ORIGINAL RESEARCH

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STUDY OF XAV-939 EFFECT ON CANCER STEM CELLS OF HUMAN COLORECTAL CANCER IN HETEROTOPIC MODELS IN VIVOABSTRACT

The purpose of this study was to investigate the numbers of cells with CSC markers in samples of human colorectal cancer (CRC) xenografts isolated in animals with tumors after exposure to the small molecule XAV-939, 5-fluorouracil, and to the combination of XAV-939 with 5-fluorouracil. The model was created in Balb/c Nude mice. Tumor fragments of human CRC were transplanted into adipose tissue of animals under the skin of the right thigh. The fourth xenograft generation was used for the study. Animals were divided into groups receiving XAV-939, 5-fluorouricil, their combination, and the control group. Tumor nodes were measured twice a week; at the end of the experiment, the number of cells with the CSC phenotype was determined in the tumor material of the animals. By the end of the experiment, tumor nodules in the group of animals receiving the combination of drugs were significantly smaller than in the control group. The highest levels of cells with phenotypic signs of CSCs were characteristic of samples from the control group, and for samples obtained from the group receiving 5-fluorouracil as monotherapy. Decreased numbers of these cells were observed in groups receiving XAV-939 and the combination of XAV-939 and 5-fluorouracil.

Keywords: colorectal cancer, CRC, cancer stem cells, CD44, CD133.

Background. Today, colorectal cancer (CRC) is among the most common malignancies and one of the most frequent causes of cancer-related deaths. Poor treatment outcomes are believed to be associated with a population of cancer stem cells (CSCs) characterized by a low proliferation rate and the ability for multipotent differentiation, as well as resistance to various antitumor effects [1]. This cell population is characterized by various specific markers with biological functions contributing to the formation of features characteristic of CSCs [2]. Proteins such as CD44 and CD133 are often used for CSC identification, and as prognostic markers of the CRC course [6].

The resistance of long-lived, low-proliferative CSCs to cytotoxic effects is also important, which indicates the need for the development and use of targeted drugs aimed at CSCs and disruption of their signaling, for example, the Wnt pathway, which is important in maintaining the CSC pool. In addition, a number of studies showed an association between its activation and the progression of some malignant tumors, including CRC; so, the inhibition of the Wnt signaling pathway is considered as a promising therapeutic direction in CRC [5; 11].

In recent years, researchers have actively discussed specific therapy aimed at CSC-associated signaling in combination with traditional chemotherapeutic regimens to eliminate both differentiated cancer cells and the CSC population in order to prevent possible recurrence [8]. At the moment, several dozens of different compounds, including small molecules, are at various stages of preclinical and clinical studies [2]. The small molecule XAV-939 is considered as one of Wnt signaling inhibitors [7]. Studies on its possible mechanisms of action reported that SW480 CRC cells treated with XAV-939 demonstrated stabilization of the protein complex, including the proteins Axin, APC, GSK-3b, Ck1a, and consequently blockade of abnormally activated Wnt signaling pathway [3]. In addition, studies on the H446 cell culture showed that, in comparison with other inhibitors of the Wnt pathway, XAV-939 exhibits strong specificity for this signaling without affecting other molecular pathways [4]. However, the mentioned studies were performed on cell cultures, and therefore it is a necessary task to study the effect of the Wnt signaling pathway inhibitor XAV-939 on CSCs in *in vivo* models obtained by xenotransplantation of clinical specimens, which are largely able to reflect the complex biology and behavior of human tumors in response to a therapeutic strategy.

In this regard, the aim of this study was to investigate the numbers of cells with CSC markers in samples of human CRC xenografts isolated in animals with tumors after exposure to the small molecule XAV-939, 5-fluorouracil, and to the combination of XAV-939 with 5-fluorouracil.

Material and methods. *Tumor material.* Subcutaneous xenografts were created using a transplantable strain of human CRC obtained at the National Medical Research Centre for Oncology. The primary tumor material was obtained from a patient diagnosed with T4N1M0 splenic flexure cancer during surgical treatment (resection of the transverse colon). The patient provided written consent to the transfer of biological material.

Recipient animals. The experiment was performed in 20 immunodeficient Balb/c Nude mice (females). The animals were obtained from the SPF-vivarium of the Institute of Cytology and Genetics of the Siberian Branch of the Russian Academy of Sciences (Novosibirsk). The mice were 5–6 weeks old, with an average weight of 22 g. The animals were kept in the SPF vivarium of the Experimental Laboratory Center at the National Medical Research Centre for Oncology. All manipulations during the experiment were in compliance with the ethical principles

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established by the European Convention for the Protection of Vertebrate Animals used for experiments or for other scientific purposes (ETSN 123, Strasbourg, March 18, 1986). The study protocol was approved by the local ethics committee of the National Medical Research Centre for Oncology.

Creation of a tumor model. The fourth generation of subcutaneous xenografts was used for this study: fragments of the third generation tumor xenograft were implanted under the skin of the right thigh in recipient mice. Animals were anesthetized using injection anesthesia with the veterinary drugs Xila 20 mg/kg and Zoletil-100 50 mg/kg.

Investigated drugs, doses, methods and modes of their administration, distribution of animals into groups. The studied drug XAV-939 (Sigma-Aldrich) – orally at a dose of 25 mg/kg, 5-fluorouracil – intraperitoneally at a dose of 25 mg/kg, 0.9% NaCl - intraperitoneally, sterile water – orally; administered twice a week. The control group received carrier substances in the same mode: 0.9% NaCl solution, sterile water.

Tumor-bearing animals were divided into 4 groups, each of which included 5 animals: group 1 – 5-fluorouracil (5-Fu); group 2 – XAV-939; group 3 – XAV-939+5-fluorouracil (XAV-939+5-Fu); group 4 – control.

Animals were divided into groups depending on the sizes of tumor nodes at the time of the beginning of the drug administration - 50±20% mm^3. When randomizing animals, we were guided by the minimum scatter of the average values of the volume of tumor nodes in groups.

Analysis of the growth of human CRC xenografts. Tumor nodules were measured twice a week, their sizes were calculated by the formula V=a×b×c×π/6, where V is the tumor volume (mm^3), and a, b, c are linear measurements of the ellipsoid in three planes (mm).

CSC determination by flow cytometry. The numbers of cells with CSC markers CD45-44+, CD45-133+, CD45-44+133+ were determined in samples of human CRC xenografts isolated from tumor-bearing animals using the FacsCanto II flow cytometer (Becton Dickinson, USA) with two lasers with fluorophore excitation wavelengths of 488 nm and 633 nm, with the ability to use up to 6 monoclonal antibodies in one tube simultaneously. A set of monoclonal antibodies was used for this study: CD44 FITC (BD Pharmingen, USA)/CD133 APC (BD, USA)/CD45 APC-Cy7 (BD, USA).

Statistical analysis. The data were analyzed using the Statistica and Excel program package. The Shapiro–Wilk test was used to verify the hypothesis of the normal distribution, and the differences between the groups were evaluated by the Mann-Whitney non-parametric-criterion.

Results and discussion. The experiment revealed the dynamics of growth of xenotransplants in three experimental groups and the controls. The volumes of the tumors in the group receiving 5-fluoruracyl and in the group with XAV-939 did not significantly differ from the control group. The volumes of tumor nodes in the experimental group with XAV-939+5-Fu were 335.2 mm^3, which was statistically significantly lower than the volumes of tumors in the control group – 609.3 mm^3 ($p<0.05$). The data demonstrating the dynamics of the growth of tumor nodes are presented in Figure 1.

Note: * - differences are statistically significant by the Mann-Whitney test ($p<0.05$).

The level of cells with the CD45-44+ phenotype in the control group was 7.9% [7.7%; 8.1%], and 8.1% [7.9%; 8.5%] in the group with 5-Fu. The percentage of cells carrying the CD44 molecule in the group with XAV-939 amounted to 3.7% [3.4%; 3.9%], which was statistically lower than the number of cells of this phenotype in the samples of the control group ($p <0.05$). The content of cells with the CD45-44+phenotype in the group with XAV-939+5-Fu amounted to 1.9% [1.6%; 2.1%], and it was statistically lower, both as in the group with XAV-939 monotherapy, than the number of CD45-44+ cells in the samples of the control group ($p<0.05$).

Experimental data on the numbers of cells with the CD45-44+ phenotype are presented in Figure 2.

Note: * - differences are statistically significant by the Mann-Whitney test ($p<0.05$).

An analysis of cells with the CD45-133+ phenotype demonstrated that their content in the control group was 8.1% [7.9%; 8.4%]; in the group with 5-Fu – 6.4% [6.3%; 7.7%]; in the group with

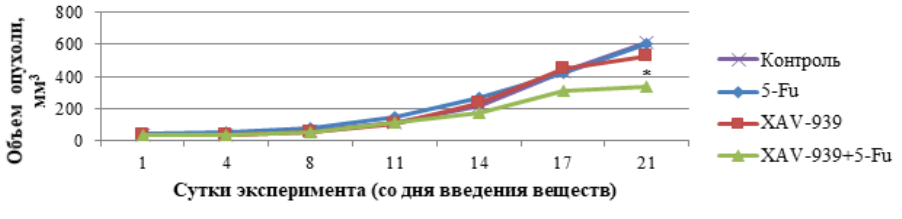

Fig. 1. The dynamics of tumor growth in experimental and control groups.

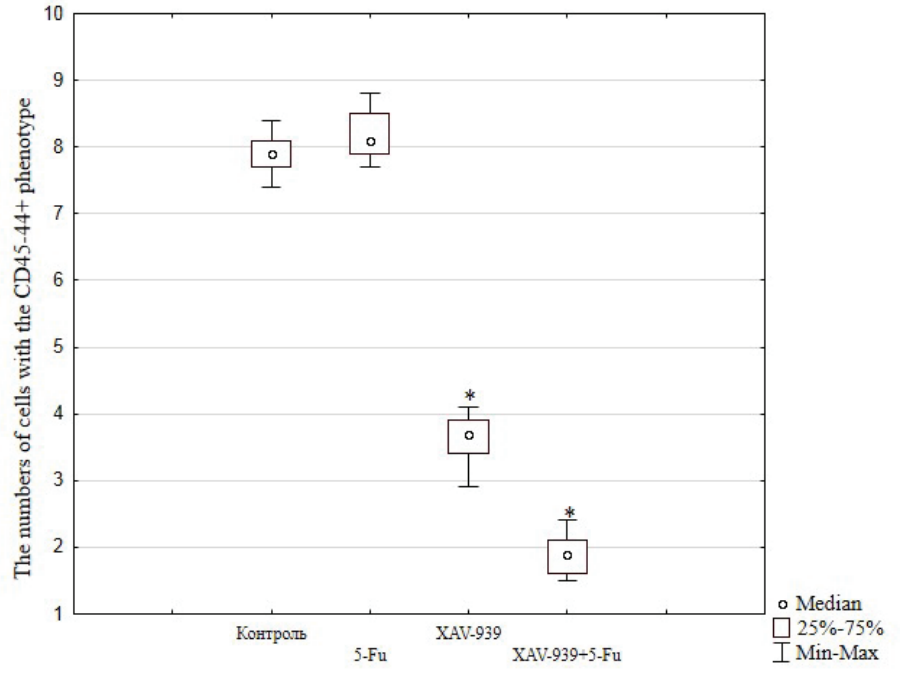

Fig. 2. The numbers of cells with the CD45-44+ phenotype in experimental and control groups.

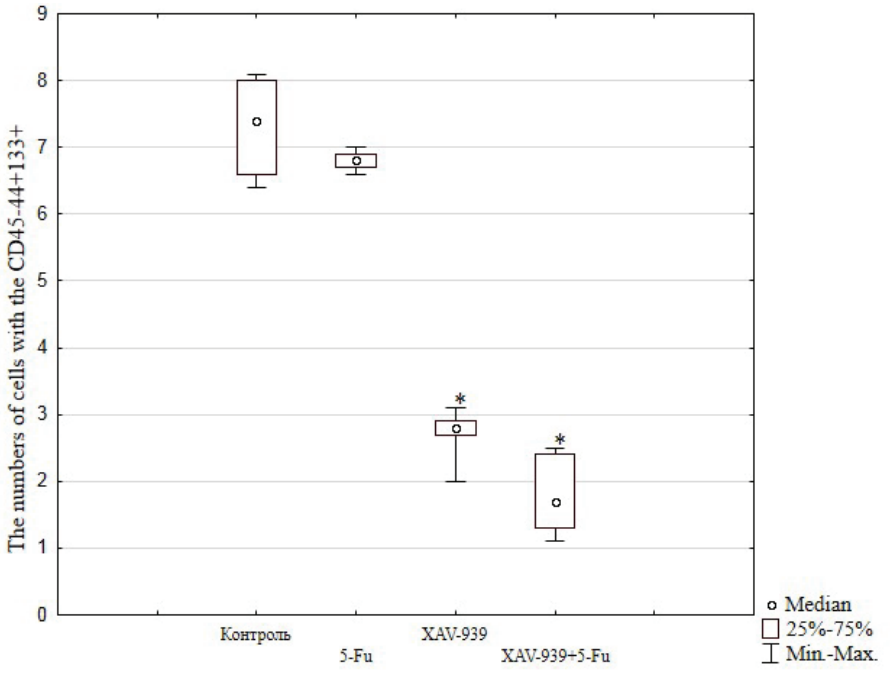

Fig. 3. The numbers of cells with the CD45-133+ phenotype in experimental and control groups.

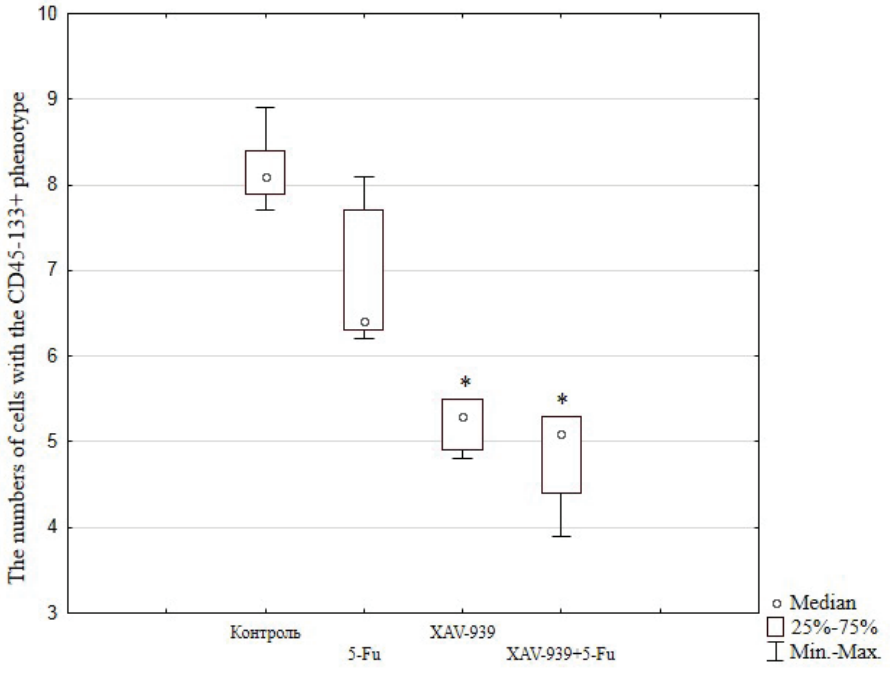

Fig. 4. The numbers of cells with the CD45-44+133+ phenotype in experimental and control groups.

XAV-939 – 5.3% [4.9%; 5.5%]; in the group with XAV-939+5-Fu – 5.1% [4.4%; 5.3%]. Both in the group with XAV-939 monotherapy and in the group with XAV-939+5-Fu, the number of CD45-133+ cells was statistically significantly lower than in the control group (p=0.0122).

The numbers of cells with the CD45-133+ phenotype in experimental groups are presented in Figure 3.

Note: * - differences are statistically significant by the Mann-Whitney test (p<0.05).

The number of cells with the CD45-44+133+ phenotype in the control group was 7.4% [6.6%; 8%]; in the group with 5-Fu – 6.8% [6.7%; 6.9%]; in the group with XAV-939 – 2.8% [2.7%; 2.9%], and in the group with XAV-939+5-Fu – 1.7% [1.3%; 2.4%], which was was statistically significantly lower than in the control group (p<0.05).

The data on the numbers of cells with the CD45-44+133+ phenotype are presented in Figure 4.

Note: * - differences are statistically significant by the Mann-Whitney test (p<0.05).

An analysis of the scientific literature of recent years demonstrates a significantly increasing interest in CSCs, their biological functions and the role in carcinogenesis [7, 10, 11]. A number of studies on the prognostic potential of the CD44 and CD133 CSC markers in CRR reported that higher expression of these proteins is associated with a high risk of tumor recurrence and metastasis [9, 10]. According to Spelt et al. (2018), patients with tumors characterized by a high content of cells with the CD45-133+ phenotype had poor survival, compared to patients without this marker [9]. On the contrary, the CD44 knockdown suppressed invasion, proliferation and migration of tumor cells. In addition, the study on SW620 CRR cell culture showed that the expression of the CD44+ and CD133+ markers was associated with the phenomenon of drug resistance to cytostatics [3].

Our study revealed that the highest levels of cells with phenotypic signs of CSCs were characteristic of samples from the control group and from the group receiving 5-fluorouracil as monotherapy, which, together with the absence of a significant decrease in the volume of tumor nodes in this group, can be considered as a sign of resistance to this drug, which is consistent with the trends described in the literature that characterize CSCs and their dynamics. Decreased numbers of cells expressing CSC markers in groups receiving XAV-939 and the combination of XAV-939 and 5-fluorouracil can be considered as confirmation of the Wnt signal path inhibition, which might help overcome the resistance of tumor cells to standard chemotherapy, and this was confirmed by a decrease in the volume of tumor nodes in the group of animals receiving the combination of drugs.

Conclusions. This study demonstrated the reduction in the number of cells with the CSC phenotypes CD45-44+, CD45-133+, CD45-44+133+ in the samples of xenotransplants in animal groups receiving the XAV-939 tested substance, as well as a combination of XAV-939 and 5-fluorurationils, which can be considered a sign of the Wnt signal path inhibition. The data characterize XAV-939 as a promising substance for further studies on its effectiveness in combination with standard therapy against CSCs in CRR.

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PHYSICAL DEVELOPMENT OF WOMEN IN MAGADAN REGION IN AGE AND ETHNIC ASPECTS

Anthropometry is known to be a qualitative measure that reflects the level of health state of a population. For the first time, an assessment study on physical development of women in age and ethnic aspects was conducted in the territory of Magadan Region. This research aimed at studying age dynamics in basic anthropometric indicators among women of different ethnicity from different regions of residence, at mature and old ages.

Materials and methods. In the year of 2022 we analyzed data of medical records of one thousand and sixty-four women from Severo-Evensk District and the city of Magadan. The following anthropometric indicators were included in the general database: Body Length, Body Mass, and Waist Circumference, with further calculating the Body Mass Index. The subjective sample was divided into six groups according to the ethnicity, as well as based on the age. The main indicators of physical development were evaluated by standard research methods.

Results. From the obtained data we could see a reduction in subjective Body Length variables with those of Body Mass, Body Mass Index, and Waist Circumference growing with increasing age, from the middle age (maturity) to the elderly period of ontogenesis. In the settlement of Evensk, the percentage of middle-aged women diagnosed with obesity was 40% among Aboriginals and 24% among Caucasians growing up to 68% and 47%, respectively, in old women. The obesity incidence among women of Magadan made up 25% and 45%, respectively.

Conclusion. The observed characteristics indicate unhealthy tendencies, which the increased Body Mass Index suggests owing to shorter Body Length and significantly bigger Body Mass indices progressing from mature to old ages. To a greater extent, obesity is experienced by women of the Aboriginal population of Magadan Region. All the examinees show Waist Circumference measurements that excess the normal standards, which indicate the development of such an unfavorable factor as abdominal obesity.

The age- and ethnicity-based somatometric status was also specified for Magadan Region women, which should be considered when forming the region-related standards of physical development.

Keywords: anthropometric indicators, ethnicity, human population, women of the Magadan region, obesity.

Introduction. According to the WHO, obesity is recognized as the epidemic of the XXI century due to the constant spread of this disease [27]. For some experts' estimates, the incidence of obesity among the female population is expected to 50% increase by the year of 2025 [24]. To reveal abnormalities in physical development, anthropometric indicators have been used in clinical healthcare practice for many decades as a way to identify some diseases, classify impairments and evaluate further treatment [29]. They are also integrative characteristics that ascertain the quality of life, respond to environmental, social, and hygienic changes [18]. The anthropometric method is inexpensive, non-invasive and universally applicable for assessing the size, proportions and composition of the human body – all these vary throughout human life [31, 5].

The growth of the elderly population is an inevitable consequence of social and economic development and improvement of medical technologies. [22]. This group is characterized by a higher risk of many diseases including cardiometabolic ones [14, 33]. Being a way to detect overweight and obesity, the careful monitoring of physical development and BMI indicators throughout life also appears to be a predictor of a number of diseases including cardiovascular and metabolic diseases in old age [28].

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