: To investigate the role of dendritic cells (DCs) in the initiation of antigen-specific immunity in cancer immunotherapy.

Materials and methods. Understanding the regulation of molecular interactions between the T cells and the tumor cells, coupled with improved T cell engineering technologies and the discovery of tumor-specific antigens, has resulted in the emergence of new cancer immunotherapies with unprecedented clinical efficacy. These treatments aim to activate and propagate tumor-specific CTLs to kill primary cancer cells. The most effective modern methods of cancer immunotherapy include the use of immune checkpoint inhibitors, cancer vaccines based on tumor-specific antigens (based on peptides or RNA), ex vivo immunotherapy with the method of adoptive transfer or native cells (clones of CD8+ cytotoxic T lymphocytes, TIL cells from tumor-infiltrating lymphocytes), or the T cells engineered to express the T-cell receptor (TCR) or chimeric antigen receptor (CAR) [1,2,3].

The concept that the immune system can be employed through vaccination to destroy malignant cells has been reproduced more than once in animal models, but sometimes it has been challenged by some results from human trials [4].

Results. Dendritic cells (DCs) are central to the initiation of antigen-specific immunity. Dendritic cells are professional antigen-presenting cells (APCs), which can capture and present various foreign proteins and tumor antigens on the cell membrane to activate T and B lymphocytes. The mechanism of action of DC vaccines is to stimulate and maintain an immune response aimed exclusively at eliminating tumor cells in the body.

Clinical trials have shown that the modified tumor cells are safe and make a positive effect on the anti-tumor immune memory. One of the main advantages of whole tumor cell vaccines is their ability to present the full spectrum of tumor-associated antigens to the patient’s immune system [5].

Conclusions. The success of cancer immunotherapy depends on the induction of immune effector mechanisms associated with the generation of tumor-specific cytotoxic T lymphocytes with high avidity. To further improve their antitumor efficacy and provide a more reliable, long-term disease control, a better understanding of the interactions between the immune system and the tumor, as well as of the tumor immune evasion strategies, is required. Overcoming immune tolerance pathways in the tumor microenvironment, which may reduce the effectiveness of immunotherapeutic approaches, is a major challenge in the field of tumor immunology and immunotherapy. In this context, an optimization of the immune system's therapeutic potential depends on a combination of different approaches, mainly cancer vaccines, which synergistically enhance the antitumor T cell response.

Keywords: Dendritic cells, Cancer immunotherapy, DC vaccines, Antitumor T cell response
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THE INFLUENCE OF A PERIPHERAL TUMOR ON THE BEHAVIOR OF ANIMALS
AND THE STRUCTURE OF NEURONS IN THE PREFRONTAL CORTEX

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Introduction. Melanoma is the most aggressive of
the common forms of skin cancer. Proinflammato-
ry cytokines produced by the tumor and delivered
via the circulatory and lymphatic systems can lead to
damage to the structures of the central nervous sys-
tem. Damage to the blood-brain barrier and the en-
dotheilum of blood capillaries can cause dysfunction
of neurons in the cerebral cortex, induce changes in
neural plasticity, cognitive functions and result in the
development of depression. Depression is observed
in 20% of the cancer patients, and it affects quality of
life, treatment effectiveness and survival. Depression
has been linked to damage to the prefrontal cortex,
which plays an important role in many brain func-
tions, including cognition, emotion regulation, mo-
tivation and sociability.

The aim hereof is to evaluate the behavior of an-
imals, as well as identify the ultrastructural features
of pyramidal neurons of the prefrontal cortex when
modeling the peripheral tumor growth.

Materials and methods. Two groups of male mice
of the C57BL/6 line (n=5) were used in the exper-
iment: intact animals (the reference group) and an-
imals with a tumor. The B16 melanoma cells were
injected subcutaneously into the right inguinal fold
at a dose of 1*106. On day 17 after the introduction
of the tumor cells, the animal behavior was tested.

In the Open Field test, animals were placed in a cir-
cular arena with a diameter of 60 cm and allowed to
freely explore the space for 5 minutes. The length of
the path, the time the animal spent in the center of
the arena, the number of washes, and the numbers
of their vertical stands were assessed. The Forced
Swimming test was carried out in cylinders 30 cm
high, which were filled with water. The mouse was
immersed in water for 6 minutes, and the time of
mobility of the animal was recorded. Fragments of
the prefrontal cortex were duly prepared according
to standard techniques for transmission electron mi-
croscopy. Using the morphometric analysis, the vol-
umetric density (VV) of organelles in the cytoplasm
of neurons was calculated. The statistical signifi-
cance of differences between the studied parameters
was determined using the Mann-Whitney U test.
Differences were considered statistically significant
at p<0.05.

Results. When assessing motor activity in the Open
Field test, in the group with tumor growth modeling,
an increase in the length of the traveled distance by
29% was observed that indicated an elevation in the
anxiety of the animals. In the group of the animals
with the tumor, other signs of activity were more often

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noted: the animals jumped and clung to the edge of the arena. In the Forced Swimming test, the mobility time of animals in the group with the tumor growth decreased by 16%. A reduction in the mobility time in this test indicates the development of depressive-like behavior.

When analyzing the ultrastructure of the pyramidal neurons of the prefrontal cortex in the animals with the peripheral tumor, a 3.43-fold increase in the volumetric density of mitochondria with destructive changes in the cristae was observed that amounted to 47.6% of the total number of mitochondria in that group. An expansion of the lumens of the endoplasmic reticulum (ER) cisterns was observed, the volumetric density of which was 3.25 times higher than the indicator in question in the reference group. The volumetric density of free polysomal complexes has decreased by 74%.

**Conclusion.** The study suggests that the animals with the peripheral tumor growth develop anxiety-depressive behavior. At the same time, in the neurons of the prefrontal cortex of the brain there are an increase in mitochondria with destructive changes, an expansion of the lumen in the ER cisterns and a decrease in the number of polysomal ribosomal complexes that may indicate a reduction in the energy function of the cell, the development of ER stress and disruption of protein synthesis and folding.

**Keywords:** B16 melanoma, Depressive-like behavior, Neuronal ultrastructure

**MicroRNA miR-204-5p IS A REGULATOR OF APOPTOSIS IN DACARBAZINE-RESISTANT MELANOMA CELLS**

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**Introduction.** Therapy for skin melanoma in its disseminated form is ineffective, among other things, due to the development of tumor cell resistance, mediated by various mechanisms. The acquisition of a stable cell phenotype is associated both with genetic and epigenetic changes mediated by the regulatory non-coding microRNA molecules.

The aim hereof is to investigate the ability of microRNA miR-204-5p to influence changes in the cell cycle and apoptosis of melanoma cells resistant to the chemotherapeutic alkylating agent dacarbazine, used in standard chemotherapy for melanoma.

**Materials and methods:** Cultivation of the SK-MEL-2 melanoma cells (ATCC® HTB-68”), determination of the half-maximal (50%) inhibitory concentration (IC50) based on the colorimetric method for assessing the metabolite 3-(4,5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT), transient transfection into melanoma cells SK-MEL-2 of a synthetic analogue of microRNA miR-204-5p (mimetic), study of cell cycle phases (identification of G0 phase) and detection of cell apoptosis by flow cytometry, immunofluorescence assay, assessment of gene expression by real-time PCR.

**Results.** Transfection of the microRNA miR-204-5p mimetic into skin melanoma cells exposed to dacarbazine at a concentration of 4xIC50 has resulted in an increase in the share of apoptosis of the latter that confirms the involvement of the studied microRNA molecule miR-204-5p in the regulation of dacarbazine-induced apoptosis, but however it has not affected the phases of the cell cycle, in particular, the exit of the G0 phase.

**Conclusions.** Generally, the results of this study have demonstrated that the microRNA miR-204-5p can influence the resistance of cutaneous melanoma tumor cells to chemotherapeutic drugs, mainly via modulating apoptosis.

These data suggest that modulation of microRNA miR-204-5p levels may be considered as a feasible therapeutic approach to combination treatment of melanoma.

**Keywords:** Melanoma, Cell cycle; Dacarbazine, microRNA, Chemoresistance
Introduction. Escape from cell death is one of the most prominent features of tumor cells and is closely related to the dysregulation of Bcl-2 family proteins. Among them, the anti-apoptotic protein Mcl-1 (myeloid cell leukaemia-1) acts as a master regulator of apoptosis in various human malignancies. The Mcl-1 protein, whose main function is to protect tumor cells from apoptosis, is not the only product of the anti-apoptotic gene MCL1. On the contrary, another product of this gene, the shortened Mcl-1S protein, formed by alternative splicing, serves as a negative regulator of Mcl-1. Increased levels of Mcl-1S lead to inhibition of apoptosis-protective Mcl-1 and, consequently, tumor cell death by apoptosis (Senichkin, 2018). Mcl-1 inhibitors are currently being developed, which opens new perspectives to fight the hitherto untreatable addiction of cancer cells (Bolomsky et al., 2020).

Previously, we (Polukonova et al., 2014, 2018, 2021, et al.) established the concentration-dependent ability of Gratiola officinalis extract to induce tumor cell death by apoptosis, while low concentrations of the extract could lead, on the contrary, to the survival of tumor cells by stimulating the autophagy process.

Aim: as part of a study to investigate in detail the mechanisms of antitumor activity of Gratiola extract, to study the relative expression of the anti-apoptotic gene MCL1 in human kidney cancer cell culture A498 under the effect of the extract at different concentrations.

Materials and Methods. Gratiola extract at concentrations of 0.02 mg/ml, leading to the highest number of LC3B-positive cells, and 0.3 mg/ml, leading to the highest number of apoptotic cells, was investigated. Autophagy was assessed with Autophagy LC3-Antibody Based Kit; apoptosis induction was performed with Annexin-V FITC Apoptosis Kit; RNA isolation was performed with Rneasy Plus Micro Kit; cDNA synthesis on RNA matrix by RT² First Strand Kit; real-time PCR reaction was performed with RT² Profiler PCR Array Human Cell Death PathwayFinder.

Results. In control and when human kidney cancer A498 cells were exposed to Gratiola extract at a concentration of 0.02 mg/ml, no apoptotic cells were formed and MCL1 gene expression was lower than at 0.3 mg/ml. At 0.3 mg/ml, death of A498 cells by apoptosis was observed and MCL1 gene expression was markedly higher. Apparently, at an extract concentration of 0.3 mg/ml, another shortened protein, Mcl-1S, encoded by the same gene, is synthesized during alternative splicing. Increased levels of Mcl-1S protein may lead to inhibition of apoptosis-protecting Mcl-1 and, consequently, tumor cell death by apoptosis.

Conclusion. A concentration-dependent mechanism of tumor cell apoptosis induction under the action of Gratiola extract was revealed: due to the formation of Mcl-1S protein (a negative regulator of Mcl-1 protein), eventually leading to tumor cell death by apoptosis.

Keywords: Gratiola extract, Apoptosis. Anti-apoptotic gene MCL1, Mcl-1S protein

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MOLECULAR GENETIC CHARACTERISTICS
OF OVARIAN CANCER IN CRIMEAN PATIENTS

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Introduction. The etiology of hereditary ovarian cancer is most often attributed to structural or functional inactivation of some tumor suppressors. An identification of the molecular genetic basis of the disease has prognostic significance, and it also influences the selection of the most effective therapeutic tactics. The rate of the occurrence of polymorphisms is variable and depends, among other things, on ethnicity and geography.

The aim hereof is to establish the range of mutations in tumor suppressor genes associated with the formation of a hereditary predisposition to malignant neoplasms of the breast and ovaries in female patients of various ethnic groups, residing permanently in the Republic of Crimea.

Materials and methods. The study group (n = 50) consisted of patients with ovarian cancer who underwent various treatment options, including surgery and/or drug antitumor therapy in Simferopol since February 2022 to March 2023. Criteria for inclusion in the study upon the patient’s informed consent were as follows: 1) age of recorded onset of the disease up to 50 years and included; 2) familial cancer; 3) primary multiple synchronous and/or metachronous tumor lesions; 4) neoplasia of both ovaries; 5) permanent residence in Crimea and self-identification of patients as a certain ethnic group (Slavs or Crimean Tatars), provided that relatives in two or more generations belong to the same ethnic group under study.

Upon admission of the above patients to the hospital, the patients' venous blood was collected, from which DNA samples were isolated using the ExtractDNA Blood kit (Evrogen, Russia). Mutations were detected by polymerase chain reaction in real time with an analysis of melting curves using primers of the commercial HRR-screening kit (Testgen, Russia): BRCA1 (c.5382insC, c.185delAG, c.300T>G, c.1961delA, c.4153delA, c.4675G>A, c.5251C>T, c.5161C>T, c.3819delGTAA), BRCA2 (c.961_962insAA, c.3749dupA), PALB2 (c.1592delT), CHEK2 (c.470T>C, c.1100delC, c.444+1G>A, c.893_897del).

Results. In the prevailing Slavic subgroup (n = 42), 7 mutations were found in 7 patients: BRCA1 c.5382insC, c.300T>G, c.185delAG with an occurrence of 2.4% each, c.4153delA with an occurrence of 4.8% and 2 mutations in the CHEK2c.470T>C gene with an occurrence rate of 4.8%. In addition, one of the patients with the CHEK2c.470T>C mutation was also identified as having the BRCA2c.6174delT mutation during a preliminary study.

Conclusion. The identified mutations are typical for the European and Slavic ethnic groups, although the genetic variant CHEK2c.470T>C is quite low-penetrant. In the Crimean Tatar group, the studied polymorphisms were not identified, despite the presence of signs of the hereditary nature of ovarian cancer. Continuation of molecular genetic studies with an increase in the sample numbers and a comparative analysis of the results of similar studies of patients with breast cancer will expand knowledge of the etiological basis of ovarian cancer and breast cancer in the Crimean population.

Keywords: Hereditary ovarian cancer, Genetic polymorphisms, BRCA1, CHEK2
OCTALCIUM PHOSPHATE CERAMICS FUNCTIONALIZED WITH CISPLATIN AND Zoledronic ACID: BIOLOGICAL ACTIVITY IN VITRO AND IN VIVO EXPERIMENTS


Introduction. Giving an additional specific antitumor activity to biomaterials used to provide the local release of medical drugs into the bone defect in oncology, i.e. their functionalization is a promising, sought-after area in the modern biomaterials’ science.

Aim. To develop a technology for functionalizing ceramic material based on octacalcium phosphate ceramics (OCP ceramics) with antitumor drugs cisplatin (Cis OCP) and zoledronic acid (Zol OCP), separately and in their combination (Cis+Zol OCP), and evaluate its biological activity in experiments in vitro and in vivo.

Materials and methods. The incorporation of the drugs onto the surface of OCP ceramics, developed by the Research Institution of Metallurgy and Material Sciences at the Russian Academy of Sciences, was carried out by keeping OCP granules in aqueous solutions of Cis (1.0 mg/ml) and Zol (0.2, 0.5 and 1.0 mg/ml) (Sigma-Aldrich). The amount of the incorporated drug was determined by measuring its concentration in the incorporation solution before and after keeping OCP ceramics therein, using inductively coupled plasma atomic emission spectrometry (ICE 3000 spectrometer, Thermo Fisher Scientific, USA) and high-performance liquid chromatography (Agilent 1260 Infinity, Agilent Technologies, USA). In vitro studies of cytocompatibility, targeted osteodifferentiation, expression of osteogenic differentiation genes, and cytostatic activity of OCP ceramics were carried out on inoculable lines of human osteoblast-like cells MG-63, human breast cancer (BC) cells MCF-7 and a primary culture of human bone marrow-derived mesenchymal stem cells (MSC). The biocompatibility of samples of the OCP ceramics was studied in vivo on a model of its subcutaneous implantation in BDF1 mice, the osteoconductive properties were explored on a model of a marginal defect in the tibia of Wistar rats, and specific antitumor activity was investigated on a model of subcutaneous inoculation of a inoculable tumor strain of breast cancer in mice CA-755 with simultaneous implantation of samples of OCP ceramics.

Results. Optimal conditions for the incorporation of Cis and Zol into OCP ceramics have been developed. It has been shown that the functionalization of OCP ceramics with Cis in combination with Zol slightly reduces the share of OCP-bound Cis (Cis OCP), but provides an even, prolonged release of Cis for one month or longer. In addition, Zol enhanced the osteoinductive properties of the OCP ceramics against donor BM MSCs and gave it an osteoclast inhibitory effect. The Cis OCP samples demonstrated their cytostatic effect on the MCF-7 tumor cells; the combined functionalization of the OCP-ceramics with Cis and Zol has resulted in the longest duration of its cytostatic effect. The Cis OCP ceramic samples are biocompatible and retain their osteoinductive properties in vitro and in vivo; the presence of Zol in OCP has produced a temporary aseptic inflammatory reaction and caused a delay in reparative osteogenesis in the bone defect. The antitumor effect by Cis OCP and Cis+Zol OCP samples on the Ca-755 mouse breast cancer strain has been shown, which is more pronounced as compared with intravenous administration of Cis.

Conclusion. The results obtained indicate the promise of using OCP ceramics as a platform for drug functionalization for further use in reconstructive plastic surgery in oncology.

Keywords: OCP ceramics, Functionalization, Cisplatin, Zoledronic acid, Biological activity in vitro and in vivo
The 20th century was skeptical about magneto-biology. But the early experiments conducted by the American couple Barnothy, M. F., & Barnothy, J. M., who presented their data on the possibility of inhibiting the growth of tumors using strong magnetic fields, excited the world, and in the 60s last century, 3 international symposiums were held in Chicago, Rome, and Moscow to discuss that topical issue. By that time, a great progress in that scientific area was made by researchers from the Rostov Scientific School of Experimental Oncology, who in 1960, for the first time in the USSR, published their own data on the mechanism of the influence of MP on the growth of novocaine-sinestrol sarcoma in rats and successfully reported thereon at the anti-cancer congress in Moscow. That area in science has gained great power because of the discovery of the pattern of development of general nonspecific adaptive reactions by L.Kh. Garkavi, M.A. Ukolova and E.B. Kvakina (Scientific Discovery Certificate No. 158, 1975), as an integral mechanism for increasing the nonspecific antitumor resistance by a human organism. The merging of those two research areas, like two deep rivers, made it possible to discover a whole hierarchy of mechanisms of the influence by magnetic fields, ranging from the subcellular to the systemic level, as well as develop a strategy and tactics, principles and technologies of magnetic therapy of tumors for translation into the clinical medicine. In that impressive advancement, a huge role was played by the fundamental research completed by Elena B. Kvakina, who was passed the magnetic therapy “torch” by her scientific advisor and mother, Maria A. Ukolova, and who together with Lyubov Kh. Garkavi, passionately worked at creating of the theory of human adaptation reactions.

Elena B. Kvakina conducted her thorough, accurate studies on the mechanism of the antitumor effect produced by MP. It has been established that with the MP central (the brain-targeted) and local (the tumor-targeted) impact, by a direct and a reflex effect, an increase in the functional state of the central nervous system takes place, in particular it refers to the hypothalamus, which causes an activation of its autonomic and endocrine parts. A change in the excitability of the nervous structures of the hypothalamus under the influence by AMF has been detected. Even a single AMF exposure resulted in lowering the threshold and led to an increase in the excitability of the hypothalamus that correlated with the data obtained with electroencephalographic research methods by Yu.A. Kholodov. Tissue respiration, oxidative phosphorylation and aerobic glycolysis of the hypothalamus with adjacent areas of the thalamus in rats of different groups, as well as the morphostructure of the hypothalamus under the brain exposure to AMF with the tumor regression cases were investigated. At the same time, a large number of thyrotrophs and luteinizing gonadotrophs were revealed in the adenohypophysis in rats with tumors, which resolved after the AMF head-targeted exposure. The functional state of the thyroid gland, the adrenal cortex, the thymic-lymphatic system and many other aspects has been investigated, confirming the activation nature of the magnetic action. In general, a huge database has been accumulated and comprehended confirming the safe and effective way of the contact-free exposure to MP that has played a decisive role in the translation of the developed intensity, frequency and exposure algorithms to the clinical departments at the Rostov-on-Don Oncology Center. The developed wave technologies were used in the complex treatment of skin cancer, lung cancer, malignant brain tumors, breast cancer, intestinal cancer and even hemangiomas in children under and over one year of age. The developments and advancements of the above researchers have been properly patented and doctoral and master’s theses have been successfully defended. Activation electromagnetic therapy of tumors is a unique product that has no analogues
THE EFFECT PRODUCED BY IL-2 AND IL-15 ON THE PROLIFERATION OF T CELLS IN VITRO IN PATIENTS DIAGNOSED WITH BREAST CANCER


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**Introduction:** Over the past decade, the use of adoptive immunotherapy has emerged as a promising approach to treatment of various malignancies, including breast cancer. Cytokines are involved in the development, differentiation and homeostasis of the T cells. Despite the similarities in signaling pathways, the γc cytokines shape the T cell responses differently: IL-2 plays a major role in the development and maintenance of the regulatory T cells, while IL-15 stimulates the proliferation and cytotoxic functions of the CD8+ T cells and NK-cells that leads to an enhanced antitumor response. It is of interest to study the combined effect of these cytokines in an experiment.

**Aim:** To evaluate the combined effects produced by cytokines IL-2 and IL-15 on the T cell proliferation in vitro in patients diagnosed with breast cancer.

**Materials and methods:** The material for the study covered 10 blood samples taken from patients with breast cancer, followed by isolation of mononuclear cells (MNC) in Ficoll-Urografin density gradient centrifugation. Cells were cultivated in equal seeding fractions of 500 thousand cells/ml in 6-well plates for 15 days at 37°C with 5–10% CO2. Anti-biotin magnetic particles MACSiBead and antibodies to CD2, CD3 and CD28 (Miltenyi Biotec, Germany) were used to activate the T cells. An induction of lymphocyte proliferation was performed on the fourth, eighth and twelfth days by adding cytokines - IL-2, IL-15 and the combination of IL-2 / IL-15 (MiltenyiBiotec, Germany) with the use of a negative control. Cytokines were added at a concentration of 40 ng/ml. Daily counts of viable lymphocytes were completed using an analyzer (Eve, NanoEnTek Inc, Korea), and morphology was conducted using an inverted microscope (AxioImagerA.2 Zeiss, Germany). Statistical data processing was carried out using Microsoft Office Excel and Statistica 10.0. The statistical significance of differences in the data between groups was assessed utilizing the Student’s t-test. The number of cells on the first day of cultivation corresponded to the seeding dose and was taken as 100%.

**Results:** In samples with the addition of the IL-2/IL-15 combination, an increased proliferative activity, as compared to the reference, and the formation of conglomerates were noted, which possibly indicates the influence of γc-cytokines on the processes of maturation and differentiation of T-lymphocytes during expansion under the in vitro cultivation conditions. After the expiration of the cultivation period, all experimental samples statistically significantly exceeded the reference, namely, the presence of IL-2 led to an increase in the number of viable cells by 2.25 times, due to the effect made by IL-15 there was the increase by 2.9 times recorded, and in case of the addition of the IL-2/IL-15 combination the increase by 2.33 times was reported.
Conclusions: Thus, we can assume that IL-15 deficiency reduces the level of expansion of CD8+ T-lymphocytes, but is not critical for their formation in their culture.

Keywords: Expansion of lymphocytes, Breast cancer, Activation of human T-lymphocytes, IL-2, IL-15.

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ASSESSMENT OF THE PROGNOSTIC POTENTIAL OF EXPRESSION AND ABERRATIONS IN THE NUMBER OF DNA COPIES OF THE HOMOLOGICAL RECOMBINATION SYSTEM GENES IN PATIENTS WITH NON-SMALL CELL LUNG CANCER

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Introduction. Numerous studies have shown that the presence of homologous recombination deficiency (HRD) in breast tumors may be a good marker of the effectiveness of chemotherapy with DNA-damaging agents such as platinum-based and anthracycline-containing medication regimens. However, the formation of HRD due to disruptions in the BRCA1/2 genes is typical not only for breast cancer and ovarian cancer, but also for other cancer localizations, in particular for non-small cell lung cancer (NSCLC). Since the use of platinum-based drugs in patients with NSCLC is a standard treatment protocol, studying the expression profile of homologous recombination genes and the presence of chromosomal aberrations therein will allow us to fully study HRD in these patients and identify new prognostic markers.

Following this way, we outline the aim of this work to assess the relationship between the expression and aberrations of the DNA copy number in the main HR genes and the metastasis-free survival (MFS).

Material and methods. The study included 104 patients with stage IIB IIB NSCLC, with central or peripheral localization. All patients underwent pneumonectomy or lobectomy. After surgery, adjuvant platinum doublets chemotherapy was performed. Surgical material after chemotherapy (tumor tissue) was used as the study material. DNA was isolated from the samples followed by the microarray examination with the use of CytoScanTM HD Array to assess the presence of chromosomal aberrations. RNA was also isolated followed by quantitative PCR to evaluate the expression of the genes as given below: BRCA1, BRCA2, ATM, BARD1, BRIP1, CDK12, CHEK1, CHEK2, FANCL, PALB2, PPP2R2A, RAD51B, RAD51C, RAD51D, RAD54L and PARP1.

Results. To identify the relationship between the expression of the studied genes and the survival, statistical characteristics of the sample according to the level of expression were applied. As a result, two analysis groups were obtained: the patients with overexpression of genes (expression over 1) and a hypoexpression group (expression under 1). Further, using the Kaplan-Meier method, it was found that the expression level did not have a statistically significant effect on the metastasis-free survival rates. Only the relationship at the level of a pronounced trend for the RAD51C gene was shown. Over-expression is associated with high survival rates (log-rank test p=0.1). An assessment of MFS indicators depending on the presence of aberrations in the number of DNA copies of the genes under study showed that the normal copy number in the PPP2R2A gene determined 86% 5-year survival, compared with the group of patients with an amplification (50%), at p = 0.01. A similar result, but
at the level of a pronounced trend, was shown for BARD1 (p=0.1). The BRCA2 deletion was associated with a low survival rate of 53% (log-rank test p = 0.0003), similarly to the case with the PALB2 deletion (BMW rate 50%, p = 0.05), compared with the group of patients with the normal copy numbers of these genes. An interesting result was shown for BRCA1: with the deletion of this gene, 100% MFS was found, with the amplification - 81%, with the normal copy number - 84%, log-rank test p = 0.03.

Conclusions. Thus, as a result of the study, the role of the expression and chromosomal aberrations of homologous recombination genes in the long-term outcomes of treatment of patients with non-small cell lung cancer was shown. The data obtained are of great practical significance in terms of assessing the sensitivity and formation of tumor resistance to DNA-damaging chemotherapy drugs, widely used in the treatment of cancer of various localizations that may help in personalized administration of the above drugs.

Keywords: Expression and chromosomal aberrations of homologous recombination genes, Non-small cell lung cancer (NSCLC), Metastasis-free survival (MFS)

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THE PATH TO GOOD HEALTH IS THROUGH MITOCHONDRIAL TRAINING. INTERMITTENT HYPOXIC TRAINING AS THE MAIN METHOD.

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All human health, from the cellular level to overall well-being, is inextricably linked to the health of our mitochondria. This article delves into the concept of mitochondrial training, focusing on Hypoxic Intermittent Training (HIT) as a central method for improving mitochondrial function. It is becoming increasingly clear that various diseases, stress, shock, inflammation, aging, and organ failure are primarily the result of respiratory problems that directly affect mitochondrial health. The regulation of metabolism is closely related to gas exchange, and the composition of the gases we breathe regulates our metabolism. Our main goal is to promote efficient mitochondrial function, a key biological imperative.

A fundamental principle states that when energy intake is high (as indicated by elevated glucose and oxygen levels), mitochondria tend to function inefficiently, generating excessive amounts of superoxide. This observation has led to the use of IHT and intermittent fasting as leading drug-free treatment and rehabilitation methods. The main goal of IHT is to determine the optimal fluctuations in low oxygen levels leading to maximum efficiency of mitochondrial respiration.

Regular IHT and high-intensity interval training (HIIT) show remarkable potential for improving mitochondrial bioenergetics in tissues and organs. In a state of IHT, the body adapts to produce the necessary energy with limited oxygen availability. The main regulator of this adaptation is hypoxia-inducible factor (HIF-1), which within 2-3 minutes triggers numerous metabolic changes, increasing the body’s ability to use oxygen more efficiently, including mitochondrial biogenesis and genetic changes. Notably, similar changes occur during both IHT and HIIT. These changes include increased oxygen intake in the lungs, increased production of erythropoietin (EPO), increased capillarisation, enhanced mitochondrial biogenesis and rejuvenation, decreased heart rate and blood pressure, increased release of human growth hormone, increased lipid and glucose metabolism and reduction of oxidative stress. In addition, IHT offers many additional benefits, including improved nitric oxide balance to prevent diabetic complications and prevent atherosclerosis, stimulation of glucose transport and insulin synthesis, normalization of thyroxine levels, detoxification through improved cytochrome p450 syn-
thesis, and improved mood through dopamine balance and serotonin, as well as improved sleep quality. An important difference and advantage of IHT over HIIT is the quick and effective removal of excess lactic acid during sessions/trainings. Lactic acid removal creates an optimal gas exchange environment for mitochondria, including maintaining pH balance, carbon dioxide and nitric oxide levels. Essentially, through controlled fluctuations in the level of inhaled oxygen, we tune the mitochondria’s ability to operate at peak efficiency, much like achieving resonance in music. Our goal is to compare these two methods and show unique possibility of using HIIT and IHT together and their application in patients with various pathologies, athletes and non-athletes.

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MOLECULAR CELLULAR EFFECTS BY PROTON AND γ-IRRADIATION IN THE B16 MURINE MELANOMA MODEL IN VIVO

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Introduction. Proton and photon (γ-) radiation is widely used in modern clinical practice for the treatment of malignant tumors of various locations. The relative biological effectiveness (RBE) of accelerated protons is about 1.1, as follows from the results of experimental studies of clonogenic survival of tumor cells in vitro. But however, despite the low RBE of proton radiation, a number of clinical studies have shown a significant increase in relapse-free and overall survival of patients after proton therapy compared with that after standard radiotherapy using photon radiation. The mechanisms, on which the differences recorded in vivo are based, are of significant scientific and practical interest, but have been poorly studied.

The aim of the work is to study the features of the effect by proton and γ-radiation on murine melanoma line B16 in vivo at the molecular and cellular levels.

Materials and methods. Irradiation of the primary focus of melanoma at a dose of 10 Gy was performed once on day 10 after the tumor cell transplantation, when the tumor became visible macroscopically. The local γ-irradiation was performed with the Luch-1 system using a 60Co source. Proton irradiation was performed using the Prometheus proton therapy system with a method of the Bragg Peak Modification. Autopsy samples of tumor tissue were collected 2 and 9 days after irradiation. With the use of flow cytometry, assessed were the number of tumor stem cells (TCCs) with the SP (side population) method, as well as the level of vascularization of the primary lesion according to the criterion of the relative number of CD31+CD146+ endothelial cells. Utilizing real-time PCR, we have analyzed the expression of genes of various functional groups, which are responsible for control of the cell cycle and proliferation; regulate cell death, epithelial-mesenchymal transition (EMT) and other processes potentially associated with the radiosensitivity of malignant neoplasms. The study was approved by the Commission for Bioethical Control over the Care and Use of Laboratory Animals at the Federal State Budgetary Institution “National Medical Research Center of Radiology” at the Ministry of Health of Russia (Approval No. 1-SI-00014 dated 09/08/2020).

Results. A number of indicators revealed significant differences in the biological effects produced by the ionizing radiation. In particular, it has been found that after the γ-quanta irradiation there is an increase
in the relative amount of TSCs 2 days after irradiation (p < 0.01 according to the Mann-Whitney test), while after exposure to protons such an effect is not available. The relative number of CD31+CD146+ endothelial cells in samples taken on day 2 after irradiation is statistically higher after the γ-irradiation compared to that after the action of accelerated protons (p=0.056). It is noteworthy that changes in the expression profile of genes involved in the regulation of stem cell properties and EMT are generally consistent with the quantitative changes in TSCs.

**Conclusion.** The data obtained allow us to conclude that the specific effects made by proton and photon radiation on the pool of TSCs and the vascularization of the primary tumor site may, at least partially, explain the differences in the clinical effectiveness of these types of ionizing radiation. The scientific and practical significance of the discovered effects is discussed in the report.

**Keywords:** Proton therapy, γ-radiation, TSC, Vascularization, Gene expression

**SYSTEMIC MECHANISMS OF DEVELOPMENT OF THE NONSPECIFIC ADAPTATIONAL REACTIONS AND ANTI-TUMOR RESISTANCE**

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**Introduction.** It is known about numerous regulatory disorders in the body during the development of a malignant process. However, in this case, the natural mechanisms of the anti-tumor resistance remain poorly understood. The concepts on the systemic processes during the development of general nonspecific adaptational reactions of the body (AR): stress and anti-stress ARs, discovered by G. Selye and Russian scientists L.Kh. Garkavi, E.B. Kvakina and M.A. Ukolova, have contributed to the development of effective technologies for preventive and complementary therapy for the purposes of clinical oncology.

The aim of the study was to carry out a comprehensive analysis of the changes in the neuroendocrine and immune systems during the development of ARs under the normal conditions, during the tumor growth and the action of anti-stress factors in experiments.

**Materials and methods.** Experiments were carried out on 530 white outbred mature rats of both sexes: on the animals without a tumor and those with transplanted sarcoma 45 (S-45) and Pliss lymphosarcoma. For the purpose of our experiment, blood parameters were assessed in 110 human individuals, both in healthy subjects and cancer patients. Low-intensity electromagnetic radiation of the infra-low-frequency (ILF) and extremely high-frequency (EHF) ranges, pulsed electric fields (SCENAR equipment), and immune and metabolic therapy substances were used as anti-stress factors. We studied the level of catecholamines (CA), the activity of corticogene-sis enzymes in the adrenal glands, and the content of serotonin (5-HT) in the epiphysis (pineal gland). The blood levels of histamine (H) and 5-HT, thyroid hormones and cortisol were assessed. We investigated the micropicture of the tumor, thymus and the spleen, hematological indicators of the nature and intensity of AR, considering the dynamics. The functional state of blood leukocytes was assessed by the activity of dehydrogenase enzymes, the intensity of phagocytosis and oxygen-dependent reactions. The methods of cytology, cytochemistry, histology, fluorescent histochemistry, immunology, radioisotope analysis and experimental oncology were used. Statistical processing of the results was carried out utilizing parametric and non-parametric criteria and cluster analysis.

**Results.** The results of studying the indices of bi-aminergic processes have essentially supplemented...
the understanding of the neuroendocrine mechanisms associated with the development of the AR of acute and chronic stress and the anti-stress AR of training, calm and elevated activation. Signs of activation of the 5-HTergic systems during the development of AR of calm and elevated activation and “switching-on” of the epiphysial-adrenal system with a decrease in the level of reactivity of AR of elevated activation, as well as a decrease in the activity of the monoaminergic systems with the development of the persistent anti-stress AR have been revealed. Under the tumor growth, a disruption of the relationship between the BA level and the character of AR has been observed.

In case of the anti-tumor effectiveness of anti-stress factors, a partial recovery of the above relationship has been noted, associated with signs of pronounced activation of blood leukocytes, lymphoproliferative processes and intercellular interactions in the organs of the immune system, infiltration of tumor zones by immune cells with pronounced markers of their interaction with tumor cells. The adaptational reactions (AR) upon the impacts of high efficiency, as well as the changes in tumor-bearing animals with signs of a state of activation areactivity have been described.

Conclusion. The results obtained by us allowed us to formulate the concepts on the multi-level systemic regulatory processes during the development of AR and their influence on the state of nonspecific anti-tumor resistance by the body.

Keywords: Malignant tumors, Adaptational reactions (AR), Biogenic amines, Regulatory systems, Anti-stress factors, Anti-tumor effect

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STRESS IN PATHOGENESIS OF DEVELOPMENT OF TUMOR PATHOLOGY OF THE THYROID GLAND

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Introduction. Our experience in the endocrine surgery at our clinics institution has shown a relationship between the appearance of nodules in the thyroid gland and a long-term exposure to stress. At present, the complicated geopolitical circumstances play a role of an active stressor, and until recently, it was also the COVID-19 pandemic, which caused a change in lifestyle, heavy material losses, and loss of loved ones. Oncogenic activity of cells is reflected in an imbalance of markers of proliferation and apoptosis. Many researchers associate thyroid pathology with molecular alterations as diagnostic markers (Styazhkina S.N., 2017; Romashchenko P.N. et al., 2021).

The aim of our research work is to study the role of stress in the development of tumor pathology of the thyroid gland in order to improve diagnostic approaches in this area.

Materials and methods. Using immunohistochemistry methods, cell cycle markers (Ki-67 mitosis and FAS-R apoptosis) of thyrocytes were studied in a model experiment of immobilization stress in albino Wistar rats and in various diseases of the human thyroid gland, based on the material of the preoperative and surgical stages of morphological diagnostics.

Results. Stress delays the differentiation of thyroid tissue in rats, reduces the thyrocyte proliferation index that is accompanied by a progressive increase in the expression of an apoptosis marker, and it causes uneven differentiation of the gland, when hypertrophic follicles are formed among the areas of the immature tissue, and the volume of the gland increases causing a goiter. In this case, the cells express a silencing death receptor. In humans with papillary carcinoma, toxic goiter, autoimmune thyroiditis and adenomatous goiter, a multidirectional expression of the markers of mitosis and apoptosis was revealed upon testing the materials of fine-needle aspiration puncture biopsy. Our cluster analysis in different groups of patients with autoimmune and proliferative diseases made it possible to mathematically determine the ra-
tio of quantitative indicators of the expression levels of these markers to assess the risk of malignancy of the glandular tissue.

**Conclusion.** Knowledge of the critical role of stress as an initiating factor in the development of thyroid pathology and the available data on the functional characteristics of the thyroid gland can serve as the basis for further study, development and implementation of methods of prevention and complex therapy of thyroid pathology in clinical practice from the standpoint of limiting the effects of stress and molecular diagnostics of its effects.

**Keywords:** Thyroid gland, Stress, Molecular diagnostics, Oncomorphology

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**VIROLOGICAL RESPONSE AND EFFECTIVENESS OF CHEMORADIATION THERAPY FOR ANAL CANCER: RELATIONSHIP AND PREDICTIVE CAPABILITIES**


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**Introduction:** The effectiveness of chemoradiation therapy (CRT) is conventionally assessed in terms of achieving clinical complete response (CCR), overall and event-free survival. Anal cancer (AR) is considered as one of the cancer diseases with a proven etiological agent: the human papillomavirus (HPV). The search for biomarkers to predict the effectiveness of CRT is always relevant, and an assessment of virological response (VR) may be useful for this purpose.

**Aim:** To compare rates of clinical complete response (CCR) and virological response (VR) to CRT for anal cancer.

**Material and methods:** 35 patients with squamous cell AR were examined, incl. 31 females (57.1±8.8 years) and 4 males (55±9.5 years). There were HPV+ detected in 28 (80%) and HPV in 7 (20%) patients. All patients received radical CRT and underwent treatment without interruption with moderate radiation reactions. Antiviral therapy was not administered. Smears from the surface of the tumor were taken before CRT, on the day of its completion, and 3, 6, 9 and 12 months after CRT. To determine HPV DNA, the AmpliSens® HPV HCR genotype-titer-FL kit was used. The presence of CCR at 6 months and 3-year overall and event-free survival rates were compared. We assessed rapid virological response (RVR - sustained disappearance or reduction of HPV DNA by more than 2 lg at the end of CRT course), slow virological response (SVR - sustained disappearance of HPV DNA at 6 months after CRT), virological load fluctuation or reappearance (VRA - reappearance of HPV DNA) and lack of virological response (LVR). All patients were divided into two groups: those with the presence of stable VR (group 1 – with RVR and SVR) and the patients without sustainable VR (group 2 – with LVR and VRA). Statistical data processing was carried out using Microsoft Office Excel and Statistica 10.0. To compare the qualitative characteristics of the samples, Fisher’s exact test was used. Differences were considered statistically significant at p <0.05.

**Results:** CCR was achieved in 6 HPV patients (85.7%) and in 20 HPV+ patients (71.4%) (p>0.05). The 3-year overall and event-free survival rates did not depend on the HPV status. RVR developed in 13 HPV+ patients (46.4%), SVR was detected in 4 patients (14.3%), VRA was revealed in 5 patients (17.9%), and LVR was recorded in 6 patients (21.4%). When assessing CCR, a significant difference was obtained between RVR and LVR (84.6% vs 33.3%, p=0.046). The difference in the incidence rate of CCR when comparing SVR and LVR tended to be significant (100% vs 33.3%, p=0.071). In the patients with RVR, a 3-year event-free survival was higher than in all others (91.7% vs 53.8%, p = 0.035) as against the patients with VRA (91.7% vs 25%, p = 0.027) and the patients with LVR (91.7% vs 40.0%, p=0.053). Among the HPV+ patients, CCR was achieved in 15 individuals (88.2%) of group 1 and in 5 individuals (45.5%)
of group 2 (p=0.022); a 3-year overall survival was recorded to be 16 (94.1%) in group 1 and 9 (81.8%) in group 2 (p>0.05), and an event-free survival was reported to be 15 (88.3%) and 3 (27.3%, p=0.0018), respectively. That is, the best rates of CCR and the event-free survival were achieved in the patients with stable VR. With VRA, the rates both of the overall and the event-free 3-year survival were however not statistically significant, but lower than in case of the complete absence of VR. VRA was reported before the recurrence. In two of the five patients, VRA occurred 3 months after CRT, and 6 months later they underwent surgery. In three patients, VRA occurred 6 months after CRT; in one of them, a relapse was detected 9 months after CRT; in another one it was revealed 18 months after CRT. Apparently, VRA occurs earlier than the recurrence develops, and, therefore, its detection may allow identifying earlier a negative response to CRT and adjusting the treatment.

Conclusions: Patients with VR more often achieve CCR and have a higher 3-year event-free survival. When assessing the prognosis of the effectiveness of CRT, it is advisable to take into account the stable VR and evaluate it simultaneously with the assessment of the CCR.

Keywords: Anal cancer, Human papillomavirus, Virological response