DISCOVERY OF ADAPTATIONAL REACTIONS: A NEW MILESTONE IN THE DEVELOPMENT OF ONCOLOGY

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The celebration of the 90th anniversary of the National Medical Research Centre for Oncology at the Ministry of Health of the Russian Federation in the past 2021 expanded its time frame due to other anniversaries that are inextricably linked with the scientific activities of the Rostov Cancer Centre. First of all, this is the 115th anniversary of the birth of M.A. Ukolova, the founder of fundamental research at the Centre for Oncology, the 65th anniversary of the establishment of the experimental department, and the 45th anniversary of the discovery of “Pattern of development of qualitatively differing general nonspecific adaptational reactions of the organism” made by Garkavi L.Kh., Ukolova E. B. and Kvakina E.B. [1].

The intellectual power of Lyubov Garkavi, the fantastic expertise and talent of Elena Kvakina, the brilliant, top leader and top organizer Maria Ukolova have contributed to expand the boundaries of the knowledge and offered to the world a discovery of the patterns of development of adaptational reactions depending on the intensity of a stimulus. It was a breakthrough, a new philosophy in biology and medicine, based on the ideas of the nervism theory by I.P. Pavlov, the laws of dialectics, synergetics, self-organization of complex systems, phase transitions and, of course, the theoretical heritage by the titans of thought: Selye, Anokhin, Simonov, Lazarev, Kavetsky, Arshavsky, Kryzhanovsky, Dilman and many other outstanding scientists of the 20th century. It is destined by fate that the Rostov Cancer Research Institute (today referred to as the National Medical Research Centre for Oncology) has become a platform and a trigger centre that launches the process of the emergence and development of the theory of adaptational reactions.
It would seem difficult to imagine how they succeeded in the identification of the subtle mechanism responsible for an integral control of the organism as a whole in presence of a tumor progression. But nothing helps to get closer to the truth as much as the obstacles which appeared at the Cancer Research Institute in their experiments, starting from the fact that at the beginning of the establishment of the experimental department there were no appropriate conditions for keeping tumor-bearing animals, there was no diagnostic and therapeutic equipment, there was no bank of tumor cell strains available. But there were bright minds of young scientists, their observation ability and full dedication to research, who dared to penetrate into the Holy of Holies of the neuroendocrine regulation: the hypothalamus, in order to discover the relationship between the quantity (strength, measure) of a direct or remote impact on this command centre of the system control and the integral qualitative response by the body, namely, the development of a general adaptational reaction of nonspecific nature.

The discovery of the patterns of development of adaptational reactions of the body, the theory and practice of managing complex systems based on the principles of activation therapy have become the visiting card of the scientific team and fundamental research of the National Medical Research Centre for Oncology in Rostov-on-Don, which has accumulated over several decades unique experience in experimental oncology and in the transfer of developed technologies to clinical practice. Those developments have been well in advance of the officially formulated idea of translational medicine. Completed governmental assignments, defended candidate and doctoral dissertations and theses, patents, hundreds of articles on solving problems of increasing nonspecific antitumor resistance, - all these works have become an integral part of the Russian national oncology, offering a new view of considering the relationship between the tumor and the body from the standpoint of the theory of adaptational reactions [2].

When presenting the basics of this theory, it is necessary to return to the prototype of those generally accepted simple true statements, according to which our genotype is not a chaotic set of molecular genetic structures, but it is a unique algorithm for the periodic repetition of the four nitrogenous bases only: guanine, cystein, uracil and adenine. No matter how the level of self-organization rises, the principle of discreteness of the particular and the integration of the whole remains applicable. The outer shell of an atom of various chemical elements is filled with electrons and, according to the criteria of the outer shell electrons, the periodic system of chemical elements with different properties is formed. At the level of the organism and its response to effects produced by the external and internal environment, a mechanism of periodic repetition of several, similarly named, general nonspecific adaptational reactions (GNAR) is implemented at different levels of reactivity.
The discovery of the archetype of the stress response by Hans Selye is one of the most outstanding achievements in biology and medicine. The implementation of such an archetype of reaction in response to a strong and dangerous impact is carried out with the participation of the command systems of the brain, regulatory influences of the pituitary gland regarding the release of ACTH, suppression of the executive endocrine and immune organs, at high energy expenses and the dominance of catabolism processes over anabolism. As a result, the resistance decreases and, with prolonged exposure to a stressor, an unfavorable outcome may occur.

The stress program is only a part of the adaptational response that forms the nonspecific basis for pathology, including cancers. To ensure health and increase resistance, including the antitumor resistance, there are other regulatory programs in a human organism available, involving the use of the same CNS structures and the regulatory systems (the neuroendocrine and the immune systems), which are sensitive and respond appropriately to physiological, middle-intensity or even weak influences and stimulation. These programs are the core and the essence of the opened by L.Kh. Garkavi, M.A. Ukolova, E.B. Kvakina discrete archetypes of the physiological adaptational responses of training (to a relatively weak stimulus), calm and elevated activation (medium strength, intermediate between the weak and strong stimulation). These latter are distinguished by the most harmonious and synchronized increase in the functional activity of all organism systems. Thus, the targeted design of the intensity of stimulation applied to the body shall be based on the control of the desired response that should lead to a rise in its nonspecific resistance.

The human brain, like the entire organism as a whole, has the ability to generate more than just the four adaptational reactions. Obeying the laws of self-organization of complex systems in an environment, where and when the intensity of influencing factors can vary in the widest range, a living organism is furnished with a multi-level periodic system of adaptive reactions. The alternation of reactions of the same name in the sequence T, CA, EA, S at one level of reactivity and the transition to another level (lower or higher) occurs with a mandatory entry into the inter-level zone of AREACTIVITY, where the systems seem to be silent, giving no response. Such a zone of areactivity is of great biological importance for maintaining negentropy and homeostasis under transitional conditions.

The theory of adaptational reactions is based on the description of the features of the development of each existing biological archetype of nonspecific adaptational reactions and the unique natural periodic system of their repetition that offers ample opportunities for the selection of the desired reaction at the required level of reactivity, taking into account age, sex, nosology and the stage of a specific treatment. Over the years of work, the researchers of the Rostov Centre for Oncology have developed the strategy, tactics and principles of activation therapy, defined simple signal criteria for assessing adaptational reactions, as well as mathematically justified programmed modes of dose
variation of different stimulating factors (weak electromagnetic fields from ultra-low frequencies to EHF range, pharmacological neurotropic gas mixtures with xenon, herbal medicines, metabolic support products based on succinic acid, cAMP, selenium, and much more).

Despite the genuine interest of scientists from America, Japan, Israel and other countries, this powerful store of knowledge has found its port of destination primarily in Russia. For more than 30 years, our Oncology Centre has been promoting the accompanying therapy for various cancers at the stages of combination treatment. It has been shown that the inclusion of accompanying activation therapy in the form of central, local, extracorporeal or other actions and effects significantly improves the indicators of the state of the central nervous system, the hormonal and immune status in cancer patients, improves the quality of life and survival and also contributes to the effectiveness of special antitumor treatment. It is a transparent system of regulation of homeostasis.

At present, great importance is prioritized to the functional rehabilitation of cancer patients: international congresses are held, rehabilitation measures are being developed in various countries, the International Model of Rehabilitation in Oncology is presented in a versatile and qualified way [3], but however the mechanisms of integral response patterns cannot be found in these high technologies and unique systems. Behind the simplicity of the theory of health, the strategic keys to practical health remain unnoticed, the acquisition of which will open the path to a long and healthy life.

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NEW CHALLENGE TO CANCER AND LONGEVITY

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Medical Corporation “Saisei Mirai”, Osaka, Japan
“Saisei Mirai” has been focusing on cancer treatments and has developed several new treatments, including GcMAF (Gc protein-derived Macrophage Activating Factor) and oral MAF (Macrophage Activating Factor) which are immunotherapies developed by “Saisei Mirai”. We are collaborating with 6 universities here in Japan to develop new and better treatments mainly for cancer. At the same time, GcMAF and oral MAF showed good effects for many infectious agents, autism spectrum disorder (ASD), chronic fatigue syndrome (CFS), atopic dermatitis, hair loss, multiple sclerosis, autoimmune diseases, injuries and so on. First, I'll explain our cancer treatments.

A. Local destruction of cancer tissue
   1. Sonodynamic therapy
   2. HIFU (High Intensity Focused Ultrasound)
   3. Tumor Treating Fields therapy

B. Immunotherapy
   1. GcMAF injection
   2. Oral MAF produced from bovine milk
   3. Hyper T/NK cell therapy
   4. Autologous cancer vaccine
   5. Coley vaccine (Fever therapy)
   6. Dendritic cell therapy

C. Other treatments
   1. New liposomal P53 and PTEN gene therapy
   2. High dose vitamin C therapy
   3. Ozone therapy
   4. Hypoxia therapy
   5. Hyperbaric oxygen chamber

Originally our aim was to destroy local cancer tissue in combination with various immunotherapies, which is a very good combination in my opinion. Recently something very new and very interesting was added to our aim.

Usually, we use GcMAF injection and oral MAF for many patients and what happened to them was amazing and beyond my understanding in the beginning. During the cancer treatments and anti-aging treatments, we have observed some very good changes in many patients, which was not only that they had increased energy, but they also had silky and beautiful skin and increased hair volume with new black hair from gray hair, and they were looking younger and healthier. Although these cosmetic changes didn't happen to terminal cancer patients, they but happened to many other patients.
Given these clinical observations, I considered many times why rejuvenation and regeneration had occurred in so many patients in such a short time and then suddenly I saw a light. This must be associated with telomere length, because it's now emerging that telomere shortening is associated with age-related degenerative disease, increased risk of cancer and life shortening. That's why I decided to start a clinical trial in Japan using MAF capsules for longevity by measuring telomere length.

I'm going to tell you in detail about our preliminary clinical trial results using MAF Triple for longevity in the latter part of my speech. They are simply amazing as we succeeded in extending telomere length and I believe that extended telomere length will contribute to reducing the risk of getting age-related degenerative disease as well as the risk of developing cancer.

At the end, I'd like to add one thing that the name MAF (Macrophage Activating Factor) is good but it's not enough to explain all of the MAF capsule's abilities and functions. Given the fact that MAF capsules can extend telomere length, the name MAF can be changed to TEF (Telomere Extending Factor) in a broad sense.

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RADIODYNAMIC AND CONTACT RADIATION THERAPY OF INOCULATED TUMOR IN AN IN VIVO EXPERIMENT

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Introduction. In recent years, interest in the use of such physical factors as magnetic fields, ultrasound, and hyperthermia to activate Photosensibilizers (PS) has increased in experimental oncology. One of the promising scientific areas is radiodynamic therapy (RDT), a new method of therapy based on the combined use of PS and ionizing radiation [1]. The first PS with the radiosensitizing properties proven in the in vitro/in vivo experiments were 5-aminolevulinic acid, Hematoporphyrin, and Photofrin II [2, 3]. The main mechanisms of the implementation of the antitumor response of RDT remain poorly studied. According to Shaffer M. et al., on the one hand, PS (for example, "Photofrin II"), under the influence of ionizing radiation, can enhance the radiolytic effect due to reactive oxygen species (hydroxyl radical, superoxide anion and singlet oxygen) formed in the tumor cell upon exposure to the proper radiation [2]. On the other hand, the exposure to ionizing radiation leads to sublethal and lethal damage to tumor cells. Further, sublethal changes are, as a rule, reversible, associated with the implementation of the mechanisms responsible for restoring the functions of the tumor cell. In case of activation of the PS "Photofrin II" by
ionizing radiation, the oligomeric components of this PS, interacting with intermediate free radicals (hydroxyl radicals) formed in the tumor cell under radiation, prevent the development of these processes, and, therefore, this combination leads to the production of antitumor effects. The result of such interactions is the initiation of the processes of apoptosis and autophagy, leading to the death of tumor cells [1-4].

The results of experimental studies on the radiosensitizing effect of PS, published in 2019–2021, are presented in a number of scientific reports [1-3]. In the framework of some pilot projects, testing of the RDT method has begun in patients with various malignant neoplasms of the cervix and bladder, pelvic sarcomas, melanoma, gliomas, including inoperable cases. The obtained preliminary data show good tolerability of the above method (no serious adverse reactions found), acceptable antitumor efficacy (an increase in the rate of objective responses and an increase in the % reduction in tumor volume, which made it possible to transfer them to a resectable state) [5-8]. Taking into account all of the above, the study of the radiosensitizing properties of PS of the chlorin series seems to be a topical promising issue. This research work is a continuation of the studies, the results of which were published earlier [9], and it is aimed at optimizing the modes and parameters of the combined use of ionizing radiation in contact radiation therapy (CRT) and PS PHOTOLO.

The aim of the study was to investigate the antitumor efficacy of the RDT method with chlorin PS in combination with CRT in an experiment on laboratory animals with transplanted tumors.

Materials and methods. The study was performed in 35 white outbred rats of both sexes (hereinafter referred to as the rats) (animal breeding facility at the N.N. Alexandrov National Cancer Center), weighing from 170 to 300 g, aged 2.5–3 months. The duration of quarantine before inclusion in the experiment was 14 days. The rats were kept under standard conditions of food and water ration ad libitum, with 12-hour illuminance, at a temperature of 19–22°C and a humidity of 55–60%, in individual cages, 5 individuals in each. The studies were carried out in accordance with international legislation and the regulatory legal acts in force in the Republic of Belarus applicable to conducting experimental studies with laboratory animals, namely, the European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes (Strasbourg, France, dd. March 18, 1986) as amended in accordance with the provisions of the Protocol (E.T.S. No. 170 of December 2, 2005) and TPC 125-2008 (02040) “Good Laboratory Practice” (Resolution No.56 of March, 28, 2008 issued by the Ministry of Health of the Republic of Belarus). The study was approved by the Ethics Committee at the N.N. Alexandrov National Cancer Center (Extract from the Protocol No. 180 dated February 25, 2022). Before the experiments, the rats were anesthetized (neuroleptanalgesia: 0.005% fentanyl solution + 0.25% droperidol solution, in a ratio of 2:1, 0.2 ml per 100 g of body weight, intramuscularly). Pliss lymphosarcoma (PLS) (Russian Collection of Cell Cultures, Institute of Cytology RAS, St.
Petersburg, Russian Federation) was used as a tumor strain. A tumor model in rats was established by subcutaneous passivation in vivo: injection of 0.5 ml of a suspension of tumor cells in a Hank's solution under the skin of the left inguinal region. After inoculation, the rats were randomly assigned to study groups, 5 animals in each group. The rats with PLS were involved into the experiment on the 6th day after the average diameter of transplanted tumors had reached a size from 3 to 5 mm. As a radiosensitizing agent, we used the PS agent of the chlorine series PHOTOLOON (Belmedpreparaty, Minsk, Republic of Belarus), which represents a trisodium salt of chlorin e6 with povidone with a K-value of 17 (Registration number 16/11/886 dated 08.11.2016) (Pharmacotherapeutic group: drugs used in photodynamic and radiation therapy; ATC code: L01XD). The injection of the above PS agent was carried out once by intravenous infusion using a special catheter into the tail vein in a darkened room at a dose of 2.5 mg/kg of body weight. Radiation therapy was carried out by the contact method (CRT) with microSelectron-HDR V3 Digital equipment (Elekta, Sweden) using γ-radiation (192 Ir). For superficial CRT on the surface area of the transplanted tumor, the Leipzig applicator was used, fixed on the tumor surface with soft rubber holders. The study groups were composed as follows: group 1 as intact reference (IR) to cover rats with transplanted tumors, which were not injected with PS and did not undergo radiation (n=5); group 2 with 1 session of CRT at a single focal dose (SFD) of 2 Gy (n=5); group 3 with 2 sessions of CRT on the 1st and 2nd days of the experiment at a SFD of 2 Gy (n=5); group 4 with 3 sessions of CRT on the 1st, 2nd and 3rd days of the experiment at a SFD of 2 Gy (n=5); group 5 with PS 2.5 mg/kg + 1 RDT session 2.5-3 hours after the end of the PS infusion at a SFD of 2 Gy (n=5); group 6 with PS 2.5 mg/kg + 1 RDT session 2.5-3 hours after the end of the PS infusion at a SFD of 2 Gy + 1 CRT session on the 2nd day of the experiment at a SFD of 2 Gy (n=5); group 7 with PS 2.5 mg/kg + 2 CRT sessions on the 2nd and 3rd days of the experiment at a SFD of 2 Gy (n=5 ). The total focal dose (TFD) in groups 2 and 5 was 2 Gy; in groups 3 and 6 the TFD values was 4 Gy, and in groups 4 and 7 the TFD value was 6 Gy.

The effectiveness of therapeutic actions and effects was estimated according to the generally accepted criteria applied to experimental oncology, which characterize the dynamics of changes in the volumes of the tumors: the average volume of tumors (Vav., in cm³) and the coefficient of tumor growth inhibition (TGI, in %).

The minimum biologically significant TGI was 50%. The rate of complete regressions (CR) of tumors was assessed 60 days after the beginning of therapeutic interventions in the absence of visual and palpatory signs of the tumor growth. After the end of the observation period, the rats were sacrificed using the euthanasia method (aether pro narcosi) in compliance with humane methods of manipulations with laboratory animals. Statistical data processing was carried out using
the Origin Pro (version 7.0) and Statistica (version 10.0) application softwares. Data are presented as M referred to as mean value and m (SEM) referred to as error of the mean. The significance of differences was assessed using the Mann–Whitney U test. Differences were considered as statistically significant at p<0.05.

Results. The efficiency of the inoculation of the tumor strain was 100%. No complications that led to the death of rats with the introduction of the PS agent and radiation of tumors were recorded. The obtained experimental data demonstrating immediate and long-term results are presented below. Vav. in the IR group on the 14th day of the experiment was 44.42±5.88 cm$^3$. Irradiation of inoculated tumors (CRT) in groups 2-4 with CRT without prior administration of the PS agent allowed obtaining a moderately pronounced inhibition of the growth of inoculated tumors: 38.88±5.65; 30.25±7.24 and 3.41±6.29 cm$^3$ (p=0.51; p=0.16 and p=0.033 in relation to the IR group values). The TGI indicators in the groups amounted to 12.47%; 31.90% and 47.30%, respectively. The rate of CR of the tumors was recorded to be 20%, 20% and 20%, respectively. Irradiation of inoculated tumors in groups 5-7 with CRT after preliminary administration of the PS agent led to a more pronounced inhibition of the growth of the inoculated tumors: 29.38±2.24; 24.45±4.52 and 13.55±4.55 cm$^3$ (p=0.036; p=0.021 and p=0.0016 in relation to the IR group values). The TGI indicators in those groups amounted to 33.86%; 44.96% and 69.50%, respectively. The rate of the complete regression (CR) of the tumors was reported to be 0%, 20% and 40%, respectively.

Conclusions. The limited global experience in the combined use of PS and ionizing radiation and our own evidence data obtained testify to the fact that RDT is a new promising area in the scientific research in experimental oncology, and that the PS agent PHOTOLON has radiosensitizing properties, when using certain irradiation parameters, that is confirmed by the data on the dynamics of the growth of the inoculated tumors and the rate of the objective responses to the therapy. And the combined use of the two methods (RDT and CRT) allows summing up their antitumor effects, thereby optimizing the therapeutic effect produced on the inoculated tumors in the in vivo experiment.

References


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PDX MODEL OF BC IN THE STUDY OF PLASTICITY OF TUMOR CELLS IN THE PROCESS OF METASTASIZING

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Introduction. Mouse models using primary patient’s tumors (patient-derived xenograft, PDX) are an effective tool for studying the mechanisms of metastasizing. Tumor plasticity, i.e. the phenomenon of cell phenotype change in response to dynamic microenvironmental conditions is one of the key processes basic to metastasizing.

The aim is to study the plasticity of tumor cells in metastasizing using the PDX model of breast cancer as an exemplary case.

Materials and methods. Balb/c nude mice (♀, 8 weeks) were used to develop a xenograft model. In the 1st experiment, a suspension of primary tumor cells obtained from the surgical material of breast cancer was injected subcutaneously into the back area with the DMEM medium and Matrigel in a volume of 100 μl. In the 2nd experiment, a fragment of the primary tumor was implanted into the mammary gland area with suturing; after the formation of the vascular network, it was removed. Phenotyping was performed by flow cytometry (NovoCyte 3000 ACEA Biosciences, Agilent, USA) using antibodies to CD45, EpCam, CK7/8, CD44, CD24, N-cadherin.

Results. Lymphogenic micrometastases were found only after injection of the tumor cells, after 2-4 weeks in 5/10 mice, and hematogenous metastasis was detected only after the implantation of the fragments in 1/10 mice. The identical tumor cell phenotypes were found in the primary tumors, the xenografts, and the hematogenous metastasis. However, the number of the CD45-EpCam+CK7/8+CD44-C24-N-cadh+ and CD45-EpCam+CK7/8-CD44-C24-N-cadh+ cells in the primary tumor and the hematogenous metastasis increased from 2.53 cells/ml to 27.42 cells/ml and from 1.44 cells/ml to 27.39 cells/ml, respectively. The xenografts represented encapsulated formations made from cells with moderately pronounced polymorphism, with ovoid and irregularly shaped nuclei and weakly expressed cytoplasm, without a clear boundary. The hematogenous metastasis revealed in spatiun retroperitoneale was represented by diffuse fields of sharply anaplastic tumor cells with ovoid and irregularly shaped nuclei with finely dispersed chromatin and the presence of small nucleoli.

Conclusion. The phenotypic composition of the primary tumor, the xenografts and the metastasis was found to be identical, but some differences were observed at the quantitative level of the tumor cell populations.

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THE ROLE OF NUTRITIONAL RESTRICTION IN THE PATHOGENESIS OF MALIGNANT TUMORS

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Cancer is one of the leading causes of death in developed countries. Cancer incidence is growing, and it may be associated with longer life expectancy that leads to higher risk of cancer development. Metabolic changes are among the most important aspects of the tumor progression, which appear both in the tumor cells themselves and in the tumor microenvironment, affecting later the entire organism as a whole. Abnormal metabolism of tumor cells, including activation of aerobic glycolysis and enhancement of anabolic pathways, is important in metastasizing, medical drug resistance, and in the implementation of tumor stem cell viability programs [2].

In this context, a priority is given to studies aimed at the inhibition of enzymes of metabolic processes by small molecules or some exogenous factors. Overcoming metabolic plasticity is one of the strategic goals in anticancer therapy [3].

Restriction in caloric intake causes reprogramming of metabolic processes both in the tumor cells and in the cells of the tumor microenvironment [1], and therefore it can have an effect on the development and progression of malignant neoplasms. Elucidation of new mechanisms for the development of cancer is important in the context of limiting the influence of risk factors responsible for malignant neoplasms affecting the human body, as well as in searching for some new factors involved in the progression of the tumor growth.

The exact effects and mechanisms of influence of dietary restriction on carcinogenesis remain poorly understood. However, the possible management of food caloric content may be of practical importance in the context of carcinogenesis.

The area of our research is to study the mechanisms of functioning of tumor cells under conditions of dietary restriction in experimental models of B-16 melanoma. The key signaling pathway JAK-STAT, which is involved in the regulation of proliferation, growth, cell survival, and immune response, has been selected for this study.

New data on the effects of dietary restriction in an in vivo experimental model will contribute to the understanding of fundamental aspects of carcinogenesis and aging that can be applied to design and development of new methods for predicting risks of developing cancer and for using a combination of anticancer drugs and dietary restriction to achieve effective outcomes in neoplasm treatment.

References


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IMPLEMENTATION OF THE PRINCIPLES OF ACTIVATION ELECTROMAGNETOTHERAPY IN EXPERIMENTAL ONCOLOGY

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Introduction. Identification of an important mechanism of homeostasis associated with the existence of a periodic system of general nonspecific adaptational reactions of the body (AR) [1-3] as well as the study of the conditions for the development of the anti-stress AR of high levels of reactivity as the most favorable AR for an organism, allowed determining the requirements for the parameters of external regulatory actions, acting factors and regimes of their application. As a result, the principles of activation therapy (AT) were formulated: it is a treatment technology aimed at the physiological mobilization of the mechanisms of general nonspecific (including antitumor) resistance of the body. Among the AT factors, one of the leading places is occupied by electromagnetic radiation (EMR), which has a wave nature, to which the CNS structures as well as regulatory and metabolic processes of lower levels are highly sensitive [1, 3].

Activation electromagnetic therapy as a variant of AT involves the use of the following principles of AT:

- a low intensity of acting factors;
- a change in the exposure during the course of AT in accordance with one of the regimes of AT (randomly, based on actual shifts in the leukogram, "by the exponent");
- impact on the brain structures and sensitive areas of the body;
- use of bio-effective frequencies;
use of low-frequency modulation of a high-frequency signal;
use of poly-frequency radiation;
application of the "double impact" regime with localization of EMR firstly on the CNS structures, and then on the tumor or peritumoral area;
complex (mixed or combined) use of EMR of different frequency ranges;
combination of EMR exposure with metabolic therapy agents ("substrate support").

The aim of the study was to explore the protective and antitumor potential of activation electromagnetic therapy in experiments in vivo.

Materials and methods. In the course of many years of research on laboratory tumor bearers, white outbred rats and linear rats (Wistar) and mice (C57Black), EMR of infra-low frequency (ILF), super high frequency (SHF) and extremely high frequency (EHF) ranges, as well as coherent and incoherent optical spectrum radiation were used by us in experiments [1, 3]. The experiments were carried out in mice with B16 melanoma and rats with transplanted tumors such as sarcoma-45, sarcoma M-1, ovarian tumor (ascitic form), Pliss lymphosarcoma, Guerin's carcinoma as well as with carcinogen-induced tumors. For poly-frequency factors in the ILF range as well as for amplitude and frequency modulation, signals with bio-effective frequencies (including the frequencies of Schumann resonances) and their corresponding multiple frequencies (0.03, 0.09, 0.3, 1.7, 3.4, 7.8, 15.6 Hz) and also some other bio-effective frequencies are applied. The microwave length EMRs (SHF, EHF) had bio-effective carrier frequencies of 1 GHz (the so-called "resonance radiation") and 42.2 GHz, previously identified by other researchers. In a number of experiments, complex (mixed or combined) actions and effects of EMR of various ranges were used: EMR SHF and EMR ILF, optical spectrum (coherent and incoherent radiation) and EMR ILF, as well as the combined effect of EMR EHF and pulsed electric fields, EMR EHF or EMR ILF and biologically active substances (vitamin-amino acid complexes "Aviton", salts of succinic acid). The ranges of power (energy) characteristics of the impacts were as follows: magnetic induction (in the case of EMR ILF) 0.001 - 100 mT, power flux density (in the case of EMR microwave or SHF) of $10^{-3} - 10^{-2}$ mW / cm² / s. Impacts were provided with the use of equipment "Polyus", "Gradient", "Yav", "Ariadna", "Spectr", "Rikta", "SCENAR". To assess changes in the organs of the immune and endocrine systems and in the tumor tissue, methods of histology, histochemistry and electron microscopy were applied. The nature and the tension of AR were determined by hematological parameters [1-3]. In statistical processing we used the Student's, Wilcoxon-Mann-Whitney and Pearson's criteria ($\chi^2$).

Results. The application of the AT principles made it possible to significantly enhance the antistress effect of the used EMR that contributed to an increase in their antitumor and protective influence compared to the influences of the same factors in case of their use in other regimes (by a factor of
That was expressed in an increase in the percentage of the tumor growth inhibition, a decrease in the number of metastases (B16 melanoma) and the organs affected by a malignant process due to an infiltrative tumor growth (Pliss lymphosarcoma), an increase in the life expectancy of the animals, an increase in the rate of the tumor regression, etc. Moreover, in a number of cases, using activation electromagnetic therapy as the only antitumor treatment, in some animals (17-60%) it was possible to achieve partial and even complete regression of experimental tumors. So, some cases of complete regression of large tumors (4-6 cm³) and partial regression (by 40-50%) of tumors of larger size (10 cm³ or more), which were unresponsive to chemotherapy and radiation therapy, were noted in rats with Pliss lymphosarcoma. In case of experimental chemotherapy (sarcolysin, cyclophosphamide, thio-tef), activation electromagnetotherapy considerably mitigated the toxic effect of antitumor drugs that was manifested in a decrease in the occurrence rate and severity of leukopenia, an increase in the hemoglobin level, a significant weakening of signs of structural and functional disorders in the thymus, the spleen, the lymph nodes and the endocrine glands (the thyroid, the adrenal glands, the ovaries). The results of histological and histochemical examinations of the thymus and the spleen as well as histochemical and electron microscopic examination of the tumor tissue indicated the activation of intercellular interactions in the organs of the immune system and the tumor zone, and the appearance of signs of an increase in the degree of differentiation of malignant transformation cells.

Conclusion. The use of the AT principles in the electromagnetotherapy of tumor-bearing animals leads to a significant improvement in the multi-level regulatory relationships disordered by the malignant process that provides pronounced antitumor and protective effects in the animals with the experimental tumors.

References
The immune system is responsible for control of the antigenic continuity, ensuring the friend-or-foe identification and the formation of appropriate responses by the organism, aimed either at destruction (in case of infection with infectious pathogens and transplantation of an alien graft), or protection of the "foe" to be incorporated by the host organism (under physiological pregnancy, or in case of pathology under malignant growth) [1].

The immune system closely interacts with the DNA Damage Response (DDR) system, which, together with other signaling pathways, ensures the repair of damaged genetic material. In the presence of a defect in the repair system, for example, with congenital mutations in the BRCA 1/2 repair genes, genomic instability appears as a basis for the development of cancer. This determines clonal evolution through an accumulation of driver aberrations (CNA, chromosomal rearrangements, mutations), malignancy, and progression of a malignant process, primarily associated with distant metastases [2].

Genetic disorders lead to development of an immune response to new antigenic determinants arising from DNA damage as a result of some congenital or somatic mutations. The DDR-deficient cancer cells are recognized by the immune system, and it should be noted that chemotherapy and radiotherapy of patients also have a DNA-damaging effect. Innate immunity is involved through GMP-AMP synthase signaling, a stimulator of type 1 IFN gene (cGAS-STING signaling), with activation of an adaptive response (effectors of T-lymphocytes) [3].

The expression of the IFN genes can act as a predictor of the effects of immunotherapy.

The phenomenon of tumor prevention by the immune system exists, but it is difficult to explore, since it is impossible to trace under natural conditions, because the tumor does not develop in this case.

The immune system plays an important role (in cooperation with other components of the microenvironment) in tumor progression, stimulating the prometastatic properties of the tumor cells, helping increase their mobility, entry into the bloodstream, and transition to metastasis sites. We have obtained original data on the phenotype and expression profiles of the tumor cells, which should have the attributes of “seed cells”, that is, capable of giving rise to a tumor focus. But however this requires the presence of some prepared premetastatic niches to have suitable conditions for activating the proliferation and growth of the cells entering there, or some conditions...
for awakening dormant cells and forming a secondary tumor [4]. The cells of the immune system constitute the inflammatory infiltrate of the microenvironment and are the main effectors against the tumor cell (T lymphocytes, macrophages associated with the tumor). All existing present-day cancer therapies involve the immune system as an additional effector or regulatory influence, acting either in favor of the tumor or initiating an adaptive immune response directed against the tumor. Modern immunotherapy approaches (immune response checkpoint inhibitors proposed by James Allison and Tasuku Honjo, the 2018 Nobel Laureates in Physiology or Medicine) also apparently recruit the immune system to produce an antitumor response. However, the number of patients who received a therapeutic effect upon immunotherapy is far from that expected (20-30%), and, most importantly, the evidence data have recently been obtained on the phenomenon of the development of a fatal progression at such immunotherapy, leading to a sudden death of patients. In this regard, scientists face a vital task to determine both the indications for immunotherapy and find markers for predicting its effectiveness, and, importantly, some markers which may identify the risk of hyperprogression.

Improved effectiveness of the use of checkpoint inhibitors can be associated with their possible combination with targeted drugs aimed at specific molecular disorders, epigenetic regulators, and their target in cancer patients is the tumor microenvironment, so its modification should create conditions for an antitumor response. Thus, the immune system can control the behavior of the tumor, even under various therapeutic effects, and allows controlling the effect of immunotherapy. The translation of immunotherapy approaches into clinical practice has the prospect for increasing the effectiveness of treatment based on the search for adequate markers of prognosis and prediction as well as targets for therapy.

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INTERMITTENT FASTING AND INTERVAL HYPOXIC TRAINING FOR ACTIVATION OF ADAPTIVE RESERVES OF THE BODY, PREVENTION AND TREATMENT OF DEGENERATIVE BRAIN DISEASES

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Degenerative brain diseases (DBD) and other cognitive dysfunctions may appear due to the depletion of adaptive reserves as a result of aging, stress, depression, and other pathological processes (inflammation, malignant neoplasms). The depletion of the adaptive reserves leads to disordering accurate, appropriate signaling in the brain, between the brain and the body, as well as disorders at the levels of the organs, cells and molecules.

The body's adaptive reserves ultimately determine the tolerability or the resistance to various degrees of hypoxia, which accompanies stress, trauma, depression, inflammation, and aging. Oxygen is the most important and fastest regulator of metabolism.

The fundamental concept is as follows: optimal health is activated and stimulated by intermittent stimulation, i.e. moderate functional loads [1, 2]. We offer practical programs, which combine two non-pharmacological natural methods of activation therapy, training aimed at improving the adaptive reserve of the human body: intermittent fasting treatment (IFT) and interval hypoxic training (IHT).

IFT is a diet treatment that implies periodic fasting with special preliminary preparation and control of hunger sensation, sometimes with intake of some vitamins, trace chemical elements and electrolytes. The most often used intermittent fasting regimen in our practice is a 2-3-5-day fasting regimen repeated 3-2-1 times a month.

IHT is a way to increase the organism's nonspecific resistance by adapting to hypoxia. Adaptation develops when breathing with a hypoxic gas mixture (10-13% oxygen and about 87-90% nitrogen) through a mask (at normal atmospheric pressure) in an intermittent mode: breathing for 4-5 minutes with a hypoxic gas mixture with a break of 4-3 minutes (breathing room air corresponds to the sea level), that is, 4/4 or 5/3, 6-7 cycles, with session duration of 40 - 55 minutes. The therapeutic effect of IHT is achieved by a repeated, multiple (5-7 times) decrease in oxygen saturation (SpO2) from 94-99% to 80-86% within one session.

The main measures for the prevention and rehabilitation of degenerative diseases should be to support the biogenesis of mitochondria and their efficiency as well as reduce misfolding of proteins in cells. Mitochondria do not operate efficiently and generate more superoxides, when they are supplied with large amounts of glucose and oxygen.
Our goal is to maintain the maximum (optimal?) efficiency of the mitochondrial function at the cellular and molecular levels. This is achieved by quantitatively changing delivery of oxygen and glucose that is provided with IFT and IHT.

Pathological neural networks in the brain differ biochemically from the healthy tissue in an increased amount of lactic acid. The capability of IHT (similar to breathing at high altitude) of removing and reducing the content of lactic acid in the tissues and the organs, including the brain, leads to the elimination of pathological neural networks in the brain. This explains the "spontaneous" recovery in patients with epilepsy as well as the rapid essential improvement in the condition of patients after heart attacks and strokes.

Considering any disease (and its resistance to treatment) as the appearance and building-up of some pathological neural networks or pathological dominants in the brain (A.A. Ukhtomsky), which are supported by some biochemical changes (shifts in the content of lactic acid, norepinephrine), IHT can be evaluated as a unique treatment method.

IHT may exert its influence on the key links in the pathogenesis of DBD like the Ca2+ homeostasis disorders, oxidative stress, impaired nitric oxide (NO) synthesis, excitotoxicity that inevitably leads to an ischemic cascade and a cerebrovascular accident. The protective mechanisms of adaptation to hypoxia may be associated with the limitation of oxidative stress in the hippocampus, the limitation in the decrease in the NO production induced by b-amyloid, and the increase in the density of the vasculature in the brain [3].

IFT stimulates the development of three major protective and therapeutic benefits for the brain: increased autophagy [4], increased levels of brain-derived neurotrophic factor (BDNF) [5], and ketosis [2]. Ketone bodies such as hydrox butyrate are neuroprotective.

IFT may protect neurons from oxidative and metabolic stress in animal models of Parkinson's disease [5], Alzheimer's disease [6], and stroke [7]. The activity of cytokines as a result of IFT has a neuroprotective effect. The leptin and adiponectin levels also mediate neuroprotective effects. The sirtuin family of genes and corresponding proteins, which appear due to IFT, have a wide range of effects that improve health and longevity [9, 10].

The fasting treatment triggers the mechanisms responsible for a sparing energy by the cell, since it leads to the activation of AMP-dependent kinase - AMPK. As a result of the AMPK activation, the processes of maintaining a sufficient number of stem cells in tissues are activated, and neoplasms are inhibited. Restorative nutrition is an important stage in the treatment procedure. To ensure maximum positive results, it is important to design an individual IFT program with a targeted treatment diet.

It is possible to achieve a significant reduction in stress of the endoplasmic reticulum, increase the antitoxic protection of the cell, and suppress inflammation with an addition of certain food to
activate Nrf2 and the transcription factor PGC-1a, which contribute to the restoration of mitochondria.

By combining these two methods of intermittent stimulation, we can synchronize the brain functions in a faster and more efficient manner and increase the brain's ability for plasticity and self-restoration.

Coordination of both methods is essential in order to obtain the maximum long-term favorable effects. The duration, the rate of breathing cycles and the amount of oxygen (determining the intensity of episodes/cycles of hypoxia), on the one hand, and the duration of fasting with a restorative diet, on the other hand, are important factors.

In our presentation, we introduce data from our 35-year experience in fasting treatment for in- and outpatients and our 25-year experience in IHT. These results support the idea that the selected programs make a perfect combination to treat and prevent DBD. In our opinion, this strategy can provide a practical breakthrough in clinical care and the research approach to these diseases.

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MITOCHONDRIAL DYSFUNCTION UNDER THE GROWTH OF B16/F10 MELANOMA IN C57BL/6 MICE


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Mitochondria are present in all organs and tissues of the organism being responsible for the control of various aspects of the cellular function, providing the necessary supply of ATP, regulating Ca2+ signaling, controlling levels of reactive oxygen species (ROS) etc. Mitochondria perform specialized functions unique to certain tissues. Abnormalities in mitochondria disorder the key physiological functions such as the ATP production, oxidative phosphorylation, the reactive oxygen species production, and the Ca2+ regulation. This is considered as the mitochondrial dysfunction [5]. It is known that tumor mitochondria undergo some adaptive changes to further accelerate the rapid reproduction of tumor cells in an acidic and hypoxic microenvironment [2].

Aims. The aims in our study were to investigate dysfunctional signs of mitochondria in tumor cells and the internal organs in mice under the growth of B16/F10 melanoma.

Materials and methods. We used mice of both sexes of the C57BL/6 line (n=336). We composed the following groups of the C57BL/6 mice: the intact animal group (n=42); the reference group (n=42) with a model reproducing chronic neurogenic pain (CNP); the comparison group (n=126) with subcutaneous transplantation of B16/F10 melanoma; the main test group (CNP+B16/F10) (n=126). The male and female mice of the comparison group and the main test group were injected under the skin of the back below the angle of the left shoulder blade with 0.5 ml of a suspension of B16/F10 mouse melanoma tumor cells in saline solution at a dilution of 1:20. Chronic neurogenic pain was reproduced by ligation of the sciatic nerve on 2 sides under xylazolylethyl anesthesia. B16/F10 melanoma was transplanted into the animals of the main test group 3 weeks after the wound had healed. Mitochondria were isolated according to the standard method using differential centrifugation with a high-speed refrigerated centrifuge. In mitochondrial samples of the tumor, the skin, the heart, the brain, and the liver cells, the following concentrations were determined by ELISA: cytochrome C (ng/mg protein), caspase 9 (ng/mg of protein), Bcl-2 (ng/mg of protein), AIF (ng/mg protein), calcium (Ca2+) (mmol/g of protein), and the protein concentration in mg/ml was determined by the Biuret method (Olvex Diagnosticum, Russia). The statistical analysis of the data was completed with software package Statistica 10.0.
Results. An analysis of the system of apoptosis in the mitochondria of the internal organs in the C57BL/6 mice with an independent growth of melanoma and that combined with CNP demonstrated some dysfunctional changes in mitochondria both in the tumor and the tumor-bearing organ, namely, the skin, and also in all organs, both under the independent tumor growth and that in combination with CNP [3]. The nature of the dysfunctional changes in mitochondria was attributed to the functional specificity of the organ; considering all the internal organs, the heart’s reaction was the most intensive: that was evidenced by the morphologically confirmed infarctions under the growth of melanoma with CNP and suppression of the apoptosis factors [1,4].

Apoptosis processes differed in tumor mitochondria under the independent and CNP-combined tumor growth. In mitochondria of the tumor cells in the females during all 3 weeks of the independent tumor growth, we observed the higher level of Ca2+, on average 60.0 times higher than that in the intact skin, and the higher Bcl-2 value on average by 3.8 times, while AIF was found to be 7.5 times lower, cytochrome C was recorded to be 3.9 times lower, and caspase 9 was reported to be 2.0 times lower.

In the males with the independent tumor growth, only the level of Ca2+ demonstrated an increase in its value by a factor of 78.5, while the Bcl-2 and cytochrome C values were recorded to be 2.9 times and 3.7 times lower, respectively. The CNP-linked tumor growth in the females led to a sharp increase in the Ca2+ level in tumor mitochondria at the initial stage, and then, from the logarithmic stage to the terminal stage, to its multiple drop. In the males, the level of Ca2+ in the tumor mitochondria was reduced throughout the tumor growth period with CNP on average by a factor of 3.2.

Conclusion. We believe that the results obtained may indicate the presence of a deep stress response to the growth of melanoma in its independent variant and with CNP, which begins to manifest itself from the subcellular level with the "involvement" and "subordination" of the tumor disease of all organs. While the dysfunctional mitochondrial “pathway” shows its own features depending on the sex of the animal and the variant of the pathological process.

References


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PECULIARITIES OF THE COURSE OF LEWIS LUNG EPIDERMOID CARCINOMA IN MICE INFECTED WITH MYCOBACTER TUBERCULOSIS

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Introduction. The potential relationship between pulmonary tuberculosis and lung cancer remains still poorly understood. The aim of our work was to study the features of the course of the tumor process and pulmonary tuberculosis in mice infected with Mycobacterium tuberculosis with transplanted Lewis epidermoid lung carcinoma.

Material and methods. The study was carried out on 88 C57BL/6 mice aged two months. In accordance with the purpose of the study, the following groups of animals were composed: group 1 – “intact mice” (n=12), group 2 - "the reference H37R infection" (animals infected with the M.tuberculosis H37RV strain) (n=24), group 3 - "the reference tumors” (animals transplanted with Lewis epidermoid lung carcinoma) (n=23), group 4 – “the main test group 1” (animals infected with M.tuberculosis H37RV strain, which were simultaneously transplanted with Lewis epidermoid lung carcinoma) (n=24). Animals were infected by inoculating a suspension of Mycobacteria (1x106 CFU/0.2 ml) into the lateral tail vein. Transplantation of a 10% suspension of the pulverized tumor in a 0.9% solution of sodium chloride was performed intramuscularly into the femur within 2 hours after their infection with the suspension of Mycobacteria. Weekly, starting with day 14, 6 animals...
were removed from groups 2, 3 and 4 by euthanasia, for periodic intermediate evaluation of the course of the experiment. The obtained samples of organs and tissues were subjected to morphological and bacteriological examinations. Individual and group-related parameters were assessed using the SPSS Statistica v23 software package.

**Results.** On the 7th day of the experiment, the tumor developed at the site of the primary tumor cell transplantation in all mice from groups 3 and 4 (3rd group $V=161.3\pm14.80$ mm$^3$; 4th group $V=99.50\pm8.72$ mm$^3$). The tumor size in group 4, the main test group, was smaller than it was in group 3, the tumor reference group (on the 14th day in the 3rd group $V=343.9\pm77.05$ mm$^3$; in the 4th group $V=366, 3\pm36.96$ mm$^3$; on the 21st day in the 3rd group $V=1297.00\pm180.1$ mm$^3$, in the 4th group $V=864.5\pm92.33$ mm$^3$). The significance level $p$ according to the Mann-Whitney test, when comparing the tumor volume in groups 3 and 4, was as given below: 0.001 on the 7th day, 0.319 on the 14th day, 0.046 on the 21st day, and 0.044 on the 28th day of the experiment.

In the femur, in the area of the transplantation of the tumor suspension, in a morphological examination in all animals we revealed a tumor nodule, which was characterized by extensive areas of tumor necrosis, destruction of the muscle tissue, and bone plate invasion areas. Tumor metastases in the lungs were represented by foci of various sizes, predominantly of subpleural locations. Starting from the 14th day of the experiment, all infected animals developed pulmonary tuberculosis, which was confirmed by the bacteriological examination of their lung samples and the PCR-RT test. In the morphological examination in the infected mice, the pattern of pulmonary tuberculosis was represented by areas of productive pneumonia. When comparing survival rates in groups using the Log Rank test (Mantel-Cox), it was found that the survival of the animals was determined by the presence of a tumor rather than by infection with tuberculosis ($p = 0.001$). At the same time, the survival rates in groups 3 and 4 did not differ significantly.

**Conclusions.** When studying the course of Lewis epidermoid lung carcinoma in mice infected with Mycobacterium tuberculosis, an inhibition in the tumor growth was found in comparison with the uninfected animals.

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**STUDY OF BINDING OF INHIBITOR MOLECULES TO THE ACTIVE SITE OF PROTEIN KINASES BY METHOD OF MOLECULAR DYNAMICS**

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Eukaryotic protein kinases are represented by a large superfamily of homologous proteins similar in structure of the catalytic (kinase) domain, consisting of 250–300 amino acids [3]. Most human protein kinases share a common fold consisting of an N-terminal lobe, consisting of a five-stranded β-sheet with an α-helix called the C-helix, and a C-terminal lobe comprising six α-helices. The active center is located between the two lobes and forms binding sites for adenosine triphosphate (ATP) and protein [4]. Protein kinases modify the functions of other proteins by phosphorylation, acting as elements of signaling pathways. Disorders and abnormalities in the activity of these enzymes give rise to the pathogenesis of many malignant neoplasms.

Based on this fact, the targeted synthesis of some substances capable of selectively modifying the activity of protein kinases is an urgent task. The study of the process of interaction of molecules with the catalytic center of protein kinase by the method of molecular dynamics makes it possible to search for chemical structures of substances capable of inhibiting this enzyme [2]. Taking into account the high similarity of the structure of the catalytic domains of various human protein kinases, it is of scientific interest to develop a technique for modeling the molecular dynamics of the ligand-enzyme system, which allows predicting the spectrum of inhibitory activity against a set of protein kinases.

Using the method of molecular dynamics, the process of binding of inhibitor molecules to the active sites of phosphoinositide-dependent protein kinase-1 (PDPK1), mitogen-activated protein kinase-1 (MAPK1), and phosphoinositide-3-kinase (PI3K) was simulated. The PI3K kinase is not a protein kinase, however, this object is of interest in this study, because the substrate of the enzyme is also an ATP molecule. The rectangular modeling area included an active center of the enzyme and a region of space with water molecules and a ligand molecule. Modeling by the method of molecular dynamics was carried out with the Bioeureka software [1]. We used the quantum-chemical ab initio method, the density functional theory (ub3lyp) and the 6-311G** basis set to preliminarily calculate the charges of atoms in the ligand molecules. To increase the efficiency of scanning the conformational space, the approach of accelerated molecular dynamics was applied [5]. Temperature was controlled out separately for water, protein, and ligand molecules. The simulation was conducted for 500 ns with a step of 2 fs. During the modeling of molecular dynamics, the binding of ligands to amino acids of the active center in various conformations was observed. For each of the targets, a ligand was selected with a known location in the active center according to X-ray diffraction analysis (XRD).
The location of those ligands in the enzyme according to the simulation data was compared with the location according to the X-ray diffraction data by calculating the root-mean-square deviation (RMSD) of the atomic coordinates. The parameters of the modeling technique were chosen so that the RMSD value for individual conformations of the ligand was less than 1 nm. When the inhibitor was bound to the active site, the average value of RMSD was estimated. When the staurosporine molecule was bound to the PDPK1 active center, the RMSD value was 0.309 ± 0.028 nm, and the minimum was 0.236 nm. For the binding of the ulixertinib molecule to the MAPK1 active site, the RMSD was 0.468±0.163 nm, and the minimum was 0.178 nm. The average value of RMSD for the binding of the PQR530 inhibitor molecule to the PI3K active site was 0.973±0.253 nm, and the minimum value thereof was 0.496 nm. Thus, the developed technique allowed modeling the spatial arrangement of ligand molecules in the active center of the kinases selected for the study according to experimental XRD data.

This modeling technique can be successfully used to explore the spectrum of inhibitory activity of substances against protein kinases and design the structures of new targeted drugs with antitumor activity.

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GD2-SPECIFIC CAR-T CELLS CO-EXPRESSING THE IL15 MEMBRANE FORM EFFECTIVELY LYSE GD2-POSITIVE CELL TARGETS
Genetically modified T cells expressing chimeric receptors (CAR-T) have become an important tool for the treatment of the CD19+ hematological tumors. Due to a single-chain variable fragment of the antibody as a part of the CAR receptor, the cells acquire their specificity against selected tumor-associated antigens. The 3rd generation CAR also includes transmembrane, co-stimulatory domains, and tyrosine activation motifs derived from CD3z. This design allows the T-cells to recognize antigens in an MHC-independent manner and provides activation and release of cytotoxic cytokines upon recognition of the target. There are several limitations to the effective use of the CAR-T cells for the treatment of solid tumors, among them tumor stroma suppressive activity, poor persistence, and migration of effectors in a tissue.

The aim of our project is to improve the CAR-T cells targeting at GD2 and L1CAM neuroblastoma antigens by co-expression with membrane-anchored cytokines.

Results. At the present time, we have succeeded in achieving a stable 60-90% efficiency of lentiviral transduction of the primary T cells with an anti-GD2 CAR construction of the 3rd generation. At the same time, the CAR+ cells are equally represented by the CD4+ and CD8+ populations. To increase the persistence of the CAR-T cells, bicistronic co-expression of anti-GD2 CAR with the membrane form of IL15 (up to 40% CAR+; 30% IL15+; 30% IL15Ra+) was performed. Although the expected benefits of the 4th generation CAR receptor remain to be tested in in vivo models, its expression correlates with a better T cell survival in vitro. Both the IL15/IL15Ra positive and negative anti-GD2 CAR-T cells have been shown to specifically kill the GD2+ neuroblastoma and the glioma target cells (IMR-32, T98G) at an E:T ratio of 5:1 - 20:1. At the same time, degranulation, analyzed by the level of the surface CD107a, in contrast to anti-CD19 CAR-T, was practically not observed in short incubations.

Conclusion. The GD2-targeted CAR-T cells armored with the membrane form of IL15 specifically kill the GD2+ tumor cells in vitro and may serve as a promising tool for immunotherapy of neuroblastoma, ganglioneuroblastoma and glioma.

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STUDY OF THE ANTITUMOR ACTIVITY OF THE LIPOSOMAL FORM OF THE GLYCOLYSIS INHIBITOR IODOACETATE IN THE LEWIS LUNG CARCINOMA MODEL

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Topicality. The altered glucose metabolism in cancer cells, known as the Warburg effect, is a new area of research of anticancer agents [1]. To date, many compounds have been identified that address the glucose metabolism. However, many of them failed at the early stages of clinical trials, demonstrating systemic toxicity [2, 3]. The glycolysis inhibitor iodoacetate (IA) has a good antitumor effect, but, like other glycolysis inhibitors, it has an unsafe profile. Liposomes (LS) are a good element to provide the required targeted delivery and reduce the systemic toxicity of drugs.

The aim of our study was to identify the effect of iodoacetate encapsulation in liposomes made on its antitumor properties and assess the systemic toxicity thereof.

Methods. LS were prepared by extrusion. The dose of IA encapsulated in drugs was quantified using high performance liquid chromatography. The toxic effect was assessed by biochemical blood markers. The antitumor and antimetastatic activity was studied in a Lewis carcinoma model using short-term (4 days) and two-week long-term regimens.

Results. Our quantitative analysis of IA in drugs showed that at the maximum allowable drug concentration of 10 mg/ml, the maximum concentration for the drug injected into the vein was 8 mg/kg. An analysis of the biochemical parameters revealed that IA was toxic to various organs, but did not have a toxic effect on the liver. Encapsulation in drugs led to the elimination of all its toxic properties. The effectiveness of IA in free form to inhibit the growth of the primary focus reached 15% with its short-term use. In this case, inhibition of the growth of metastases was found at the level of 45%. In the treatment group with drugs, the therapeutic effect produced on the primary focus increased to 25% that was a moderate indicator of the antitumor effect for that model. However, the liposomal form thereof also reduced the antimetastatic efficacy of IA in short-term use. The use of drugs with IA in the long-term regimen resulted in the inhibition of the tumor growth in animals by 40-50% considering both the primary focus and metastasis.

Conclusions. The liposomal form of iodoacetate has a safe profile compared to the free form thereof. Its liposomal form has a moderate antitumor activity, and their antimetastatic effect is manifested under the conditions of the long-term use. The liposomal form of iodoacetate can be considered as a promising adjunctive therapy in treatment of malignant tumors.

References

LITHIUM EFFECT ON AUTOPHAGY AND CELL DEATH IN SKIN MELANOMA

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Melanomas are a group of tumors produced by malignant melanocytes, and despite the fact that the recent advances in molecular therapy and immunotherapy of skin melanoma significantly help improve the outcome of the disease, nevertheless, spontaneous mutations and dysregulation of various signaling pathways contribute to the tumor aggressiveness and the development of the drug resistance. Autophagy is a conservative intracellular mechanism that maintains cell homeostasis by processing damaged organelles. The role of autophagy in carcinogenesis, including melanoma, is ambiguous: on the one hand, autophagy contributes to the maintenance of intracellular homeostasis and cancer progression, but on the other hand, it can be aimed at activating processes leading to cell death. In certain cases, autophagy can enhance apoptotic signals, in particular, it is known that prolonged autophagy initiates the cell death. Currently, the growing number of studies confirm the possibility of using lithium as an antitumor agent in experimental oncology due to its capability to influence the signaling pathways, which govern the growth and development of tumor cells.

**Aims.** It seems promising to investigate the capability of lithium to stimulate autophagy and apoptosis in skin melanoma cells to enhance cell death.

**Material and methods.** To induce tumor growth, 1 * 10^6 cells of B16 skin melanoma were injected subcutaneously into the right inguinal region of C57BL/6 male mice. In the experiment, 2 groups of animals (n=5) were used: the mice with an intact tumor (the reference group) and the mice receiving daily lithium carbonate (Li2CO3) at a dose of 300 mg/kg for 7 days *per os*. Autopsy material (tumor) was prepared according to the standard method for performing histological examination and transmission electron microscopy. Tumor tissue cryosections were analyzed with immunofluorescent staining using primary anti-PCNA antibodies. For the immunohistochemical examinations, formalin-fixed and paraffin-embedded tumor sections were deparaffinized and rehydrated, subjected to thermal extraction of epitopes, and sequentially incubated with primary and secondary antibodies. Stained sections of the tumor were evaluated by two investigators using an
immunoreactive score. Weighted scores for expression of LC3 beta, LAMP1, Bcl-2, Bad, and caspase 3 were calculated using the formula as follows: degree x intensity of staining. Differences were assessed using nonparametric Mann–Whitney tests with the Statistica 6.0 statistical software.

Results. When evaluating the expression of the proliferation marker protein PCNA, a significant decrease in the number of proliferating cells in the tumor was shown. Lithium considerably increased the formation of autophagic vacuoles and the expression of autophagy markers LC3 beta and LAMP1 in the tumor cells. In sections stained with hematoxylin-eosin in the group of mice, who received lithium, multiple focal areas of cells with the eosinophilic cytoplasm, swollen nuclei and karyorrhexis were detected that were markers of the necrotic changes. Quantification of the identified areas of necrosis showed that lithium enhanced the necrotic death of the cancer cells in vivo. Lithium also increased the expression of proapoptotic proteins, but however no decrease in the Bcl-2 expression was observed. The Bad/Bcl-2 ratio for the reference group was recorded to be 0.77, and for the group receiving lithium, it was reported to be 1.

Conclusion. Thus, lithium carbonate, the conventional drug for the treatment of bipolar disorders, is a compound that inhibits proliferation and induces death of the melanoma cells via the induction of autophagy, necrosis, and apoptosis, likely associated with caspase activation. The obtained evidence data allow achieving a better understanding of the mechanisms of cell death of the melanoma cells with the introdution of lithium. Considering the accumulated experience of research in the use of lithium in cancer, as well as taking into account the fact that patients treated with lithium demonstrate a lower incidence rate of melanoma, further research on the therapeutic potential of lithium in cancer is required. The proper understanding of the precise mechanisms of the action of lithium may be useful for further development of combined chemotherapy regimens to address some specific intracellular signaling pathways and improve the anticancer treatment in melanoma.

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SELF-DEPENDENT ANTITUMOR EFFECTS PRODUCED BY METAL-CONTAINING NANOPARTICLES IN IN VIVO EXPERIMENTS
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At present, the scope of research of the medical and biological effects produced by metal-containing nanoparticles (NPs) is very impressive. At the same time, the vast majority of scientific research therein is carried out to cover three main areas: visualization of neoplasms (improvement of image contrast in MRI), targeted delivery of various antitumor factors (cytostatics, vaccines, etc.) and
magnetic fluid hyperthermia [1–3]. In the last decade, the fourth area of exploration is forming, associated with the study of the self-dependent effects of metal-containing NPs, which can be considered as a new option in cancer immunotherapy. At the same time, domestic researches carried out at the National Medical Research Center for Oncology in the period 2007-2016 [1, 3] allowed to obtain fundamental results earlier than foreign works of a similar essence came out [2].

The aim of our research was the study of self-dependent (without the use of any other, specific, antitumor agents) effects produced by various metal-containing NPs on transplanted tumors and the body of laboratory tumor-bearing animals.

Material and methods. The experiments were carried out in outbred rats of both sexes (500) and outbred male mice (40), as well as in C57BL/6 mice of both sexes (150). We used transplanted sarcoma 45, Pliss lymphosarcoma, Guerin's carcinoma, murine sarcomas 37 and 180, melanoma B16. The effects produced by NPs of magnetite (10 ± 2 nm), Cu (70–80 nm), Fe (30–50 nm), and ZnO (18–20 nm) NPs were studied. We began injecting of NPs after the formation of the tumors, when they reached the sizes, at which their spontaneous regression was considered to be unlikely, in some cases, even with the larger tumor sizes (with a volume exceeding 3 cm$^3$). Various single doses of the metal-containing NPs (0.25–35.5 mg/kg) and various methods of their injection - peritumoral, intratumoral, intraperitoneal - were used, with a total number of the NP introductions of 4-10. In separate experiments on white outbred rats and mice of the C57BL/6 line, weak (up to 3.2 mT) infra-low-frequency (up to 9 Hz) electromagnetic radiation (ILF EMR, Gradient-4M device) was applied to the brain (in rats) or the organism as a whole (in mice) in the regimes of activation therapy. Changes in the blood count and tissues in the animals were analyzed using cytology, histology, histochemistry, immunohistochemistry, electron microscopy, biochemistry, and flow cytometry. The adaptational status of the animals was assessed by their hematological parameters and the morphofunctional state of their immune system organs (the thymus and the spleen). The Statistica 6–10 software was used for statistical processing of the results. The Wilcoxon-Mann-Whitney and Pearson ($\chi^2$) tests were used.

Main results. The tested NPs, used as monofactors, had their antitumor effect in 33-100% of the animals in different sets of the experiments. The antitumor effect produced by NPs was manifested in an increase in the survival (at least by a factor of 1.5), inhibition of the tumor growth (by 33–78%), partial (by 30–60%) and complete regression of tumors, tumor growth arrest, and various combinations of the above effects. The form and the intensity of the effects depended on the type of NP, the type of the tumor, the dose, the method of introduction, the biological kind and the sex of the animals, the use of weak ILF EMR, and the season of the year. In the white outbred rats, in general, more pronounced antitumor effects were obtained as compared with those found in the mice. The high efficiency of NPs at minimal doses was demonstrated. The histochemical and
electron microscopic examinations showed signs of increased lymphoproliferative processes in the organs of the immune system, activation of intercellular interactions involving macrophages in the tumor zone, as well as death of malignant cells by apoptosis, autophagy, and necrosis. ILF EMR, which had a systemic antistress effect, enhanced the antitumor effect of magnetite ILF in rats with Pliss lymphosarcoma, which increased regression rates by 20-30%.

The results of flow cytometry indicated the dominance of the subpopulation of the CD3+ T-lymphocytes within the tumor zone with the detected growth inhibition and regression. The delayed onset of the regression of Pliss lymphosarcoma, the dynamics of such regression, the recorded large and very large sizes of the completely regressed tumors (10-30 cm$^3$) in the absence of toxic reactions indicated the development of the antigen-presentation processes in those cases and the death of the tumor cells by apoptosis as a result of effective tumor-specific T- killing.

Based on the obtained evidence data and the available literature data on the relationship between the macrophage phenotype and the ratio of the activity of iron transport proteins ferritin and ferroportin, a hypothesis was formulated that the antitumor effect of iron-containing NPs is due to a change in the polarization of tumor-associated macrophages M2 → M1 [3, June 2016]. The hypothesis was confirmed by the results of the studies of the tumor-growth-preventing effect made by ferrumoxitol, which were conducted by the group of H.E. Daldrup-Link [2, e, September 2016]. Similar, but somewhat less pronounced, effects were obtained in the rats with Pliss lymphosarcoma after the use of copper NPs.

The novelty of our obtained evidence data is supported by 6 patents for inventions.

The study results and their interpretation differ from foreign ones in the following:

1. The injection using metal-containing NPs was applied to the fully developed tumors, while foreign researchers started the injection of NPs in parallel with the introduction of tumor cells [2].
2. Foreign researchers associate the antitumor effects produced by the iron-containing NPs with the free radical processes only, due to the Fenton reaction initiated in the tumor-associated macrophages, which have undergone M2 → M1 polarization [2]. Based on the data on the dynamics of regression of the large Pliss lymphosarcoma tumors, we have made an assumption that, upon the polarization of the tumor-associated macrophages under the influence made by magnetite NPs, there is a possibility of the restoration of their ability to recognize tumor cells and effectively destroy them with the T-killers by apoptosis.
3. Studies of the antitumor effects of Cu and ZnO NPs were unique for a long time and did not have foreign analogues.

**Conclusion.** The results suggest using biogenic metal NPs as factors in antitumor immunotherapy aimed at overcoming the mechanisms of malignant cell evasion from immune surveillance.

**References**


PLASMONIC PHOTOTHERMAL THERAPY IN EXPERIMENTAL ONCOLOGY: PROBLEMS AND PROSPECTS
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Despite great advances in the treatment of cancer, the effectiveness of standard chemotherapy and radiation therapy for some malignant neoplasms remains insufficient. Plasmonic photothermal therapy (PPT) with plasmon resonance gold nanoparticles has been an intensively researched area in therapy in recent years [1, 2]. Successful PPT with nanoparticles for tumor treatment requires solving a number of problems associated with the potential toxicity and the bio-distribution of nanoparticles, including the development of the most efficient method of nanoparticle delivery, as well as optimization of their therapeutic protocol.

The aim of our study was to develop an effective and safe method of plasmonic photothermal therapy in rats with inoculated PC-1 cholangiocarcinoma.

Materials and methods. Gold nanorods (GNRs) coated with polyethylene glycol (PEG) were used in the study; the length and the diameter of the rods were 41 ± 8 nm and 10 ± 2 nm; the concentration of gold was 400 μg/mL, and their optical density was 20 at 810 nm. 24 white outbred male rats with inoculated cholangiocarcinoma RS-1 were divided into 4 groups (n=6) as follows:
the comparison group covering the rats without any exposure; the group with a single intravenous injection of GNRs and a single applied PPT procedure; the group with a double intravenous injection of GNRs and two PPT procedures, and the group with three intravenous injections of GNRs and three PPT procedures; in all groups, a dose of 2 mg/kg of GNR was used per injection. Prior to the injection of GNRs, the rats were scanned with Doppler-sonography using the Voluson E8 Expert Ultrasound Machine (GE Healthcare, USA) and applying the VOCAL software (Medison-Kretz) with the determination of the tumor volume and the vascularization index. A day after the last injection of GNRs, PPT was performed according to the protocol previously developed for intratumoral administration of GNRs [2] using the 808-nm IR laser LS-2-N-808-10000 (Laser Systems, Ltd., Russia) with a power density of 2.3 W/cm². The temperature of local heating of the tumor was measured using the IRI4010 IR thermograph (IRISYS, UK).

Half of the animals were removed next day after the therapy completion, and in the remaining animals, the tumor growth was monitored for 3 weeks. To assess an accumulation of gold by atomic absorption spectroscopy (AAS) and morphological examinations, tumor samples were taken. The microvascular density in the tumor was determined in the field of view of the histological specimen using the digital image analysis system of the medical microvisor of type µVizo-103 LOMO.

Results. The Doppler sonography showed that when the tumor reached a certain volume in a range from 5 cm³ to 10 cm³, the vascularization index increased with an increase in the tumor volume. When analyzing the temperature indicators of local heating of the tumor, it was revealed that the temperature rose above 60ºC for an efficient PPT procedure, only when the value of the tumor vascularization index reached at least 0.15. There was a relationship between the vascularization index and the gold content in the tumor after repeated intravenous injections of GNRs. At lower values of the vascularization index, there was a smaller accumulation of gold in the tumor tissue with multiple intravenous injections of GNRs.

A positive correlation was found between the index of vascularization and the index of microvascular density of the tumors which was consistent with the data of other authors [3].

Conclusion. The study demonstrated that an efficient PPT procedure with repeated intravenous injections of GNRs required a well-formed vascular network in the tumor.

References


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INNOVATIVE APPROACHES TO DEVELOPMENT OF GEROPROTECTORS-PHYTOADAPTOGENS FOR PREVENTIVE ONCOLOGY AND AGE-RELATED PATHOLOGIES
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At the present stage, the problems of preventive oncology are becoming more and more important, especially, at the stages of blastomogenesis, recurrence and metastasizing of tumors. Preventive measures can also be aimed at regulating the stress syndrome, improving the efficiency and safety of chemoradiotherapy, and improving the quality of life and rehabilitation processes for cancer patients. Promising in this aspect are approaches, which take into account the unique capabilities of natural antitumor geroprotectors: phytoadaptogens (PhA) [1]. The use of appropriate methods and techniques, including computer methods, makes it possible to identify probable pharmacotherapeutic effects as well as the mechanisms of action of individual phytocomponents and their combinations, which makes it possible to optimize the use of PhA, taking into account the pleiotropy of actions. Scientifically substantiated and relevant are studies of phytocomplexes based on the principle of a rational combination of structurally diverse biologically active substances and providing unique synergistic effects that cannot be obtained with each individual ingredient separately. Moreover, the use of several adaptogens in the same pharmaceutical composition allows you to influence the body without inducing addiction. Taking into account the centuries-old experience of traditional folk medicine in many countries, the results
of domestic and foreign studies, considering the capability of PhA to correct adhesive dysregulation, which is the key factor in blastomogenesis [2,3], we have studied innovative approaches to the design and development of complex geroprotectors-phytoadaptogens with high efficiency and safety. Our research opens up prospects for the design and development of geroprotective drugs for the prevention and treatment of neoplasms, age-related pathologies as well as increasing human life expectancy.

References

STUDY OF BIOLOGICAL ACTIVITY OF DOXORUBICIN CONJUGATE WITH N-ACETYL-D-GALACTOSAMINE HYDRASONIC LINKER
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Molecules containing the N-acetylgalactosamine residue are capable to bind to the asialoglycoprotein receptor, a protein presented on the surface of the liver tumor cells; therefore it is suggested that doxorubicin conjugates containing such fragment can be delivered to hepatocellular carcinoma cells as target, and then, upon hydrazone link splitting, in acidic environment of the tumor release the active substance.

The aim was to study the biological activity of the conjugate, which was a doxorubicin derivative with an N-acetylgalactosamine residue attached with a hydrazone linker, in experiments in vitro and in vivo.

Material and methods. The cytotoxicity of the synthesized conjugate was assessed in the cultivated Huh7 cells using the MTT test in co-incubation for 72 h in the concentration range from 4×10-2 µg/ml to 10 µg/ml. The toxicity was tested in vivo in the female DBA2 mice by an intraperitoneal injection of the test conjugate at a dose of 20 mg/kg, 25 mg/kg and 33 mg/kg (water for injection
used to dilute) followed by observation of the animals for 28 days. The DBA2 strain mice were intraperitoneally transplanted with the lymphocytic leukemia L1210 to study the antitumor activity of the above agent. The conjugate was administered at a dose of 24 or 12 mg/kg once 24 hours after the tumor transplantation. The efficacy was assessed by the criterion of increased survival (IS), and tolerability was evaluated by changes in the body weight of mice, some external signs, and the number of animals’ deaths from toxicity.

Results. The IC50 values in the cultivated Huh7 cells were recorded to be 0.08±0.02 µg/ml. The doses tested in the mice did not show any toxic effects within 672 hours after the single dose use; no death cases in the mice were reported, and no special features were recorded in the autopsy. The maximum tolerated dose was determined to be above 33 mg / kg for the intraperitoneal administration. In the model of the lymphocytic leukemia L1210, the IS value was 60% (p<0.05) at a conjugate dose of 24 mg/kg.

Conclusion. Thus, the obtained results show the prospects for investigations of the doxorubicin conjugate as an antitumor agent.

EVALUATION OF CYTOTOXICITY AND PRO-APOPTOTIC EFFECTS OF NEW CYP17A1 INHIBITORS
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The CYP17A1 inhibitors are used to treat castration-resistant prostate cancer. However, the existing shortcomings (side effects and low bio-availability) make the search, synthesis, and evaluation of the biological properties of new agents relevant.

The aim of our research work was to investigate the cytotoxic and proapoptotic properties of new compounds: alsevirone and LA-91.

Materials and methods. The antitumor agents alsevirone [1] and LA-91 were synthesized by the Laboratory for the Synthesis of Physiologically Active Compounds at the Institute of Biomedical Chemistry named after V.N. Orekhovich. The abiraterone substance (Baoji Guokang Bio- Technology Co., Ltd, China) was used as the positive reference. An inhibition of the CYP17A1 activity was assessed by changes in the rate of the enzymatic reaction for the formation of
androstenedione from progesterone in the homogenate of rat testicles. Cytotoxicity was assessed using the MTT assay with calculation of IC50 values. Prostate cancer cells 22Rv1, DU-145, PC-3, and LNCaP were incubated with alsevirone, LA-91 and abiraterone at specified concentrations (0.7-100 µM) for 72 hours. Proliferation and apoptosis markers were assessed using the Muse Cell Analyzer with special assay reagent kits Ki-67, Annexin V & Dead Cell Kit, Caspase-3/7 Kit, Bcl-2 Activation Dual Detection Kit, Multi-Color DNA Damage Kit.

Results. All studied agents contributed to the inhibition of the enzymatic activity of CYP17A1, whereas at a concentration of 5 µM the most active compound was LA-91, and the amount of androstenedione produced was 2 ± 0.5% compared with the reference (15 ± 3% of the reference after incubation with alsevirone, and 17±3% of the reference with abiraterone). Compound LA-91 was more cytotoxic than abiraterone in all cell cultures, and the IC50 values were recorded as follows: 5.4±1.8 µM (DU-145), 2.6±0.4 µM (22Rv1), 3.7 ±0.6 µM (LNCaP), 2.3±0.6 µM (PC-3).

Alsevirone demonstrated its cytotoxic activity comparable to that of abiraterone. It is interesting to note that alsevirone at a dose of 0.3 µM increased the content of phosphorylated histone H2A.X (H2A.X cells (+)) by 1410% of the reference value, while after the incubation with a toxic dose of LA-91, the level of the cells, containing H2A.X (+), reached 92.5%, and that after the use of abiraterone was 53.3% of the reference value. Alsevirone stimulated apoptosis and was accompanied by the caspase 3/7 activation comparable to that of abiraterone. So, 0.06 µM of alsevirone induced an increase in the number of cells with activated caspases 3 and 7 to a level of 286% of the reference, as compared to the same amount of abiraterone with 196% of the reference. Alsevirone also contributed to a significant increase in the expression of the phosphorylated form of Bcl-2 to a level of 352% of the reference compared to 181% of the reference value after the incubation with abiraterone at a dose of 0.3 µM.

Conclusions. Thus, the new inhibitors CYP17A1 successfully decrease the enzymatic activity to a degree comparable to that by made abiraterone. The cytotoxicity of LA-91 is higher than that of alsevirone and abiraterone. Alsevirone promotes the apoptotic cell death, and its mechanism of action differs from that of LA-91 and abiraterone, and it is associated with double-strand breaks in the DNA molecule.

References
ENERGY PHENOTYPES OF BT20, BT474, MDA-MB-453 UNDER ACTIONS AND EFFECTS MADE BY BERBERINE
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Breast cancer (BC) is among most cancers worldwide. To date, research is being actively conducted to study substances that can influence on the phenotype of the tumor cells. In chemotherapy with various drugs, the tumor cells have an ATP deficiency that contributes to a decrease in their proliferative activity. In the course of numerous pharmacological studies, the natural compound berberine isolated from the roots of plants of the genus Berberis sp. [1] has engaged the researchers’ attention as a candidate for this purpose.

The aim of our study was to explore the actions and effects produced by berberine on the energy phenotypes of breast cancer cell lines BT20, BT474 and MDA-MB-453.

Methods. We used immortalized breast cancer cell lines BT20, BT474, and MDA-MB-453 as material for our study. The cells were cultivated in the DMEM environment (Gibco, USA) with an addition of 10% fetal bovine serum (FBS) (HyClone, USA) at 37°C and 5% CO₂. The studied lines were seeded at a density of 2 × 10⁴ per well in the Seahorse XFp Analyzer microplate in DMEM with 10% FBS; berberine was added to the experimental wells to a final concentration of 2.5 μM or 10 μM [2]. The energy phenotype was investigated with the Agilent Seahorse XFp Extracellular Flux Analyzer (Agilent, USA) using the Seahorse XFp Cell Energy Phenotype Test kit (Agilent, USA) according to the Manufacturer's protocol. The statistical analysis was performed using Student's t-test. The significance level adopted in the study was α = 0.05.

Results. According to our research, berberine inhibited dose-dependently the basic level of cell respiration in all the studied lines, without having a significant effect on the reserve of oxidative phosphorylation. A decrease in the oxygen consumption was accompanied by an increase in glycolysis in the MDA-MB-453 cells, however, in the BT20 and BT474 cell cultures, the level of glycolysis did not differ significantly between the reference and the tested experimental samples. In addition, in all samples BT20, BT474 and MDA-MB-453 without berberine addition, suppression of mitochondrial ATP synthase by oligomycin and an increase in the level of glycolysis were found.

Conclusion. Thus, berberine contributes to the formation of a low energy phenotype with reduced ability to resist stress that makes this adjuvant a good candidate for breast cancer chemotherapy.

References
EFFECACY OF ANTISENSE OLIGONUCLEOTIDE HUSH-11 WITH THIOPHOSPHATE IN TREATMENT OF MELANOMA IN MICE
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Melanoma is one of the highly aggressive cancer diseases. Melanoma accounts for about 2% of the total cancer incidence in Russia. In a large number of cases, melanoma shows its potential to metastasize and becomes a cause of death.

Aims. Our research team focused on the design and development of an anti-melanoma drug based on antisense oligonucleotides (ASOs) with thiophosphate for the use both in the postsurgery treatment and the targeted therapy.

Results. Our pilot experiments showed the effectiveness of blocking the enzyme glucose-6-phosphate dehydrogenase (G6PD) using the thiophosphate antisense oligonucleotide Hush-11, which significantly reduced the cell index in the cancer cells in the cell culture of the Clone M3 melanoma murine line. The measurements were taken with the xCELLigence RTCA DP Analyzer. The determination of the cell index was carried out in 10 repetitions of the experiments performed in real time within 24 hours. The most active action made by the antisense oligonucleotide Hush-11 (5'-AGC-TAT-CTC-CG-3'), at a concentration of 7000 ng per 6000 cells, was observed 8 hours after the beginning of the experiment. The cell index in the reference sample was 0.2680 ± 0.092 s.u. that exceeded the average values in the experimental group by 70.1 ± 1.84% (p <0.01). Thus, our studies demonstrated the inhibitory effect produced by the antisense oligonucleotide Hush-11 on the proliferative activity of the Clone M3 melanoma cells. In addition, an injection of the antisense oligonucleotide Hush-11 into mice with the inoculated Clone M3 melanoma resulted in the significant tumor reduction compared to the untreated reference group. The average tumor size in the groups at the beginning of the experiment was 0.26 ± 0.03 cm²; on day 7 of the experiment, significant differences were observed between the mean tumor area recorded in the reference group (0.32 ± 0.07 cm²) and the group receiving Hush-11 (0.14 ± 0.04 cm²) (p <0.05). Currently, an ointment base formula is being developed for locoregional tumor therapy in order to avoid
injections as a possible damaging factor near the tumor that may induce dissemination of cancer cells.

**Conclusion.** The studies showed that *in vitro* the ASO Hush-11 significantly reduced the cell index in the melanoma cells compared to that in the reference group. The use of ASO Hush-11 in experiments *in vivo* contributed to a reduction in the tumor size within 7 days.

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**PATHOMORPHOSIS AND MECHANISMS OF DEATH OF TUMOR CELLS IN CELL CULTURES AND INOCULATED TUMORS UNDER THE INFLUENCE OF DRUG HEDGE HYSSOP EXTRACT**

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At present, the incidence of cancer tends to dramatically increase, and the existing advanced methods of cancer treatment are not always effective [1]. In addition, the disadvantages of the antitumor drugs used are their toxic effect made on the intact organs and tissues of the body, as well as the development of tumor resistance to them [2]. All this makes it necessary to search for new, safer and more effective drugs. In this case, plant-derived medicines have minimal side effects, so they are given special attention today. Plant-based drugs can be used both for the treatment of tumors as monotherapy and also in combination treatment to protect normal cells (including bone marrow stem cells) in a standard course of chemotherapy and radiation therapy [1,2, 3]. According to some researchers, the most promising group of this sort of medical drugs are flavonoids, since they produce the greatest number of biological effects, which favorably influence outcomes of treatment of neoplasms [2,3].

The aim of our research was to reveal some features of the pathomorphosis of tumors of different histogenesis, identify possible mechanisms of the tumor cell death and the resistance of tumor cells in experiments in cell cultures of human tumors (*in vitro*) and inoculated tumors in animals (*in vivo*) under the influence made by the flavonoid-containing hedge hyssop extract (Gratiola officinalis).

**Materials and methods.** The methodology of our research work based on a combination of experimental and analytical methods, as well as on a systematic and complex analysis of the already available data and obtained fresh evidence data. The methods and techniques used in our work were as follows: the cultural method, fluorescent staining techniques, flow cytometry methods, electron microscopy, morphological methods, morphometric methods, histochemical (Pas-reaction, WGA,
MHS staining, DNA and RNA staining according to Brache) and immunohistochemical methods (markers of Ki67, EGFR, VEGF, p53, Bax, Bcl-2, CD95 (Fas/APO-1), Fas-ligand and LC3B; statistical processing of results. In order to study the death of tumor cells, different groups of bioflavonoids were used: flavonones (extract of Helichrysum arenarium), flavonols (extract of Gratiola officinalis), anthocyanins (extracts of the anthocyanin form of Zea mays). The objects of our study were cell cultures as given below: SPEV - culture of epithelial cells of the kidney of the pig embryo; HeLa - cervical cancer; SK-BR-3, MCF-7 - breast adenocarcinoma; Jurkat - T-cell lymphoblastic leukemia; A549 - lung carcinoma; PC-3 - prostate carcinoma; HCT-116 - colorectal carcinoma; A498, Caki and Sn12c - kidney carcinomas; tissues of inoculated tumors (sarcoma 45, kidney cancer PA and liver cancer PC-1); cells of the internal organs and the brain, fixed in 10% buffered neutral formalin solution and embedded in paraffin. For the statistical data processing, the normality of the distribution of the indicators in the groups was tested using the Shapiro-Wilk test.

To compare the parameters in the groups, the Cramer-Welch test (T) was applied.

Results. Regardless of the histological structure of the inoculated tumor in the animals, with the introduction of hedge hyssop extract, grade 2-3 pathomorphosis of 2-3 developed in the tumor: the proliferative activity decreased (Ki67; EGFR), the cell cycle stopped and the cells entered the G0 phase (Ki67), angiogenesis (VEGF) was blocked, and apoptosis was activated (p53, Bax, CD95 (Fas/APO-1), Fas-ligand), and protective autophagy was blocked (LC3B). The features of the pathomorphosis in the inoculated sarcoma 45 involved the replacement of the tumor tissue by the fibrous tissue, and in case of kidney cancer PA the development of giant cell pathomorphosis.

Conclusion. Thus, in our experiments in vitro and in vivo, as a result of a comprehensive morphological explorations of structural changes and alterations in the tumor cells under the action made by the hedge hyssop extract, we found that apoptosis caused the typical cell death, realized through the caspase-3 activation mechanism, and that the expression of the LC3B protein, an autophagy marker, was a morphological criterion and predictor of the tumor resistance to antitumor drugs.

References
GALLO-ELLAGI-TANNINS OF CHAMAENERION ANGUSTIFOLIUM: ANTITUMOR ACTIVITY

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Currently, in Russia and abroad, there is a growing interest in the use of gallo-ellagi-tannins of fireweed (Chamerion angustifolium) as a source of antitumor and immunomodulating agents [1]. Fireweed (Chamerion angustifolium) is a popular tea; it is typical of the flora in many regions of Russia, and therefore studies on its chemical composition and biological activity are relevant.

The aim of our research work was to study the possibility of isolating and using gallo-ellagi-tannins as adjuvant for immunization (obtaining a B-cell response) in an experiment in mice.

Materials and methods. Gallo-ellagi-tannins with antitumor properties (the chanerol substance) were isolated from inflorescences and shoots of Chamerion angustifolium from the Onagraceae family, according to the original method by the N.N. Blokhin Research Institute [2]. We used selective extraction methods and chromatographic techniques for separating the obtained compounds.

To test the possibility of using tannins as an adjuvant, immunization experiments were carried out in 21 mice. For comparison, the following compositions were prepared: a) 25 µg chamenerin I + PRAME, b) 25 µg chamenerin II + PRAME, c) 25 µg chamenerin III + PRAME, d) 25 µg of the sum of higher oligomeric tannins of Chamerion angustifolium + PRAME, e) Al(OH)₃ + PRAME used as the positive reference for the conventional adjuvant and the antigen presence f) pure PRAME protein used as the positive reference for the antigen presence, g) the negative reference without immunization. The B-cell response of the compounds was assessed by the intensity of staining of the PRAME-expressing K562 cell line with the sera of the immunized mice.

Results. According to the results of flow cytometry, antibodies contained in the mice sera stained the K562 cells with different intensity. The staining intensity of the K562 cell line was highest when using the sera from the mice immunized with dimeric and trimeric gallo-ellagi-tannins (chamenerin I and chamenerin II). Serum from the mice immunized with chamenerin I stained the cells more intensely than the sera from the reference mice (p = 0.0282). Serum, obtained from the mice immunized with chamenerin II, stained cells more intensely than the reference, but the differences tended to be significant (p = 0.0933). The rest of the data did not significantly affect (p>0.1) the results of the experiment.

Conclusions. Thus, the drug chanerol based on the dimeric tannin derived from the inflorescences and shoots of the fireweed plant (Chamerion angustifolium) contains admixed trimeric and higher oligomeric tannins. An analysis of the biological activity demonstrated that the most pronounced B-
cell response in mice against the PRAME protein was achieved with the use of dimeric and trimeric
gall-ellagi-tannins as an adjuvant for immunization.

The offered method for isolating the oligomeric hydrolysable macrocyclic tannins allows obtaining
purified compounds for preclinical studies in the required quantities.

The researches were carried out within the framework of the state task of the Research Institute EDTT Federal State Budgetary Institution named after N.N. Blokhin and state task of Federal State Budgetary Institution "VILAR" (FGUU-202-0009, FGUU-2022-0010).

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ABNORMALITIES IN NATURAL LIGHT REGIMEN AS TRIGGER OF TUMOR GROWTH
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One of the regulators of physiological rhythm patterns in humans and animals is the change in the circadian cycle implying the day-night alternation. Exposure to light at night has been found to be directly related to serious behavioral problems, as well as to the health state and the development of malignant neoplasms. Epidemiological studies show an increased risk of breast and colon cancer in night shift workers and a reduced cancer risk found in blind women. Currently, a fairly large number of the population is exposed to light pollution (light at night). Therefore experimental studies, revealing the role of abnormalities in the natural light regime in carcinogenesis, become topical.

A specific photoperiodism is observed in Karelia: a long daylight period in the spring-summer period (the season of the “white nights”) and a short daylight period in the autumn-winter period (with day light duration of 4.5 hours). Seasonal fluctuations in illuminance as a natural abnormality
in the circadian rhythm pattern, as well as light pollution as an artificially produced disturbance are considered certain modifiers of the tumor growth.

The aim of our study was a comparative investigation of the long-term effects produced by different light regimes on spontaneous carcinogenesis in female rats.

Materials and methods. The experiments were carried out in 220 in-bred female rats, initially delivered by the Research Institute of Oncology named after N.N. Petrov. All animals received standard prepared laboratory food and water with free access to them. The research work was performed in compliance with the international principles specified by the Helsinki Declaration, adhering to the guidelines for the use and care of laboratory animals in research.

The study lasted up to the natural death of the animals. To measure the illuminance in testing facilities, the F-107 luxmeter (Russia) was used. All in-bred rats aged 25 days were randomly divided into 4 groups. The first group of the female rats was exposed to a standard fixed light regime (12-hour light conditions 750 lux: 12-hour dark conditions; LD). The second group of the animals was exposed to natural light (NL), and in this case, the features of the annual photoperiodicity of the North-West of Russia were taken into account. Multiple measurements of illuminance were taken at different times of the day and night within the annual cycle. Illuminance in the test room changed during the day, and its values at the level of the cells were recorded to be 50-200 lux per m² in the morning, up to 1000 lux in the afternoon on a clear day and 500 lux on a cloudy day, and 150 to 500 lux per m² in the evening. The third group was kept under constant illuminance (750 lux; LL) 24 hours a day. The females of the fourth group were under the conditions of day light deprivation (DD), while the illuminance in the room was 0–0.5 lux per m².

Animals dying during the experiment were dissected. All internal organs were examined during autopsy. Neoplasms were classified according to the IARC recommendations as fatal or incidental. All tumors and major internal organs suspected of the tumor growth were examined microscopically after the standard histological processing. The histological classification of the tumors proposed by the IARC was applied.

Results. Abnormalities in the natural light regime produced a statistically significant modifying effect on the development of spontaneous tumors in the female rats. So, in the NL group of rats, the incidence of neoplasms significantly increased compared to the LD group, mainly due to a double increase in the incidence of benign breast tumors. It should be noted that three cases of uterine adenocarcinoma were revealed in the NL group, while in the LD group no such tumors were observed. Housing of the female rats under the DD conditions significantly suppressed the development of all tumors, and mainly the breast tumors, compared to the reference cases. The NL and LL regimens promoted the development of all or malignant tumors only, while the DD regimen suppressed spontaneous carcinogenesis in the female rats. In the long-rank test, the differences
between the curves of the dynamics of the development of all or only malignant tumors between the reference and all other groups were highly significant (p<0.001). The highest index of tumor multiplicity (the number of tumors per tumor-bearing rat) of 1.63 was recorded in the LL group, and the lowest index of 1.07 was reported to be in the DD group. Fatal malignant neoplasms most often developed in rats in the NL regimen, least often they were found in the DD regimen. The most common neoplasms were benign tumors, accounting for 81.8% of all neoplasms. Among them, fibromas and fibroadenomas of the breast prevailed (53.8% of all tumors). The second largest group of the benign neoplasms included glandular-fibrous polyps, fibromas and uterine fibromyomas (14.4% of all tumors). Among the malignant tumors, which accounted for 18.8% of all neoplasms, hemoblastoses, carcinomas of internal organs and sarcomas were approximately equally shared (37.5; 37.5 and 25%, respectively). Malignant mesenchymal tumors were represented by poorly differentiated sarcomas located primarily in the abdominal cavity, and stromogenic uterine sarcoma. Conclusion. Thus, housing the female rats under the LL and NL conditions led to a significantly faster development of spontaneous tumors compared to the animals under the LD conditions. Light deprivation reduced the tumor incidence rate. Apparently, a lack of melatonin or an abnormality in the rhythm pattern of its secretion is the cause of the accelerated development of neoplasms. An essential modifying factor of carcinogenesis is a disturbance in the normal natural alternation of light and dark periods, resulting in desynchronization of the organism's circadian rhythms. Exposure to light at night can be considered as one of the environmental factors leading to disordering of homeostasis and carcinogenesis. The results of our studies allow us to substantiate the fundamentally important position that desynchronosis of the activity of the epiphysis, caused by light pollution or seasonal fluctuations in illuminance, leads to an increase in the incidence rate of neoplasms. A comprehensive assessment of this sort of impacts is possible with the use of an adequate experimental model. Rats can serve as such model, since melatonin secretion in these animals has the same circadian rhythm as it is the case with humans. The longitudinal research method used in our experiment is the most adequate one, since it involves observing the same animals over a long period from youth to old age and death. This approach makes it possible to correctly compare the studied parameters in different experimental groups.

References
Hepatocellular carcinoma (HCC) is one of the most aggressive human tumors with a high mortality rate. Despite the fact that the incidence rate of this sort of tumors is high, the mechanisms of its pathogenesis and metastasis are not fully understood, and the existing therapy is often unsuccessful. There is evidence in the reference literature available that in patients with HCC, alterations occur in the microenvironment of the tumor tissue, including the extracellular matrix (ECM) that stimulates the growth and metastasis of the HCC cells. Therefore, the aim of our study was to investigate the state of the tumor ECM during the spontaneous tumor development and with lithium carbonate introduction.

**Materials and methods.** The study was carried out in male CBA mice. The hepatocarcinoma-29 (G-29) cells were used to induce the tumor process. The animals were divided into 2 groups: group 1 included the animals with the tumor growth; group 2 covered the animals with the tumor treated with lithium carbonate. The material was taken on the 30th day of the experiment. Tissue staining was performed with the use of the PAS-reaction method. The morphometric analysis of the tumor tissue was completed.

**Results.** A part of the PAS-positive tumor regions had gaps and contained erythrocytes, while the other part had no visible gaps, therefore it was impossible to evaluate their functional state. The volume density of the PAS-positive regions in the tissues in the animals with spontaneous tumor development was found to be significantly higher than in the animals treated with lithium carbonate as therapy. There is evidence in the reference literature that ECM is actively involved in HCC carcinogenesis and metastasis. In this case, proteoglycan levels increase. Apparently, lithium carbonate is capable to block these processes in the ECM, thereby suppressing the tumor growth and development.
Conclusions. The state of the ECM is an indicator of the tumor growth. A decrease in the volumetric density of the PAS-positive components in the tumor tissue with the use of lithium carbonate may be a marker of blocking the G-29 growth.

References

INFLUENCE OF HYPOTHYROIDISM ON THE HYPOTHALAMIC-PITUITARY-GONADAL AXIS IN RATS WITH GUERIN'S TUMOR
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The thyroid and the gonadal (HPG) regulatory axes are interconnected and act together as a single system and their imbalance leads to pathological responses of the organism [1, 2]. The involvement of thyroid and sex hormones in the development of the malignant process has been discussed for many decades.

The aim of our research work was to study the concentrations of regulatory peptides in the hypothalamus and the pituitary glands, as well as sex steroids in the gonads, the tumor and its perifocal zone in rats of both sexes with Guerin's carcinoma growing in the presence of hypothyroidism.

Materials and methods. Albino outbred rats of both sexes (n=30) weighing 150 g or more received Mercazolil at a daily dose of 2.5 mg/100 g of body weight (the total dose of 75 mg/100 g of body
weight) for 30 days. In blood of the animals, the levels of thyroid hormones T3 and T4 as well as TSH were determined. After persistent hypothyroidism, male and female animals of the main group were inoculated with 0.5 ml of a suspension of the Guerin's carcinoma cells in a 1:5 dilution with saline solution under the skin of the back. The reference group consisted of rats of both sexes (n=30) with subcutaneous inoculation of the Guerin's carcinoma cells at the same dose and volume as in the main group. In the rats of the main test group and in the reference group, after 14 days of the tumor growth, using the standard ELISA methods, in homogenates of their organs and tumors, we determined concentrations of the hypothalamic gonadotropin-releasing hormone (GnRH) and the pituitary tropic hormones LH, FSH as well as sex steroids: estradiol (E2), testosterone (T) and progesterone (P4).

Results. The growth of Guerin's carcinoma in presence of hypothyroidism demonstrated its own sex-related characteristics, since the central regulatory structures of the HPG axis - the hypothalamus and the pituitary glands in the males - were found to be more susceptible to changes in response to suppression of the thyroid gland function than in females. In the males of the main group, compared with the reference group, the level of LH-releasing decreased by a factor of 4, while LH in the pituitary gland increased by a factor of 1.3, but however FSH decreased by a factor of 2.2. In the females of the main group, only a decrease in the level of pituitary FSH by a factor of 3.6 was found. Also, in the male tumor-bearers, in response to hypothyroidism, in their testicles, suppression of steroidogenesis was observed: the level of T decreased by a factor of 4, P4 increased by a factor of 1.9, and the content of E2 did not change, while the ratio of E2/T increased by more than 4 times. In the gonads of the females in the main group, compared with the reference, the concentration of all steroids increased: E2 by 2 times, T by 1.8 times and P4 by 1.7 times, but the E2/T ratio did not change. However, the tendency of the changes in steroid hormones in the tumor in response to induced hypothyroidism in the females and the males was the same: the concentration of E2 decreased on average by 1.4 times, but the concentration of T increased on average by 1.8 times and P4 by 1.6 times and 3.9 times, respectively, while the ratio of E2/T decreased in the females by 2.3 times and in the males by 7.7 times.

Conclusion. Hypothyroidism as comorbidity for a malignant process makes its influence on all links of the HPG axis and also alters the local synthesis/uptake of steroid hormones by the tumor.

References
THE ROLE OF THE SYSTEM OF INSULIN-LIKE GROWTH FACTORS IN BLOOD IN SOFT TISSUE SARCOMAS


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Although soft tissue sarcomas (STS) belong to a group of rare diseases of mesenchymal origin, their characteristic tendency to recurrence and high mortality necessitates the search for some prognostic bio-markers to identify patients at risk of their recurrence [1]. In this regard, the system of insulin-like growth factors (IGF) and their binding proteins (IGFBP) is of interest, since it has been established that dysregulation of the IGF system leads to the proliferation of cancer cells, migration and resistance to chemotherapy [2].

The aim of our research was to conduct a comparative study of the content of the components of the system of insulin-like growth factors in blood of patients of both sexes with primary and recurrent soft tissue sarcomas.

Materials and methods. The study included 54 patients with histologically confirmed soft tissue sarcomas (STS), stage T2bN0M0, who were treated at the National Medical Research Centre for Oncology from 2020 to 2021. The average age of the patients was 63.1±0.9 (48 to 75) years. The group with primary STS included 12 male individuals and 12 female individuals; the group with recurrent STS covered 10 male individuals and 20 female individuals. Highly differentiated tumors (G1) were observed in 50% of the patients, the rest of them had poorly differentiated (G3) and undifferentiated (G4) tumors. The reference group included 10 males and 10 females of the same age without cancer. The concentrations of IGF-1, IGF-2 and IGFBP 2 were analyzed in blood of the patients before the beginning of their treatment, with the use of ELISA (Mediagnost Germany). The dependence of the changes on the tumor differentiation (G1 or G3,4) and on the time of recurrences was analyzed. Our statistical analysis was carried out using the Statistica 10 software with nonparametric Kruskal-Wallis and Mann-Whitney tests. Differences were considered statistically significant at p<0.05 (when 2 groups were compared), p<0.017 (3 groups), and p<0.0085 (4 groups).

Results. In primary STSs in the males and females, a decrease in the concentration of IGF-1 was revealed. In the males with G3.4, IGF-1 was reduced relative to the healthy group almost by 14 times (p=0.0000), and with G1 only by 26% (p=0.041); in females, a 20-fold decrease was
observed, independent of the tumor differentiation. Only a slight decrease in the IGF-2 level was noted that led to a drop in the IGF-1 / IGF-2 ratio in the males with G3.4 by 8.8 times and in all females by 24.3 times. With recurrent STSs in the males, there was a decrease in the IGF-1 level by 40.4% (p=0.0001) and in the IGF-2 level at G3.4 by 19.3% (p=0.0027), while at G1 the IGF-2 concentration even exceeded the level of donors by 9.4 (p=0.0017). In the females with the recurrences, there was a decrease in the IGF-1 level by 78.2-85.5% (p ≤ 0.0003) and an increase in the IGF-2 level by 20.9-57.8% (p ≤ 0.04) relative to the concentrations of these ligands in healthy women, whereas both processes were found more pronounced in case of a slow process of recurrence. IGF-2 levels in relapses exceeded the levels in primary STSs by 63-113% (p<0.004). Blood levels of IGFBP2 in all females with primary STSs increased by more than 3 times, while in recurrences the protein level increase depended on both tumor grade and the time to recurrence.

With a rapid recurrence (from 1 to 3 years), the median concentration of IGFBP2 was 2.6 times higher (p = 0.0001) than that recorded in a slow recurrence (more than 3 years), and at G3.4 relative to G1 it was recorded to be 2, 2 times higher (p=0.005). In the males, the increase in IGFBP2 was less pronounced than in the females. Thus, the dependence of an increase in the level of IGFBP2 in the females on the time to recurrence and the STS grade suggested that component of the IGF system as a biomarker of poor prognosis, as it was reported for other cancers [3; 4].

Conclusion. STSs are characterized by changes in the IGF system. The imbalance between the IGF components in blood is most pronounced in patients of both sexes with the G3,4 tumors. The increase in the level of IGFBP2 in STS in all patients and its dependence on the clinical characteristics of the disease, in particular in recurrent processes, indicate the prognostic value of this indicator.

References

PECULIARITIES OF INFLUENCE MADE BY DIABETES MELLITUS AS COMORBID PATHOLOGY ON THE SYSTEM OF SEX HORMONES AND THEIR RECEPTORS IN TUMORS OF DIFFERENT HISTOLOGICAL ORIGIN


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Improving the survival of cancer patients contributes to a growing number of patients with chronic comorbidities, including diabetes mellitus (DM). Moreover, higher risk of cancer development in patients with DM and poor prognosis determine the importance of studying the mechanisms mediating the effect of altered glucose metabolism made on the growth and metastasis of malignant tumors [3,4]. The polyetiology of both pathologies and the presence of research studies that do not confirm a direct relationship between DM and the development of malignant neoplasms indicate the complex nature of their relationship with the possible context-dependent regulation of many cellular processes associated with carcinogenesis and the development of DM [1,2]. An important role in the processes of the regulation is played by an imbalance in the system of sex hormones.

The aim of our investigations was to study the features of the influence made by DM as comorbid pathology on the course of the tumor process and the concentrations of the sex hormones and their receptors in the tumor tissue of different histological origin and the perifocal zone in different species of experimental animals.

Material and methods. Females of the white non-linear rats (n=36) and the BALB/c Nude mice (n=28) were used. The reference groups included the animals with an independent growth of an inoculated tumor (Guerin's carcinoma (GC) in the rats, and the Lewis lung carcinoma (LLC) in the mice), and the main groups covered the rodents with the tumor growth with presence of DM induced by alloxan injection. In some animals, survival and dynamics of the tumor volume were studied. In the other animals after euthanasia in the pre-terminal state (rats on the 10th day after CG inoculation, mice on the 19th day after LLC inoculation) in the tumor and its perifocal zone, the
concentrations of estradiol, testosterone, progesterone, estrogen receptors, progesterone and androgens were determined by ELISA (Casabio, China).

**Results.** The rats in the main group had GC with a tumor volume 1.5 times (p<0.05) smaller than in the reference group and multiple metastatic lesions of the internal organs that led to a reduction in their survival by a factor of 1.7 (p<0.05). In contrast, in the mice of the main group, an increase in the LLC volume was recorded by up to 13.7 times (p <0.001), metastases were not detected, and the survival was 1.5 times shorter (p <0.05) than in the reference group. GC and LLC developed in presence of DM demonstrated higher concentrations of estradiol, testosterone and progesterone than the tumors in the reference groups: they were on average 2.4-2.6 times higher (p<0.02). At the same time, the receptor concentration in GC was on average 3.6 times higher (p <0.01), and in LLC it was on average 1.8 times lower (p <0.05) than in the tumors in the corresponding reference groups. The perifocal zone of GC demonstrated very high concentrations of estradiol and androgen receptors: they were recorded to be 9.5 and 3.9 times higher (p <0.01), respectively, than it was the case in the reference group. In the perifocal zone of LLC, on the contrary, there were lower levels of estradiol, testosterone and receptors, respectively, by 2.3, 5 and on average 1.8 times (p<0.05–p<0.01) than in the reference group. In different models of the tumor growth in presence of DM, the tendency of changes in the concentrations of sex hormones and their receptors in the perifocal zone of the tumors had its own features. In the peritumoral area of disseminated GC (main group) higher levels of testosterone and estradiol were revealed: they were 1.6 and 2.7 times higher, respectively (p <0.05) than in the tumor tissue, in contrast to the respective indicators in the reference group. In the main group of mice with LLC, in contrast to the indicators in the reference group, there were lower concentrations of estradiol and testosterone in the perifocal zone as compared to the levels in the tumor tissue: they were 1.5 and 3.5 times lower, respectively (p<0, 05). At the same time, in both models, higher concentrations of hormonal receptors in the perifocal zone of the tumors remained, similar to what we observed in the independent growth of Guerin's carcinoma and LLC (the reference group).

**Conclusion.** The obtained evidence data revealed an essential role of DM as comorbid pathology in the activation of the growth and intensification of tumor metastasizing, the formation of its aggressive phenotype, and a reduction in the survival of the animals. The study of tumors of similar histological structure, but of different origin (uterine epithelium, lung epithelium), developing in different physiological contexts, contributed to the proper assessment of the impact made by DM, accompanying the malignant growth, on changes in the hormonal status of the tumor tissue and the perifocal zone, which was involved in the molecular relationship between DM and progression of the tumor process.
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XENON-OXYGEN THERAPY AS A FACTOR OF NORMALIZATION OF ADAPTIVE AND PSYCHO-EMOTIONAL STATUS IN PATIENTS DIAGNOSED WITH CERVICAL CANCER AT THE SURGICAL STAGE OF ANTITUMOR TREATMENT


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Introduction. Radical surgical treatment of cervical cancer (CC) involves the removal of the uterus with suspected metastatic foci in the regional lymphatic collectors and the ovaries. In this case, total ovarian suppression in patients of reproductive age is accompanied by polysystemic functional disorders in the form of postovariectomy syndrome (POES), which is manifested by changes in homeostasis, neuroendocrine and cardiovascular insufficiency, pronounced disorders in the psycho-emotional sphere with the formation of a persistent stress situation and the development of psychological dysfunction [1]. Most of the methods of rehabilitation therapy are limited in their use and do not always lead to a satisfactory result that promotes searching for new technologies in the rehabilitation of cancer patients at all stages of antitumor treatment. In order to achieve a balanced response by the organism of a cancer patient to stressful events of the disease progression and the...
The impact of aggressive treatment methods, the basic principles of activation therapy are actively implemented by the National Medical Research Centre for Oncology. So, guided by the pattern of changes in the dose-time parameters of activation therapy [2], in order to provide regression of the functional disorders in patients with cervical cancer, an exponential algorithm of xenon-oxygen therapy (XOT) was developed. Having nootropic, anti-inflammatory, analgesic and immunostimulating properties, xenon is effectively used in the correction of acute and chronic stress [3].

The aim of our study was to assess the dynamics of the adaptive and psycho-emotional status in patients with cervical cancer of reproductive age with clinical manifestations of POES under the influence of exponential xenon-oxygen therapy at the surgical stage of antitumor treatment.

Patients and methods. 26 patients of reproductive age, diagnosed with cervical cancer, who underwent radical surgery (extirpation of the uterus with appendages) and had clinical signs of POES, received restorative therapy as follows: 5 inhalation procedures with a xenon-oxygen mixture in an exponential dose regime with an increase in xenon dosing from 15% to 25% in the mixture and with a reciprocal decrease in the exposure interval from 25 to 10 minutes. 24 patients with similar initial data received standard postoperative treatment without XOT. The psychoemotional status was assessed according to the results of the generally accepted standardized questionnaire for the quality of life for cancer patients, namely, The Edmonton Symptom Assessment System (ESAS). The type of their adaptive response to treatment (according to L.Kh. Garkavi) was assessed using the analysis of the Schilling's leukogram and the calculated coefficient of the ratio of the total cluster of stress and antistress reactions (K = AS/S) (before surgery, on the 1st and 10th days of the postoperative period).

Results. According to our study, on postsurgery day 1, 72.8% of the patients with cervical cancer had acute stress, which was 2.3 times higher than that recorded for the initial state; the antistress / stress ratio was 0.37, which was 5.4 times less than the level recorded before treatment. On postsurgery day 10, in the group without XOT, the acute stress reaction prevailed in 67.2% of the cases; no dynamics of the antistress/stress ratio was observed. The use of XOT in the patients with cervical cancer led to diametrically opposite results, the incidence rate of the responses of the antistress type was 78.6%, the ratio of antistress/stress exceeded the value recorded on postsurgery day 1 by 6.6 times and reached the initial values, whereas balanced training reactions dominated. The positive dynamics of the adaptive status in the CC patients with the use of XOT corresponded to the subjective data obtained with the ESAS questionnaire. According to the results obtained, in the group of patients receiving the restorative therapy with a xenon-oxygen mixture, a clear regression of their vegetative and psycho-emotional disorders was observed, where a statistically
significant decrease in the manifestations of nausea by 3.9 times, depression by 2.7 times and anxiety by 2.1 times was recorded (p<0.05).

Conclusion. The use of exponential XOT, in the shortest possible time, provided a productive reformatting of the adaptive status in the cervical cancer patients with a predominance of a cluster of anti-stress types of reactions and normalization of the psycho-emotional state in response to radical surgical treatment.

References

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ACCOMPANYING TRANSCRANIAL MAGNETOTHERAPY IN COMBINATION TREATMENT OF HIGH-GRADE GLIAL BRAIN TUMORS

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Introduction. Low survival and poor quality of life in patients with high-grade brain gliomas (HGG) dictate the need to develop and promote new approaches, including accompanying transcranial magnetic therapy (TMT) with pulse-type magnetic field (PT MF) and ultra-low-frequency magnetic fields (ULF MF), characterized by their non-invasiveness and safety in applied procedures. The key point of these original wave technologies is the construction of algorithms of the actions based on the principles of activation therapy, which was developed as a result of the discovery “Pattern of development of qualitatively differing general nonspecific adaptational reactions of the organism” (Scientific Discovery Registration Certificate No. 158 issued by the Committee on Inventions and Discoveries at the Council of Ministers of the USSR, 1975) [1]. It is important that the developed programmed modes of TMT, inducing the development of general nonspecific adaptational reactions (GNAR) of a physiological type, allow co-adjusting various body systems, including the cardiac performance, and indirectly exercise a regulatory effect on some cardiovascular disorders that occur in cancer patients [2]. As a rule, surgical treatment of HGG is associated with a significant functional load on the heart, causing persistent cardiotoxicity and myocardial metabolic disorders.
The aim of our research study was to form an integral adaptational reaction of the antistressor type and correct some metabolic myocardial disorders based on a comprehensive cardiometric analysis of the cardiac performance in patients with brain HGG by applying TMT at the stage of surgical treatment.

Patients and methods. The study included primary records of 50 patients with HGG covering the main group (n=25) and the reference group (n=25). The TMT technology included a dual exposure mode that was previously reported by us (DOI: 10.1200/JCO.2021.39.15_suppl.e14027), and the treatment was carried out in patients of the main group in their early postsurgery period. 1 day before surgery, as well as on day 7 and day 15 after the HGG surgical removal, all patients underwent an electrocardiogram (ECG) with the PC-assisted cardiac analyzer CARDIOCODE with application of its proprietary software (Taganrog, Russia) and a complete blood count in order to study the integral mechanisms of the TMT effect. The Cardiocode software allowed automatically noninvasively calculating metabolism data by the levels of oxygen, lactate, and phosphocreatine in the myocardium. For the aerobic process, the value of the parameter varying in the ranges of 0.7…0.85, 0.6…0.65, 0.5…0.55 standard units (s.u.) was taken as the norm; for the anaerobic-glycolytic process the normal value was taken to be 3.0…7.0 s.u., and for the anaerobic-alactate process the normal value was taken to be at the level of 2.0…4.0 s.u. Identification of general nonspecific adaptational reactions was carried out according to the signal lymphocytic criteria of the Schilling's leukogram individually for each patient, and then a general group-related pattern of GNAR was estimated, where the percentage of similar reactions was determined: the reactions of training, calm and elevated activation, acute or chronic stress. After that, the coefficient of anti-stress and stress reactions (C=AS/S) was calculated, which served as an integral synthetic indicator of the effectiveness of the antistress effect produced by TMT. Statistical data processing was performed using the Mann-Whitney U-test.

Results. On postsurgery day 7 in the patients of the main group, the average oxygen index in the myocardium was 0.7 s.u. against the reference value of 0.5 s.u. (p=0.04); on postsurgery day 15 it was recorded to be 0.6 s.u. against 0.4 s.u. (p=0.04), respectively. The level of lactate on postsurgery day 7 in the main group was 4.3 s.u. against 17.1 s.u. (p=0.03) in the reference group; on postsurgery day 15 the lactate level was recorded to be 4.4 s.u. against the reference group value of 15.1 s.u., respectively (p=0.03). The level of phosphocreatine in the patients of the main group on postsurgery day 15 was 4.2 s.u., while in the reference group it was recorded to be 4.8 s.u. (p=0.05). In both groups, the existing wide range of the initial normotypes of reactions (training, calm and elevated activation) in the period before surgery and immediately after the surgical treatment narrowed sharply to the identical type of pathological response, i.e. the development of acute stress in 70–80% of the cases. During the period of TMT exposure, starting from the second
day after the operation and before the start of radiation therapy, there was a decrease in the rate of stress reactions by 3.4 times and the dominance of stable normotypes of anti-stress reactions, beginning with the reaction of training to elevated activation that was confirmed by an increase in C=AS/S by 3.0 times against the respective reference values. Thus, in the reference group without the TMT application, in the development of a stress state, inhibition of the aerobic energy processes in the myocardium was observed with activation of the anaerobic-glycolytic and anaerobic-alactate processes, and oxygen deficiency was compensated by an increase in the lactate and phosphocreatine levels. In the patients of the main group, after TMT, the indices of oxygen, lactate and phosphocreatine remained within the physiological norm, and the stable normotypes of the anti-stress reactions developed.

**Conclusion.** The use of accompanying TMT at the stage of surgical treatment of HGG determines the dominance of the anti-stress adaptational reactions that contributes to the prevention of the development of stress and myocardial dysfunction and to an earlier restoration of the quality of life in such patients.

**References**


**GENOME-WIDE ANALYSIS OF TRIPLE-NEGATIVE PHENOTYPE BREAST TUMORS**

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*Introduction.** Identification of the molecular genetic phenotype of breast carcinomas is an important prognostic factor of the disease, and helps to personalize the treatment of such patients. According to the evidence data from the reference literature, breast cancer (BC) with a triple negative phenotype among all molecular subtypes accounts for about 10-24%, showing the most aggressive progression and, as a result, has the worst prognosis [1, 2, 4].
The aim of the presented study was to conduct a genome-wide analysis of breast cancer with a triple negative molecular subtype (TN BC).

Material and methods. Our study included 25 patients with a morphologically verified diagnosis of TN BC, mean age 47.6±0.4 years. Prior to treatment, DNA from tumor biopsy material was isolated using the QIAamp DNA mini Kit (Qiagen, Germany). In order to evaluate the CNA landscape, we utilized the CytoScan HD Array (Affymetrix, USA) microarray. For the purpose of the bioinformatics analysis, the Chromosome Analysis Suite 4.0 software was applied.

Results. The largest number of amplifications (more than 64.0%) (in the absence of deletions) was found in the 1q21.3 locus. The highest rate of deletions (more than 56.0%) was found in loci 3p21.31, 3p21.2, 3p21.1 (in the absence of amplifications) and in locus 17q11.2. As a result from comparing the rates of the CNA incidence in groups of patients, depending on the presence/absence of an objective response to neoadjuvant chemotherapy (NCT) in them, it was shown that the presence of an objective response to preoperative chemotherapy was observed in case with a greater number of amplifications in the 3q23 region (p = 0.03). Potentially, this locus can be considered as a predictive marker of a good response to NCT in the TN BC patients. As a result from the comparison of the CNA incidence rates in groups of patients with the presence / absence of hematogenous metastasis, it was shown that the presence of hematogenous metastasis was observed in case with a greater number of the amplifications in the 5p14.2 region (p = 0.018) and with a greater number of the deleted regions in the 4q26 region (p =0.04).

Discussion. Already more than 10 years ago, Mersin et al detected that patients with TN BC had a higher risk of recurrence, with its average time slightly exceeding 1 year [3]. To date, searching for markers to predict the course of the disease as well as identification of an approach to prevent the recurrence and metastasis is crucial for improving the survival of patients with TN BC [5].

Conclusions. We assessed the genetic response of TN BC to NCT and the association with distant metastases. In the future, such data can form the basis for the development of new markers of the treatment effectiveness for patients with triple-negative phenotype breast cancer.

References
PERSONALIZED APPROACH TO ADJUVANT CHEMOTHERAPY IN PATIENTS WITH NON-SMALL CELL LUNG CANCER

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Introduction. Numerous studies have shown that differential expression and/or co-expression of several chemosensitivity genes, such as ERCC1, RRM1, TUBB3, TOP2A, and some others, in the tumor tissue are closely associated with chemoresistance and prognosis in cancer patients. Despite the fact that more and more data comes to support the idea on the important role of the presented genes in the assessment of chemosensitivity (CS), at present time there is ample room remaining for further research of the combined estimation of the CS gene expression for a personalized approach to chemotherapy in patients with lung cancer.

Thus, the aim of this research work was to conduct a prospective study of the administration of personalized chemotherapy in patients with non-small cell lung cancer.

Material and methods. The study included 85 patients with stage IIB IIIB central or peripheral NSCLC. All patients received 2 cycles of neoadjuvant chemotherapy (NCT) according to the vinorelbine/carboplatin regimen. After NCT, the above patients were subjected to surgery consisting in either pneumonectomy or lobectomy. The hospital record reference group (n=37) included patients, who received 3 cycles of ACT according to the standard scheme: vinorelbine/carboplatin. The test group in our study consisted of 48 patients, the ACT regimen for which was personalized depending on the expression of their chemosensitivity marker gene profiles. After surgery, these patients received platinum-based doublet chemotherapy according to the schemes as follows: vinorelbine/carboplatin; doxorubicin/carboplatin; gemcitabine/carboplatin; paclitaxel/carboplatin; irinotecan/carboplatin; vinorelbine/cisplatin; gemcitabine/cisplatin; paclitaxel/cisplatin. The test material was surgical material after chemotherapy (tumor tissue), RNA
from which was isolated using the RNeasy Plus mini Kit (Qiagen, Germany). The level of gene expression: BRCA1, RRM1, ERCC1, TOP1, TOP2a, TUBB3, TYMS, GSTP1 was assessed using quantitative RT-PCR. The selection of chemotherapy regimen for each patient in the personalized group was based on an assessment of the actual expression profiles of the chemosensitivity genes.

**Results.** The data in a total of 85 patients with lung cancer was analyzed. There were no significant differences in the initial characteristics between the studied groups of the patients with personalized and classical chemotherapy. The follow-up median among patients included in the study was 32.0 months (the range 2–88 months). In the reference and the main test groups, this indicator was recorded to be 27 months (2-55 months) and 48.0 months (2-88 months), respectively. Further, the indicators of metastasis-free survival (MFS) and overall survival (OS) in patients of the studied groups were assessed. The mean value of the MFS scores was 46.22±3.98 months in the personalized chemotherapy group compared to 22.9±2.65 months in the reference group. The differences were statistically significant with log-rank test p=0.05. Very good results were shown for OS. Thus, the patients with the individual selection of the chemotherapy regimens had a 96% survival rate, compared to the reference group, where the lower limit was recorded to be 48% (log-rank test p<0.0001). At the same time, the mean OS for the reference group was 26.9±2.39 months versus 58.6±2.9 months in the second group of the patients under study. It was important to note that the best MFS values in the personalized ACT group were achieved in the patients treated with paclitaxel/carboplatin. Those patients (8.3%, 4/48 cases) had a 100% survival rate. Slightly lower rates of the 5-year MFS values (83%) were observed in the group of the patients treated according to the vinorelbine/carboplatin regimen (35.4%, 17/48 cases). The scores in the reference group (50%) and the personalized gemcitabine/cisplatin group (33.3%, 16/48 cases; MFS score: 57%) were similar. The lowest MFS was observed in the gemcitabine/carboplatin ACT group: 25% only. Three of the four patients there developed metastatic disease between 2 and 16 months.

**Conclusions.** Thus, the personalized approach to administering anticancer drugs has essentially expanded and improved the conventional methods of treating non-small cell lung cancer. However, further studies of associations between the chemosensitivity gene expression and other chemotherapeutic agents are needed. However at present the use of technology for assessing the expression of the studied genes can increase the quality of life of cancer patients.

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FEATURES OF EXPRESSION OF TRANSFORMING GROWTH FACTOR BETA IN ESTROGEN-POSITIVE BREAST CANCER

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**Topicality.** Changes in the activity of growth factors and their receptors play a key role in shaping the response to treatment with anticancer hormonal drugs.

**The aim** of this work was to study the relationship between the TGFβ1 and TGFβR1 expression and the progression of breast cancer (BC) during tamoxifen therapy.

**Material and methods.** Our study included 65 patients with primary estrogen-positive breast cancer who received tamoxifen at a standard dose of 20 mg/day as an adjuvant treatment. The age of the patients was 40–79 years. Depending on the effectiveness of the treatment, the patients were divided into two groups: a tamoxifen-sensitive group (no signs of disease progression, N = 55) and a tamoxifen-resistant group (with disease recurrence, N = 10). Genotyping of polymorphic loci (SNPs) TGFβ1 (rs1800470) and TGFβR1 (rs334354) was carried out by real-time PCR. The expression profile of the TGFβ1 and TGFβR1 genes was studied using reverse transcription PCR. The expression levels of TGFβ1 and TGFβR1 were assessed by flow cytometry.

**Results.** Our genotyping analysis showed that the carriage of mutant variants of the TGFβ1 gene (rs1800470) was associated with sensitivity to hormone therapy (p = 0.019 and p = 0.039; for the genotype and allele, respectively). Disease progression while taking tamoxifen was more often observed in patients with a high level of the TGFβ1 expression in the tumor tissue (p = 0.025). The percentage of cells expressing TGFβR1 was more often observed in tumors responding to the treatment (p = 0.036). A positive correlation between the transcriptional activity of the TGFβ1 and TGFβR1 genes (r = 0.295; p = 0.039) was revealed.

**Conclusion.** Determining the genetic features and the expression profile of TGFβI and TGFβRI can be essential in evaluating the effectiveness of antitumor treatment with tamoxifen.

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CIRCULATING DISIALOGLANGLIOSIDE GD2 IN NEUROBLASTOMA DIAGNOSIS

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Neuroblastoma is one of the most common solid tumors in children, characterized by high clinical heterogeneity and low predictability of the progression course. To assess the prognosis and response to therapy, protein tumor markers are often used, such as neuron-specific enolase, lactate dehydrogenase, ferritin as well as catecholamine metabolites: homovanillic acid, vanillylmandelic acid, 3-methoxytyramine, meta- and normetanephrine. Immunocytochemical studies have shown that many neuroblastomas are characterized by high surface expression of disialoganglioside GD2. In this case, well-differentiated tumors are often characterized by a lower level of GD2. It is known that GD2 can be detached from the cell surface and transported in the bloodstream as a part of the low density lipoprotein composition. In this connection, it is assumed that the concentration of GD2 in blood may depend on the presence of the tumor, its grade and mass.

**Aims and methods.** In our study, we analyzed the level of GD2 circulating in blood with the use of high-performance liquid chromatography coupled to tandem mass spectrometry in patients with histologically confirmed neuroblastoma and ganglioneuroblastoma (n=26), ganglioneuroma (n=1) as well as in the reference group, which covered patients ( n=25) with a suspected neurogenic tumor, which, after confirmation of the histological diagnosis, were characterized both by non-tumor lesions (n=11) and various tumor diseases (n=14).

**Preliminary results** obtained with the above sampling showed a high specificity of the method in the detection of primary neuroblastoma with a plasma-related C18 GD2 lipoform cut-off threshold approximately of 35 nM. At the same time, a positive correlation was found between the concentration of the circulating GD2 and the tumor mass. As distinct from glycoproteins, gangliasides were characterized by high clearance, which makes GD2 a potentially useful marker of the response to therapy. We observed a rapid pronounced decrease in the level of GD2 during resection of a large tumor formation. In some individual cases, an increase in the level of the circulating GD2 was also observed in the disease recurrences. At present, we have been collecting the relevant data to increase the sampling sizes in order to improve the statistical significance of this study.

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**ANTIBODY-FREE CHIMERIC FLT3-CAR RECEPTOR FOR TARGETING THE FLT3 RECEPTOR, A MARKER OF POOR PROGNOSIS IN ACUTE MYELOID LEUKEMIA**

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Therapy in acute myeloid leukemia (AML) usually begins with a course of induction chemotherapy using anthracyclines and cytarabine to eliminate blast cells. Chemotherapy is a necessary step in preparing a patient for transplantation of donor hematopoietic stem cells (HSCs). However, leukemia stem cells often show their chemoresistance and initiate the AML recurrence. Specific elimination of the leukemic stem cells during the preparation of a patient for HSC transplantation may reduce the probability of recurrence. For this purpose, an active development of chimeric antigen receptors (CAR) to address various markers of AML is being in progress.

The concept of CAR T-cell therapy is based on an introduction of an artificially engineered genetic construct of a chimeric CAR receptor into a patient's T-cells ex vivo. The chimeric CAR receptor is a transmembrane protein, the extracellular part of which recognizes the molecular target: the tumor antigen. As a result of recognition of the target molecule on the cell surface, the chimeric CAR receptor is activated, triggering the T-cell killing mechanisms. A single-chain fragment of the variable part of an antibody (scFv) as an extracellular part of the chimeric CAR receptor is conventionally used, which specifically recognizes the target molecule. The scFv sequence is based on the sequence of the corresponding known monoclonal antibody. The design of the scFv sequence for the chimeric receptor includes the selection of an eligible linker to bind the variable fragments of a monoclonal antibody into one chain as well as the study of the stability of the obtained scFv protein at various pH values and temperatures, testing the affinity to the target molecule and specificity of its interaction with the latter. An original approach is to use as an extracellular part of a chimeric receptor a part or a full-length natural ligand of a tumor antigen in the case when the latter is a receptor. Being the original ligand, the extracellular part of the chimeric receptor shall be capable of specifically, with known affinity, recognizing the target molecule.

The advantage of the development of this sort of antibody-free chimeric receptors is the availability of a large body of research evidence data on the structures and functional domains of proteins that may considerably accelerate design and development of the CAR T-therapy.

The most common are at least eight types of AML. There is a variety in the immunophenotypes of blast cells in different types of AML. The blast cells in AML develop clonally, each clone is characterized by its own independent set of surface markers. In addition, the immunophenotype of the blast cells of the same type of AML in different patients may vary significantly, and it may also alter with time. In case of AML, a promising way may likely to be the development of a number of CAR T-cell approaches specific with respect to various tumor antigens. Thus, based on the results of the diagnostic immunophenotyping of the blast cells in a particular patient, it would be possible to select the appropriate variant of the CAR T cells.

**Aims and methods.** For this study, the Flt3 tyrosine kinase receptor was chosen as a molecular target. Being excessively available on the surface of blast cells in AML, Flt3 often persists in
recurrences. In ~30% of the AML cases, the Flt3 sequence carries an activating mutation (ITD), making it an attractive target molecule. To target the Flt3 receptor, the full-length sequence of the natural Flt3 receptor agonist – the Flt3 ligand was taken as the recognition extracellular part of the antibody-free chimeric Flt3-CAR receptor.

Results. In the course of our research work, the antibody-free Flt3-CAR T cells were engineered, where the Flt3 tumor antigen-recognizing function was undertaken by the full-length natural Flt3 ligand instead of scFv. The antibody-free Flt3-CAR T cells were shown to specifically eliminate the Flt3-positive THP-1 cells and did not affect the proliferation of the Flt3-negative U937 cells. The antibody-free chimeric Flt3-CAR receptor was not cytotoxic against the Flt3-positive THP-1 cells; their elimination required activation of the T-cell killing mechanisms. The recognition by the chimeric Flt3-CAR T cell receptor of the Flt3 receptor on the surface of the target cells occurred via the Flt3:Flt3 ligand interaction interface.

Conclusion. Thus, we have succeeded in designing and engineering of highly specific antibody-free Flt3-CAR T cells with clear mechanisms of activation and recognition of the target molecule.

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PRODUCTION AND LARGE-SCALE EXPANSION OF FUNCTIONALLY ACTIVE NK CELLS SUITABLE FOR USE IN IMMUNOTHERAPY OF HEMATOLOGICAL AND SOLID TUMORS

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Natural killer cells (NK) are part of the innate immune system of the body and play an important role in the immune surveillance of foreign cells and the body’s own altered cells, including the transformed ones. The NK cells, without prior stimulation, can eliminate target cells which do not express their own MHC class I molecules or express stress-induced molecules. These basic, natural functions of the NK cells as a tool in the fight against malignant tumors have attracted particular attention. For this purpose, various approaches are used to increase the number and/or enhance the functional activity of the NK cells. One approach uses cytokines to selectively increase both the number and effectiveness of the NK cell antitumor function. Another approach focuses on checkpoint inhibitors targeting the NK cell receptor. Bispecific and trispecific activators have been developed to enhance the specific immune response by cross-linking specific tumor antigens with effector cells. In addition, methods of adoptive transfer of the NK cells have shown promising results, however, a fairly large volume of cell mass is required there.
To solve this problem, it is necessary at the beginning to determine the source of biological material. We had peripheral blood at our disposal, and further work was carried out using various methods for isolating and expanding the NK cells: conventionally, used are isolation of the mononuclear cell fraction in the Ficoll density gradient and its variant RosetteSep and magnetic separation. The obtained cell fraction was cultured in the presence of the K562-mIL15-mIL21-41BBB feeder line for 3 weeks. As a result of the cultivation, significant amounts of the NK cells were obtained: an increase by 100,000 times from the initial number. The functional activity of the obtained cells was determined using flow cytometric analysis by changing the degranulation marker (CD107a) against the K562 cell line and fluorescent imaging in a cytotoxic test against tumor cell lines. The degranulation marker increased approximately by 1.5 times.

The cytotoxic test showed that after 2-3 days the number of tumor cells was reduced to almost zero. To assess the possibility of storing the obtained NK cells, they were subjected to cryopreservation. Their functional activity, after thawing, was tested in tests similar to testing of the freshly prepared cells. All functional characteristics of the post-thaw cells preserved.

Thus, the large-scale production technology of the NK cells makes it possible to obtain a highly purified population of the cytotoxic NK cells suitable for clinical use.

MECHANISMS OF RESISTANCE IN THERAPY WITH ANGIOGENESIS BLOCKERS IN COLORECTAL CANCER

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One of the fundamental events that determine carcinogenesis and its progression is angiogenesis mediated by the activation of the vascular endothelial growth factor (VEGF). The angiogenic cascade provides a comfortable micro-environment for the tumor, the creation of a metastatic niche and subsequent metastatic dissemination of neoplastic cells. The development of a recombinant hyperchimeric monoclonal antibody that selectively binds and inhibits the biological activity of VEGF - Bevacizumab, has led to a change in the therapeutic strategy for patients with cancer and, in particular, for patients with metastatic colorectal cancer (CRC), because it demonstrated an increase in their survival rate. However, after several years of the anti-VEGF therapy in patients with solid tumors, the benefits are lost due to formation of the resistance to targeted therapy with the VEGF blockers, and in some cases, due to use of the drugs in an intermittent regimen or in
periods of medication interruptions (because of secondary transient effects or choice of strategy), as well as due to the recurrent tumor growth with its rapid vascularization.

The aim of our study was to study the pathogenetic mechanisms of the resistance to therapy with angiogenesis blockers in colorectal cancer.

Patients and methods. Our sampling covered 263 patients with colorectal carcinoma divided into 2 groups: group 1- mCRC (metastatic) n=132; group 2 - nmCRC (non-metastatic) n=131. Material for the study was represented by tumor tissue fragments obtained in surgery for their subsequent histological and immunohistochemical studies (IHC) with markers CD4, CD8, CD163, iNOS, TNFa, HIFa, VEGF-A, ANG1, ANG2, TIE2; peripheral blood samples for enzyme immunoassay (ELISA) with markers VEGF-A, ANG1, ANG2, TIE2.

Results and discussion. In the studied tumor tissue specimens, a redistribution of the immune response along the Th2 pathway was established, aimed at remodeling the tumor microenvironment and the escape of tumor cells from immune surveillance mechanisms. The heterogeneity of the IHC reactions was manifested in the presence of "cold" and "hot" tumors according to the studied parameters. The cold tumors were characterized by a minimum number of cell populations of tumor-associated infiltration with positive expression, including HIFa (tissue hypoxia factor) and VEGF-A that could explain the minimal effectiveness of Bevacizumab and Aflibercept due to the absence of a potential target in the focus of the tumor growth. At the same time, high levels of the ANG1 and ANG2 expression were noted in all tissue samples, and low levels of the TIE2 receptor expression that indicated the presence of a parallel mechanism of activation of the VEGF-A independent angiogenesis. In our opinion, it is precisely the activation of the alternative neoangiogenesis that may be activated when VEGF-A is blocked in patients with hot tumors, leading to tumor progression in case of medical drug withdrawal. Low levels of TIE2 receptors in all studied samples explain the ineffectiveness of Regorafenib, the action of which is aimed at blocking tumor growth factors, including VEGFR and TIE2.

Our correlation analysis of the studied parameters in the tumor and peripheral blood of patients with CRC demonstrated the presence of a strong direct relationship between the VEGF-A parameters in the tumor and blood, while ANG2 in blood and the issue was inversely correlated with VEGF-A and TIE2 that can be applied as screening diagnostics in patients with CRC before starting therapy with angiogenesis blockers.

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ANALYSIS OF IMMUNOHISTOCHEMICAL REACTIONS OF VARIOUS PD-L1 CLONES IN PROSTATE ADENOCARCINOMA

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Prostate cancer (PC) is the most commonly diagnosed malignancy in men. The standard treatment for localized PC is radical prostatectomy, but recurrence is a significant problem in this case. The main prognostic markers of the disease recurrence are the Gleason score and the preoperative level of the prostate-specific antigen (PSA), which determines the need to identify some key molecules of the PC progression. This can be useful in identifying new prognostic markers or targeted treatments for this pathology.

The PD-1 (programmed cell death) protein and its ligand PDL-1 play a significant role in the suppression of the adaptive immune system. The anti-PDL1 antibodies are used to treat many malignancies such as melanoma, urothelial carcinoma, head and neck squamous cell carcinoma, etc. The availability of immune checkpoint inhibitors allows studying PDL-1 expressed by the tumor and immunocompetent cells. However, the significance of the PDL-1 expression in prostate cancer is still poorly understood. To date, to determine the PD-L1 status, an immunohistochemical (IHC) study is used.

Aims. Our study was aimed at comparing the results of the IHC assessment of the PD-L1 – the PC status using three diagnostic systems SP142, SP263 (manufactured by Ventana) and 73-10 (manufactured by Leica).

Material and methods. We studied 30 tissue samples obtained in radical prostatectomy in prostate cancer, performed at the Clinical Medical Multidisciplinary Center named after V.I. Vernadsky. When assessing the expression of antibodies SP263 and 73-10, the ratio of the number of positively stained tumor and immune cells relative to all viable tumor cells in the sample, multiplied by 100 (CPS), was determined. The cut-off threshold value was taken to be ≥ 1. For the SP142 antibody, the IC scoring was applied that implies the ratio of the area occupied by the positively stained immune cells to the area of the tumor, which includes all viable tumor cells, immune microenvironment cells and granulomas. The resulting number shall be multiplied by 100. The threshold value (cut-off) was taken to be ≥ 1%.

Results. When evaluating the IHC reactions performed using clones SP263, SP142 and 73-10, no significant differences were found between the above antibodies. The differences referred only to those cases, when the values of IC and CPS were close to the threshold. However, given that there
are differences and poor knowledge on this issue, we believe that further validation studies with larger cohorts are needed.

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A NEW MUTATION IN THE PALB2 GENE ASSOCIATED WITH HEREDITARY BREAST CANCER IN A YOUNG PATIENT BELONGING TO THE YAKUT ETHNIC GROUP


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Abstract. Breast cancer is the most common cancer worldwide. The oncosuppressor gene PALB2, along with such genes as BRCA1 and BRCA2, is involved in the repair of damaged DNA. The incidence of germline mutations in the PALB2 gene in breast cancer and in familial cases of pancreatic cancer is approximately 1–2% and 3–4%, respectively. A clinical case of a 39-year-old woman belonging to the Yakut ethnic group, diagnosed with breast cancer and with a family history of pancreatic cancer, is presented hereby.

Materials and methods. Genomic DNA was isolated from peripheral blood, DNA libraries were prepared using the Hereditary Cancer Solution™ kit (Sophia Genetics, Switzerland) to study the status of 27 genes (ATM, APC, BARD1, BRCA1, BRCA2, BRIP1, CDH1, CHEK2, EPCAM, FAM175A, MLH1, MRE11A, MSH2, MSH6, MUTYH, NBN, PALB2, PIK3CA, PMS2, PMS2CL, PTEN, RAD50, RAD51C, RAD51D, STK11, TP53 and XRCC2). Sequencing (2 x 150 bp) was performed using the NextSeq 500 system (Illumina, USA).

Results. According to the results of bioinformatics analysis of the NGS data, a 39-year-old woman, belonging to the Yakut ethnic group, diagnosed with breast cancer and with a family history of pancreatic cancer, was found to have a new germline mutation of the PALB2 gene with a frameshift (NM_024675: Exon1: c.47delA: p.K16FS). According to the PubMed ClinVar db, the new mutation in the PALB2 gene is located in the same codon, where probably the pathogenic splicing mutation site (NM_024675: Exon1: c.48+1delG) was previously described, which is involved in the pathogenesis of hereditary forms of breast and ovarian cancer.
Conclusion. For the first time in a 39-year-old woman, belonging to the Yakut ethnic group, diagnosed with breast cancer and with a family history of pancreatic cancer, a new, probably pathogenic, frameshifted PALB2 gene mutation (NM_024675: Exon1: c.47delA: p. K16FS) has been found.

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PROSPECTS FOR THE USE OF MOLECULAR GENETIC MARKERS TO IMPROVE DIAGNOSTICS AND TARGETED THERAPY OF MELANOMA
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Skin melanoma is a highly aggressive malignant neoplasm, the treatment of disseminated forms of which remains a serious problem, despite recent advances in the development of medical drugs based on the inhibition of the BRAF oncogene as well as on the basis of immune response regulators [1]. In addition, the question of identifying prognostic markers of metastasis and diagnostic markers of skin melanoma remains relevant. Despite advances in understanding the pathogenesis of melanoma and the development of various therapies aimed at immune checkpoints, the patient survival remains low in the progressing metastatic disease [2]. Targeted therapy and immune checkpoint inhibitors are used in the treatment of metastatic melanoma. Melanoma has intratumoral and intertumoral heterogeneity that further complicates its treatment. The most
common melanoma-associated mutation (about 50% of the tumors) is the BRAF gene mutation, which is a replacement for BRAF V600E. This mutation activates MEK/ERK kinase, enhancing melanogenesis [5]. The effect made by the inhibitors on the BRAF mutant serine-threonine kinase demonstrates a pronounced response in melanomas carrying BRAF V600E mutations, contributing both to the regression of tumor foci and an improvement in patient survival [4, 9]. However, resistance to this drug is rapidly developing, preventing its long-term effective use. The mechanisms of the acquired resistance to the BRAF V600E inhibitors include mutations in the NRAS/MEK-1 genes and overexpression of COT, EGFR, PDGFRβ, IGF1R, and MET [7, 10]. The development of the drug resistance necessitates the use of a combination of some chemotherapeutic agents blocking the MAPK signaling pathway [4, 6]. However, due to multiple genetic and epigenetic modifications as well as due to the tendency of melanomas to a high degree of clone heterogeneity, it is not always possible to achieve the desired therapeutic effect [8]. This necessitates additional molecular genetic examinations of the patient.

The aim of this study was to identify the possible dependence of the stage of the disease on the presence of mutations in the tumor tissue.

Methodology. The examined group included both female (n=13) and male (n=14) patients, the average age of which was 63.5±3.5 and 62.7±3.9 years, respectively. All patients were divided into two groups depending on the disease stage: locally advanced disease (stage I-II) in 14 patients and metastases (stage III-IV) in 13 patients. A molecular genetic study of melanoma samples to identify the presence of BRAF mutations in 17 patients with a histologically confirmed diagnosis was carried out: there were 7 females and 10 males; whose mean age was 65.5±5.1 and 62.3±3.7 years, respectively. All the patients were divided into two groups depending on the stage of the disease, but without regard to their gender: the number of patients with locally advanced disease among them was 9 individuals, and the patients with the presence of metastases were 8 individuals. To confirm the presence of the BRAF V600E mutation, the Real-time-PCR-BRAF-V600E test system was used; the analysis was carried out according to the manufacturer's instructions (Biolink, Russia). In addition to this mutation in 6 tumors (3 men and 3 women), a study was carried out for the presence of a mutation in the c-kit gene; for this purpose the c-kit D816V test system was utilized, according to the manufacturer's instructions (Gene Formula, Russia). Statistics data processing of the correlation of the occurrence with the prevalence of the process was carried out using Pearson's χ2 method.

Results. A positive mutation was detected in 53% of the cases that was consistent with the reference literature data [5]. Moreover, in the presence of metastases (stage III-IV), the mutation rate increased to 75% compared to 33% in the presence of a local process (stage I-II) (χ2=2.951; p=0.086). Mutations in the c-kit gene were not found.
Conclusion. Identification of the mutation in the BRAF gene is important for the purpose of the selection of treatment of melanoma, due to its greater rate in patients with a metastatic process. Moreover, the presence of mutant BRAF alleles may be a negative prognostic sign.

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