DOI: 10.17816/PED9349-56

VEGF DYNAMICAL CHANGES IN LABORATORY RODENTS WITH TRANSPLANTED EXPERIMENTAL TUMORS OF VARIOUS HISTOLOGICAL TYPES

© A.P. Trashkov [1], N.A. Verlov [1], V.R. Artemenko [1], V.A. Pechatnikova [1], M.A. Zelenenko [2], M.A. Pahomova [3], A.G. Vasiliev [3]

[1] St. Petersburg Institute of Nuclear Physics named after B.P. Konstantinov, Saint Petersburg, Russia;
[2] Sechenov Institute of Ephysiology and Biochemistry, Russian Academy of Sciences, Saint Petersburg, Russia;
[3] St. Petersburg State Pediatric Medical University, Ministry of Healthcare of the Russian Federation, Russia

For citation: Trashkov AP, Verlov NA, Artemenko VR, et al. VEGF dynamical changes in laboratory rodents with transplanted experimental tumors of various histological types. *Pediatrician (St. Petersburg)*. 2018;9(3):49-56. doi: 10.17816/PED9349-56

Received: 14.05.2018 Accepted: 21.06.2018

Vascular endothelial growth factor (VEGF) is one of the most important cytokines in charge of proliferation, migration and differentiation of endothelial cells in physiological and pathological processes. Because of this they are involved in pathogenesis of neoplasmatic process namely mechanisms of neoangiogenesis – development of blood-vessels' network in the tumor as well as adjoining intact tissues. Almost all contemporary antiangiogenic medicines are targeted at either VEGF or its receptors. However, there are practically no conventional models of oncologic pathology nowadays described in detail and recommended for preclinical studies. The present study focuses at changes of VEGF concentrations at various stages of disease using experimental tumors of different histological types, intensity of neoplasmatic growth and localization. Development of experimental transplantable tumors of various histological types and locations has been demonstrated to be usually accompanied by increased VEGF blood serum concentration in experimental animals; the dynamic of this increase depending upon the intensity of the tumor growth. A statistically valid decrease of VEGF level in comparison with the previous control point of the study has been demonstrated in BALB/c male mice with subcutaneously transplanted colonic adenocarcinoma on the background of active development of the tumor at the 45th day of the study. Pliss' Lymphoma, and Lymphocytal Leukemia P-388 models have been demonstrated to be optimal for the assessment of medicines' aimed at VEGF elimination pharmacological activity.

Keywords: neoangiogenesis; growth factors; VEGF; tumor; Pliss Lymphosarcoma; lymphoma; Acatol; Lymphocytal Leukemia P-388; rat ovarial tumor; preclinical studies.

ДИНАМИКА VEGF У ЛАБОРАТОРНЫХ ГРЫЗУНОВ С ТРАНСПЛАНТИРОВАННЫМИ ЭКСПЕРИМЕНТАЛЬНЫМИ НОВООБРАЗОВАНИЯМИ РАЗЛИЧНОГО ГИСТОЛОГИЧЕСКОГО ТИПА

© А.П. Трашков [1], Н.А. Верлов [1], М.Р. Артеменко [1], В.А. Печатникова [1], М.А. Зелененко [2], М.А. Пахомова [3], А.Г. Васильев [3]

[1] ФГБУ «Петербургский институт ядерной физики им. Б.П. Константинова Национального исследовательского центра „Курчатовский институт“», Санкт-Петербург;
[2] ФГБУН «Институт эволюционной физиологии и биохимии им. И.М. Сеченова» РАН, Санкт-Петербург;
[3] ФГБОУ ВО «Санкт-Петербургский государственный педиатрический медицинский университет» Минздрава России

Для цитирования: Трашков А.П., Верлов Н.А., Артеменко М.Р., и др. Динамика VEGF у лабораторных грызунов с трансплантированными экспериментальными новообразованиями различного гистологического типа // Педиатр. – 2018. – Т. 9. – № 3. – С. 49–56. doi: 10.17816/PED9349-56

Поступила в редакцию: 14.05.2018 Принята к печати: 21.06.2018

Сосудистый эндотелиальный фактор роста (VEGF) является одним из наиболее важных цитокинов, обеспечивающих пролиферацию, миграцию и дифференцировку эндотелиоцитов в норме и при патологии. Это обусловливает его вовлеченность в патогенез опухолевого процесса, в частности в механизмы неоангиогенеза — развитие сети кровеносных сосудов в опухоли и прилегающих к ней участках неизмененных тканей. Подавляющее большинство современных антиангиогенных препаратов имеют в качестве мишени VEGF и/или рецепторы к нему. Вместе с тем общепринятые, детально описанные и рекомендованные к внедрению в практику доклинических исследований модели онкологических заболеваний *in vivo* в настоящее время отсутствуют. В исследовании на экспериментальных опухо-

лях различного гистологического типа, интенсивности опухолевого процесса и локализации была изучена динамика VEGF на различных стадиях заболевания. Продемонстрировано, что развитие экспериментальных трансплантируемых опухолей различных гистологического типа и локализации, как правило, сопровождается увеличением содержания VEGF в крови подопытных животных, динамика которого зависит от интенсивности опухолевого процесса. У мышей-самцов линии BALB/c с трансплантированной подкожно экспериментальной аденокарциномой толстой кишки на фоне активного развития опухоли на 45-е сутки эксперимента наблюдалось статистически значимое снижение уровня VEGF по сравнению со значением в предыдущей контрольной точке исследования (30-е сутки). Полученные данные указывают на необходимость проведения специального исследования для уточнения механизмов такой динамики VEGF. Показано, что наиболее оптимальными для исследования фармакологической активности препаратов, нацеленных на элиминацию VEGF, являются лимфосаркома Плисса и лимфоцитарная лейкемия Р-388.

Ключевые слова: неоангиогенез; факторы роста; VEGF; опухоль; лимфосаркома Плисса; АКАТОЛ; лимфоцитарная лейкемия Р-388; опухоль яичника крыс; доклинические испытания.

The development of a wide network of blood vessels in tumorous tissues and the peritumoral region, and the high-rate maintenance of this process, which is equivalent to the growth rate of the mass of the tumor node, are important components of the pathogenesis of malignant neoplasms of any localization and initial histological type. Accumulated results of experimental and clinical studies indicate the importance of neoangiogenesis in the growth and metastatic spread of tumors, as well as the development of complications of oncological diseases (hemorrhagic and hypercoagulation syndromes, anemia) [2, 3, 5, 15, 22].

The important role of blood vessels in neoplasm development is primarily due to the impressive metabolic, proliferative, and migration needs of tumors. Despite the ability of tumor cells to exist for a long time under conditions of limited inflow of oxygen and nutrient compounds, which ensures the synthesis of high-energy molecules due to the activation of anaerobic mechanisms of energy production, any neoplastic cell still needs adequate oxygen delivery for rapid and complete implementation of the cell cycle program [4, 7, 8]. Neoangiogenesis is necessary for the progressive growth of the tumor even if restrained by natural limitations imposed by diffusion laws, that is, without initiating the invasion of blood vessels into the developing tumor, which cannot reach dimensions exceeding 1–3 mm in diameter, as reported by Jude Folkman over 45 years ago [16].

At present, numerous pro- and antiangiogenic factors have been identified and described in detail, which affect the functional activity of various cells that make up the vascular wall, such as endotheliocytes, fibroblasts, smooth muscle cells, and pericytes, and cause the degradation or organization of the endothelial basal membrane and interstitial matrix [14, 19]. These compounds are produced to a large extent directly by tumor cells, but a similar spectrum of substances possessing angiogenic and pro-inflammatory activity is also typical for migrating endotheliocytes, fibroblasts, paravasal macrophages, and neuroglia cells activated by tumor cells, which results in the formation of a double system of initiation and regulation of neoangiogenesis.

Despite the large number of described angiogenic compounds, in the pathophysiology and clinical oncology, the main attention is traditionally paid to cytokines of the family of vascular endothelial growth factor (VEGF) [1, 9, 11, 17]. VEGF was discovered in 1989 by Napoleon Farrara as a selective heparin-dependent factor in the proliferation of endotheliocytes. For this discovery, which laid the foundation of a purposeful search for methods of antiangiogenic therapy in oncology, in 2010, Farrara was awarded the Lasker-DeBakey Award in the field of clinical studies. Note that the predecessor of Farrara in the study of VEGF was Professor Harold Dvorak who, in 1983, described this compound as a factor enhancing the permeability of blood vessels.

VEGF, which is mainly produced in the norm by endotheliocytes [12], stimulates mitosis, chemotaxis, and locomotor activity of endotheliocytes and has the ability to block the mechanisms of apoptosis of these cells [10]. In addition, one of the important properties of this compound is its ability to enhance transcapillary exchange, thus increasing the permeability of the vessels of the microcirculatory bed.

The key role of neoangiogenesis in the pathogenesis of oncological diseases makes this component of the tumor process an attractive option for targeted therapeutic intervention. At the same time, the vast majority of developed medications are aimed at eliminating VEGF or blocking its receptors [6, 18]. Despite the large volume of studies conducted and the detailed pathogenetic justification, the use of antiangiogenic medications in oncology is still limited and, in some cases, empirical in nature. Some researchers also highlight the inadequate clinical efficacy of antiangiogenic therapy with a simultaneous increase in toxicity of chemotherapeutic agent in certain oncological diseases [21], a reduction in the duration of the delaying time to relapse, and an increase in

the dynamics of development of neoplasms and their invasive and metastatic potential [13, 20]. Obviously, these issues require a more elaborated study and close attention to the preclinical stage of trials of promising angiogenic drugs.

Taken together, *in vivo* test systems for experimental and preclinical studies of medications intended for target therapy of tumor-associated neoangiogenesis are not described in detail and not included in the relevant guidelines and methodological recommendations. Therefore, studying the dynamics of the serum VEGF of experimental animals in the development of malignant neoplasms of different histological types and the development rate of the pathological process appeared interesting.

MATERIALS AND METHODS

This study included 60 albino male and 42 albino female gray rats (Rattus norvegicus, John Berkenhout, 1769) that weighed 190–220 g, 48 male BALB/c mice that weighed 22–24 g, and 42 male CDF_1 mice (BALB/c (female) × DBA/2 (male)) that weighed 26–28 g. The animals were taken from a specialized nursery of laboratory animals "Rappolovo" (Leningrad region, Russia).

The content, nutrition, and withdrawal of animals from the experiment, and recovery of biological wastes were performed in accordance with Russian and international regulatory enactments regulating work with laboratory animals and principles of bioethics.

For modeling of the tumor process, the following strains of malignant tumors, obtained in the laboratory of carcinogenesis and aging (Petrov Research Institute of Oncology), were used: Pliss lymphosarcoma (PLS), transplanted ascitic ovarian tumor of rats (OT), colon adenocarcinoma (COLAD), and lymphocytic leukemia P-388 (P-388). Tumor strains were selected based on their histological type, rate of development and spread in various organs and systems, and high biological equivalence with oncological diseases registered in humans (neoplasms of blood and hematopoietic organs, ovarian cancer, colorectal cancer).

The tumor process was reproduced conventionally by direct transplantation of malignant cells obtained from tumor-bearing animals:

1) Pliss lymphosarcoma: At 10^3 cells in 0.2 ml of 0.9% sodium chloride solution per animal was administered subcutaneously into the right side of the rats.
2) Transplantable ascitic ovarian tumor of rats: 0.5 ml of ascitic fluid mixture with 0.9% sodium chloride solution in the ratio 1:4 was administered intraperitoneally.
3) Colon adenocarcinoma: 0.2 ml of a suspension of the tumor mass in a 0.9% solution of sodium chloride in the ratio 1:10 was administered subcutaneously in the right side region.
4) Lymphocytic leukemia P-388: 0.2 ml of ascitic fluid mixture with 0.9% sodium chloride solution in a ratio of 1:4 was administered intraperitoneally.

The following experimental groups were identified:

1. Control (male rats): healthy intact animals (n = 12).
2. Control (female rats): healthy intact animals (n = 12).
3. Control (BALB/c mice): healthy intact animals (n = 12).
4. Control (mice CDF_1 (BALB/c (female) × DBA/2 (male)): healthy intact animals (n = 12).
5. Pliss lymphosarcoma: animals with transplanted Pliss lymphosarcoma (n = 48).
6. Ovarian tumor: animals with transplanted grafted ascitic ovarian tumor of rats (n = 30).
7. COLAD: animals with transplanted adenocarcinoma of the colon (n = 36).
8. P 388: animals with transplanted lymphocytic leukemia P-388 (n = 30).

Blood sampling was performed by transcutaneous puncture of the heart of animals under general anesthesia (Zoletil 2.0 ml/kg body weight) in Monovette vacuum systems at 6.0 and 1.5 ml for rats and mice, respectively. Blood was processed immediately after sampling. To obtain platelet-enriched plasma, the blood was centrifuged at an acceleration of 240 g for 7 min, then transferred to another tube. The platelet-depleted plasma was obtained from the platelet-enriched plasma by repeated centrifugation at an acceleration of 1200 g for 15 min. The resulting material was transferred to Eppendorf and frozen at –20 to –22 °C until analysis. The maximum cryopreservation period did not exceed 4 months.

The preservation of serum VEGF was assessed by enzyme immunoassay using Cusabio reagent kits (CusaBio Biotech, China) in accordance with the manufacturer's instructions.

Considering the average life span of experimental animals and the dynamics of the development of experimental neoplasms established in the preliminary studies, the VEGF level was determined in animals with PLS on days 5, 10, 15, and 20 from the moment of neoplasm transplantation, on days 15, 30, and 45 in animals with COLAD, on days 4 and 8 in animals with transplanted ovarian tumor of rats, and days 5 and 8 in animals with lymphocytic leukemia P-388.

Statistical analysis was performed using SPSS software package. Data distribution was verified by cal-

culating the Kolmogorov–Smirnov criterion. The average data of independent samples was compared using Student's *t*-test (normal distribution, the variant in the sample set) and Mann–Whitney *U* test (in the distribution, a variant other than the normal one). The average data of the dependent samples were compared using the Friedman χ^2-criterion. The exact confidence intervals for the shares were calculated with the Clopper–Pearson method using the CONFINT program. A significant difference was the probability of not less than 95% ($p < 0.05$), which is the standard in biomedical research.

RESULTS AND DISCUSSION

Transplantation of all studied tumor strains was successful in 100% of cases. The average life span of rats with PLS was 27.4 ± 1.15 (median, 28.0) days, 11.3 ± 2.02 (median, 7.0) days in rats with ascitic ovarian tumor, 62.4 ± 4.45 (median, 60) days in mice with COLAD, and 10.0 ± 0.51 (median, 9.0) days in mice with lymphocytic leukemia P 388.

The analysis of serum VEGF of the experimental animals established significant differences in the dynamics of this index with the development of tumors of different histological type and the activity of the pathological process.

VEGF level in rats with Pliss solid lymphosarcoma slowly increased, as the primary tumor node grew and PLS spread in the animal's body ($\chi^2 = 10.571$, $p = 0.005$, Fig. 1). From the ligation of the neoplasm to day 15, the serum VEGF concentration of rat tumor carriers was significant, being on average, 2.6 times higher than in control animals ($p = 0.009$). Subsequently, a slight increase in VEGF content was observed in PLS rats, which was not a statistically significant difference from those recorded on day 15 (Figure 1). Such a rapid decrease in the growth rates of the level of the factor under study can be explained by a general deterioration in the state of the experimental animals by day 20 of the experiment due to tumor intoxication.

The dynamics of serum VEGF of the COLAD, a slowly growing solid tumor (Fig. 2), group, had a significantly different nature than that in the group of PLS (Fig. 1). High values of this factor, statistically significantly different from the control indices, were registered throughout the study from day 15, during the period of the onset of detection of single tumor nodes (in 11% of mice, 4 of 36 animals). By day 30, the maximum serum VEGF level of mice was reached, which was then drastically reduced by day 45 from the beginning of the experiment ($p = 0.031$, Fig. 2).

The reasons for such a change in serum VEGF concentration of BALB/c mice with COLAD remain incomprehensible within our research. From COLAD transplantation to day 45, the rate of increase in the volume of the tumor node remained sufficient, which was comparable to that of day 30 (Fig. 2). There were no clinically significant signs of tumor intoxication in experimental animals.

The VEGF level in animals with highly invasive, initially generalized (ascitic) tumors increased rapidly and reached the maximum values at the time of their

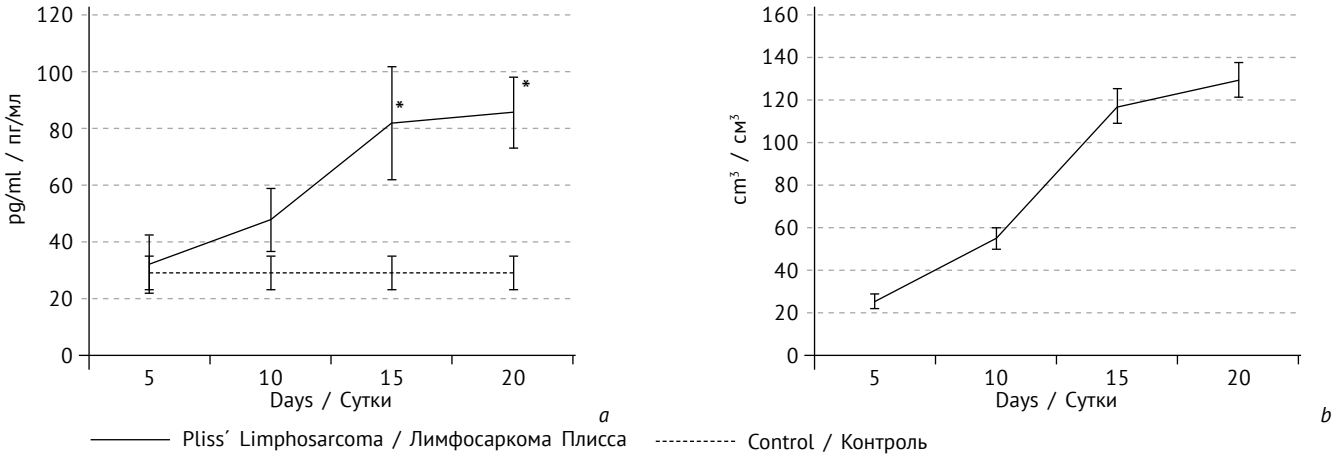

Fig. 1. VEGF concentration dynamics (*a*) in blood serum of male rats at various stages of subcutaneously transplanted Pliss' Lymphoma growth (*b*). * valid distinction from control ($p < 0,05$)

Рис. 1. Динамика содержания VEGF (*a*) в сыворотке крови крыс-самцов на различных этапах развития и увеличения размеров (*b*) трансплантированной подкожно лимфосаркомы Плисса. *Примечание:* *отличие от группы «Контроль» достоверно на принятом уровне значимости ($p < 0,05$)

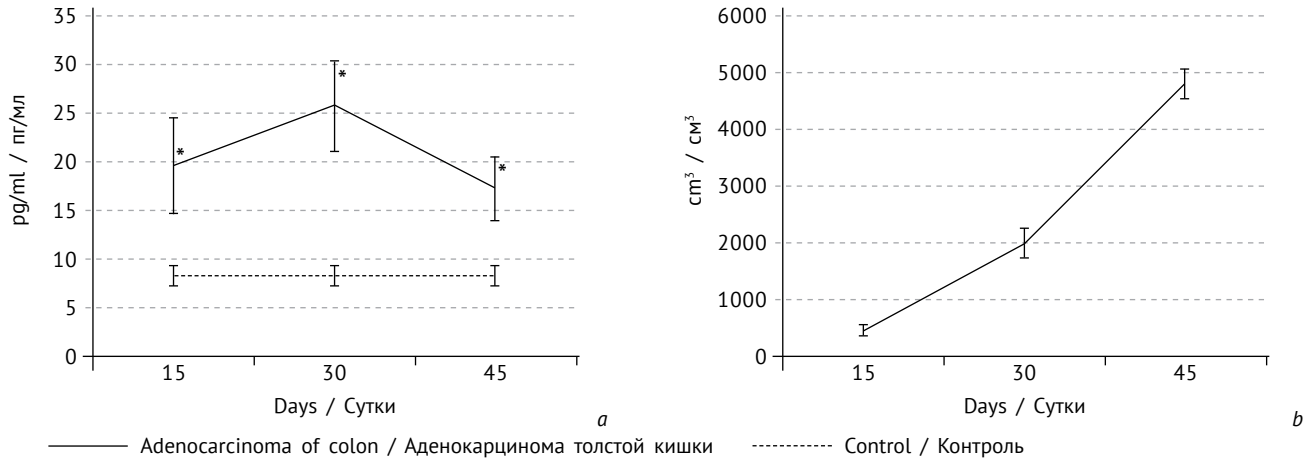

Fig. 2. VEGF concentration dynamics (*a*) in blood serum of BALB/c male mice at various stages of subcutaneously transplanted experimental colonic adenocarcinoma growth (*b*). * valid distinction from control ($p < 0,05$)

Рис. 2. Динамика содержания VEGF (*a*) в сыворотке крови мышей-самцов линии BALB/c на различных этапах развития трансплантированной подкожно экспериментальной аденокарциномы толстой кишки (*b*). *Примечание*: *отличия от группы «Контроль» достоверно на принятом уровне значимости ($p < 0,05$)

death. The concentration curves of VEGF in CDF_1 mice with leukemia P-388 and in rats with an ovarian tumor had a similar appearance (Fig. 3, 4). In mice with transplanted leukemia P-388, statistically significant differences from the control values of the index under study were observed throughout the experiment (Fig. 3). In animals with ascitic ovarian tumor, there was a moderate tendency to increase the VEGF content as the neoplasm develops (Fig. 4).

In this study, the results obtained from the experimental group were highly variable, especially at late observation terms, which can be explained by the high invasiveness and tropism of this tumor to the blood vessels. In 93% of cases, the direct cause of death of experimental animals was major bleeding into the abdominal cavity.

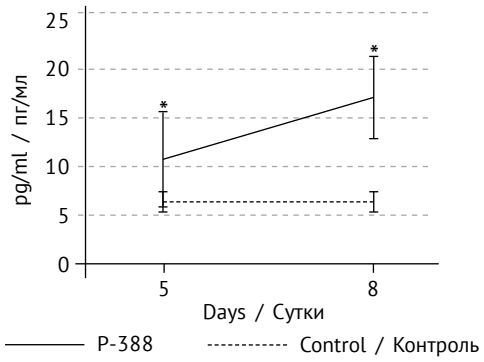

Fig. 3. VEGF concentration dynamics in blood serum of CDF_1 (BALB/c (female) × DBA/2 (male)) male mice at various stages of intraperitoneally transplanted lymphocytal leukemia. * valid distinction from control ($p < 0,05$)

Рис. 3. Динамика содержания VEGF в сыворотке крови мышей самцов CDF_1 (BALB/c (самка) × DBA/2 (самец)) с трансплантированной внутрибрюшинно лимфоцитарной лейкемией Р-388. *Примечание*: *отличия от группы «Контроль» достоверно на принятом уровне значимости ($p < 0,05$)

CONCLUSIONS

1. The development of experimental transplanted tumors of different histological type and localization is usually accompanied by increased serum VEGF content of experimental animals, the dynamics of which depend on the intensity of the tumor process.
2. In male BALB/c mice with transplanted subcutaneous experimental COLAD, along with active tumor development on day 45, a statistically significant decrease in the VEGF level was observed compared with the value at the previous control point of the study (day 30). The data obtained indicate the need for a special study to clarify the mechanisms of such dynamics of VEGF.
3. Study results recommend the transplanted tumors of PLS and lymphocytic leukemia P-388 as test systems for conducting experimental or preclinical studies of target antiangiogenesis drugs and an alternative to determine the development of neoplasms and significant changes in VEGF level associated with the intensity of the tumor process.

REFERENCES

1. Герштейн Е.С., Щербаков А.М., Алиева С.К., и др. Фактор роста эндотелия сосудов в опухолях и сыворотке больных раком молочной железы: связь

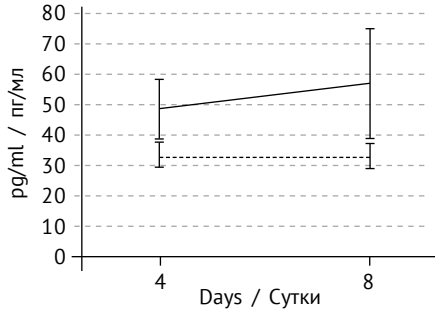

Fig. 4. VEGF concentration dynamics in blood serum of female rats at various stages of subcutaneously transplanted ovarian tumor growth

Рис. 4. Динамика содержания VEGF в сыворотке крови крыс самок с трансплантированной внутрибрюшинно опухолью яичников

с клинико-морфологическими факторами // Вестник РОНЦ имени Н.Н. Блохина РАМН. – 2005. – Т. 16. – № 1–2. – С. 26–30. [Gershteyn ES, Shcherbakov AM, Alieva SK, et al. Vascular endothelial growth factor in tumors and serum of breast cancer patients: relation to clinicomorphological factors. *Journal of N.N. Blokhin Russian Cancer Research Center RAMS*. 2005;16(1-2): 26-30. (In Russ.)]

2. Жуков Б.Н. Фотодинамическая терапия в комплексном лечении и реабилитации больных с острыми тромбозами глубоких вен нижних конечностей на фоне онкопатологии / Сборник тезисов Всероссийской научно-практической конференции «Посттромботическая болезнь»; Санкт-Петербург; 2009 г. – СПб.: СПбГУ, 2009. – С. 14–16. [Zhukov BN. Photodynamic therapy in the complex treatment and rehabilitation of patients with lower exteremities deep veins acute thrombosis on the background of oncopathology. In: Proceedings of the All-Russian scientific-practical conference «Post-thrombotic disease»; Saint Petersburg; 2009. Saint Petersburg: SPbGU; 2009. p. 14-16. (In Russ.)]
3. Зубаиров Д.М., Адрушко И.А., Зубаирова Л.Д., Свинтенок Г.Ю. Пертурбация эндотелия — причина острой гиперкоагулемии // Казанский медицинский журнал. – 2002. – Т. 83. – № 5. – С. 327–333. [Zubairov DM, Andrushko IA, Zubairova LD, Svintenok GY, et al. Pertubation of endothelium the cause of acute hypercoagulemia. *Kazan Med Zh*. 2002;83(5):327-333. (In Russ.)]
4. Мнихович М.В. Межклеточные и клеточно-матриксные взаимодействия в карциномах молочной железы: современное состояние проблемы // Российский медико-биологический вестник имени академика И.П. Павлова. – 2014. – № 2. – С. 152–161. [Mnihovich MV. Cell and cell-matrix interactions in breast carcinoma: the present state of problems. *Rossiiskii mediko-biologicheskii vestnik imeni akademika I.P. Pavlova*. 2014;(2):153-162. (In Russ.)]
5. Степанова Е.В. Характеристика ангиогенной активности опухолей человека *in vivo* // Российский биотерапевтический журнал. – 2007. – Т. 6. – № 1. – С. 6–7. [Stepanova EV. Characteristic of angiogenic activity of human tumors *in vivo*. *Rossiiskii bioterapevticheskii zhurnal*. 2007;6(1):6-7. (In Russ.)]
6. Степанова Е.В. Антиангиогенная терапия: новые возможности лечения злокачественных заболеваний // Практическая онкология. – 2002. – Т. 3. – № 4. – С. 246–252. [Stepanova EV. Angiogenic therapy: novel opportunities in the treatment of malignancies. *Practical oncology*. 2002;3(4):246-252. (In Russ.)]
7. Трапезникова М.Ф., Глыбин П.А., Морозов А.П., и др. Ангиогенные факторы при почечно-клеточном раке // Онкоурология. – 2008. – № 4. – С. 82–87. [Trapeznikova MF, Glybin PA, Morozov AP, et al. Angiogenic factors in renal cell carcinoma. *Onkourologiia*. 2008;(4):82-87. (In Russ.)]
8. Трашков А.П., Васильев А.Г., Цыган Н.В. Антитромботическая терапия в онкологии: современное состояние проблемы и нерешенные вопросы // Педиатр. – 2012. – Т. 3. – № 2. – С. 3–19. [Trashkov AP, Vasiliev AG, Tsygan NV. Antithrombotic therapy in oncology: contemporary concepts and pending problems. *Pediatrician (St. Petersburg)*. 2012;3(2):3-19. (In Russ.)]
9. Трашков А.П., Панченко А.В., Каюкова Е.С., и др. Лейкемия Р-388 у мышей линии CDF1 как тест-система опухоль-ассоциированного неоангиогенеза и гиперкоагуляции // Бюллетень экспериментальной биологии и медицины. – 2014. – Т. 158. – № 10. – С. 500–502. [Trashkov AP, Panchenko AV, Kayukova ES, et al. R-388 leukemia in CBA1 mice as tumor associated angiogenesis and hypercoagulation test system. *Biull Eksp Biol Med*. 2014;158(10):500-502. (In Russ.)]
10. Шурыгин М.Г., Шурыгин И.А., Дремина Н.Н. Влияние фактора роста эндотелия сосудов на выраженность цитолиза при экспериментальном инфаркте миокарда // Acta biomedical scientifica. – 2008. – № 1. – С. 68–71. [Shurigin MG, Shurigina IA, Dremina NN. The influence of vessels endothelium growth factor on the cytolysis intensity at experimental cardiac infarction. *Acta biomedica scientifica*. 2008;(1):68-71. (In Russ.)]
11. Anisimov VN, Popovich IG, Zabezhinski MA, et al. Sex differences in aging, life span and spontaneous tumorigenesis in 129/Sv mice neonatally exposed to metformin. *Cell Cycle*. 2015;14(1):46-55. doi: 10.4161/15384101.2014.973308.
12. Cao Y, Ji WR, Qi P, et al. Placenta growth factor: identification and characterization of a novel isoform gen-

erated by RNA alternative splicing. *Biochem Biophys Res Commun*. 1997;235(3):493-498. doi: 10.1006/bbrc.1997.6813.
13. Ebos JM, Lee CR, Cruz-Munoz W, et al. Accelerated metastasis after short-term treatment with a potent inhibitor of tumor angiogenesis. *Cancer Cell*. 2009;15(3):232-239. doi: 10.1016/j.ccr.2009.01.021.
14. Fagiani E, Christofori G. Angiopoietins in angiogenesis. *Cancer Lett*. 2013;328(1):18-26. doi: 10.1016/j.canlet.2012.08.018.
15. Folberg R, Hendrix MJC, Maniotis AJ. Vasculogenic Mimicry and Tumor Angiogenesis. *Am J Pathol*. 2000;156(2):361-381. doi: 10.1016/s0002-9440(10)64739-6.
16. Folkman J. Tumor angiogenesis: therapeutic implications. *N Engl J Med*. 1971;285(21):1182-1186. doi: 10.1056/NEJM197111182852108.
17. García-Román J, Zentella-Dehesa A. Vascular permeability changes involved in tumor metastasis. *Cancer Lett*. 2013;335(2):259-269. doi: 10.1016/j.canlet.2013.03.005.
18. Hurwitz H, Fehrenbacher L, Novotny W, et al. Bevacizumab plus Irinotecan, Fluorouracil, and Leucovorin for Metastatic Colorectal Cancer. *N Engl J Med*. 2004;350(23):2335-2342. doi: 10.1056/NEJMoa032691.
19. Liekens S, De Clercq E, Neyts J. Angiogenesis: regulators and clinical applications. *Biochem Pharmacol*. 2001;61(3):253-270. doi: 10.1016/s0006-2952(00)00529-3.
20. Paez-Ribes M, Allen E, Hudock J, et al. Antiangiogenic therapy elicits malignant progression of tumors to increased local invasion and distant metastasis. *Cancer Cell*. 2009;15(3):220-231. doi: 10.1016/j.ccr.2009.01.027.
21. Poveda A, Selle F, Hilpert F, et al. Weekly paclitaxel (PAC), pegylated liposomal doxorubicin (PLD) or topotecan (TOP)±bevacizumab (BEV) in platinum (PT)-resistant recurrent ovarian cancer (OC): analysis by chemotherapy (CT) cohort in the GCIG AURELIA randomised phase III trial. *Ann Oncol*. 2012;23(Suppl 9):LBA26.
22. Takano S, Yoshii Y, Kondo S, et al. Concentration of vascular endothelial growth factor in the serum and tumor tissue of brain tumor patients. *Cancer Res*. 1996;56(9):2185-2190.

◆ Information about the authors

Alexander P. Trashkov — MD, PhD, Head, Trials Center of Radiopharmaceutical, Petersburg Nuclear Physics Institute named by B.P. Konstantinov of National Research Centre "Kurchatov Institute", Gatchina, Russia. E-mail: alexandr.trashkov@gmail.com.

Nikolay A. Verlov — PhD, Senior researcher, Trials Center of Radiopharmaceutical, Petersburg Nuclear Physics Institute named by B.P. Konstantinov of National Research Centre "Kurchatov Institute", Gatchina, Russia. E-mail: virlov@gmail.com.

Margarita R. Artemenko — Graduate, Trials Center of Radiopharmaceutical, Petersburg Nuclear Physics Institute named by B.P. Konstantinov of National Research Centre "Kurchatov Institute", Gatchina, Russia. E-mail: shadow_ii@list.ru.

Valeria A. Pechatnikova — Researcher, Trials Center of Radiopharmaceutical, Petersburg Nuclear Physics Institute named by B.P. Konstantinov of National Research Centre "Kurchatov Institute", Gatchina, Russia. E-mail: floluttrell@gmail.com.

Maria A. Zelenenko — MD, Researcher, Experimental Pharmacology Department. Sechenov Institute of Ephysiology and Biochemistry, Russian Academy of Sciences. Saint Petersburg, Russia. E-mail: magu56110@gmail.com.

◆ Информация об авторах

Александр Петрович Трашков — канд. мед. наук, заведующий, Испытательный центр радиофармпрепаратов. ФГБУ «Петербургский институт ядерной физики им. Б.П. Константинова Национального исследовательского центра „Курчатовский институт"», Гатчина. E-mail: alexandr.trashkov@gmail.com.

Николай Александрович Верлов — канд. биол. наук, старший научный сотрудник, Испытательный центр радиофармпрепаратов. ФГБУ «Петербургский институт ядерной физики им. Б.П. Константинова Национального исследовательского центра „Курчатовский институт"», Гатчина. E-mail: virlov@gmail.com.

Маргарита Радиевна Артеменко — аспирант, Испытательный центр радиофармпрепаратов. ФГБУ «Петербургский институт ядерной физики им. Б.П. Константинова Национального исследовательского центра „Курчатовский институт"», Гатчина. E-mail: shadow_ii@list.ru.

Валерия Антоновна Печатникова — научный сотрудник, Испытательный центр радиофармпрепаратов. ФГБУ «Петербургский институт ядерной физики им. Б.П. Константинова Национального исследовательского центра „Курчатовский институт"», Гатчина. E-mail: floluttrell@gmail.com.

Мария Александровна Зелененко — научный сотрудник, отдел экспериментальной фармакологии. ФГБУН «Институт эволюционной физиологии и биохимии им. И.М. Сеченова» РАН, Санкт-Петербург. E-mail: magu56110@gmail.com.

◆ Information about the authors

Mariya A. Pakhomova — Senior Researcher, Research Center. St. Petersburg State Pediatric Medical University, Saint Petersburg, Russia. E-mail: mariya.pahomova@mail.ru.

Andrei G. Vasiliev — MD, PhD, Dr. Med. Sci., Head, Pathophysiology Department. St. Petersburg State Pediatric Medical University, Saint Petersburg, Russia. E-mail: avas7@mail.ru.

◆ Информация об авторах

Мария Александровна Пахомова — старший научный сотрудник, Научно-исследовательский центр. ФГБОУ ВО «Санкт-Петербургский государственный педиатрический медицинский университет» Минздрава России, Санкт-Петербург. E-mail: mariya.pahomova@mail.ru.

Андрей Глебович Васильев — д-р мед. наук, профессор, зав. кафедрой патологической физиологии с курсом иммунопатологии. ФГБОУ ВО «Санкт-Петербургский государственный педиатрический медицинский университет» Минздрава России, Санкт-Петербург. E-mail: avas7@mail.ru.