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CREATION OF A HETEROTOPIC PDX MODEL OF UVEAL MELANOMA

Uveal melanoma, the second most prevalent form of the disease after cutaneous melanoma, exhibits a lower prevalence rate. Treatment options for uveal melanoma may include enucleation, exenteration, radiation therapy, brachytherapy, or systemic therapy. However, these methods do not guarantee a complete cure, and half of the patients subsequently develop metastases, which still carry an unfavorable prognosis. Therefore, preliminary selection of therapeutic candidates in preclinical studies is important. The reliability and potential of preclinical study results depend on the tumor model's reflection of the fundamental biological characteristics of uveal melanoma. The objective of our study was to develop a subcutaneous PDX model of uveal melanoma, which will be utilized to generate uveal melanoma metastases.

In the course of our research, we developed a collection of three heterotopic PDX models of uveal melanoma. Histological examination of these models revealed their correspondence to the donor tumors. According to the results of our study, the first-generation tumor engraftment rate was 37.5% (3/8). We also assessed the growth dynamics of the obtained PDX models. The analysis revealed that the growth rate of the first-generation xenografts obtained from patients was low, with a doubling time of 42 days. To sustain the growth of the PDX model, the tumors obtained from the first-generation mice were systematically transplanted into the subsequent group of mice. Our findings demonstrate that the first-generation models successfully engrafted in the second-generation mice. Furthermore, it was observed that the growth rate of the tumor node was higher in the second generation compared to the first generation.

Keywords: uveal melanoma, PDX, tumor models.

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Introduction. Uveal melanoma, the second most prevalent form of the disease after cutaneous melanoma, manifests as the most common primary intraocular neoplasm [4]. This neoplasm originates from melanocyte cells residing within the intraocular structures, including the choroid, ciliary body, and/or iris. The predominant location of the disease is the choroid (90%), followed by the ciliary body (6%) and iris (4%) [8].

A multitude of factors have been identified as contributing to an increased likelihood of developing uveal melanoma. These factors include, but are not limited to, light eye and skin color, the presence of dysplastic nevus syndrome, ocular melanocytosis, and xeroderma pigmentosum [2]. Furthermore, research has demonstrated a positive correlation between prolonged exposure to natural and artificial ultraviolet radiation and the likelihood of developing uveal melanoma. [11].

Treatment of uveal melanoma may include enucleation, exenteration, radiation therapy, brachytherapy (placement of radioactive sources directly into the tumor), or systemic therapy (chemotherapy, targeted therapy) [11]. However, these strategies do not always lead to complete recovery. In fact, half of the patients subsequently develop metastases, the prognosis for which remains unfavorable [6].

Currently, there are no effective treatment options for patients with advanced or metastatic uveal melanoma. There-

fore, innovative therapeutic approaches are needed to improve treatment outcomes [1].

In recent years, preclinical models created by direct implantation of tumor material obtained from patients into immunodeficient mice (patient derived xenografts - PDX) have been considered the most preferable for oncology research, since they most accurately reproduce the characteristics of a human tumor compared to other model systems [3].

PDX models serve as an experimental tool, facilitating a deeper understanding of the molecular processes underlying diseases and identifying promising targets for therapeutic intervention. In the context of oncology research, the use of PDX models of uveal melanoma allows for a broader understanding of the mechanisms of disease progression and metastasis.

Furthermore, such models enable the testing of new therapeutic approaches and the identification of optimal combinations of treatments. Consequently, this may result in enhanced prognoses for patients afflicted with metastatic uveal melanoma [5].

In this regard, the objective of our study was to develop a subcutaneous PDX model of uveal melanoma, as well as to assess the growth dynamics and histology of the obtained tumor nodes, with the aim of further refining this model to create a metastasis of uveal melanoma.

Materials and methods. Animals and their management. In the course of the experiment, female Balb/c Nude mice aged 12-14 weeks, with an average weight of 27-30 g, were utilized. These animals were obtained from the in-house breeding vivarium of the Testing Laboratory Center of the National Medical Research Centre for Oncology of the Ministry of Health of the Russian Federation. The mice were housed in individual ventilated cages and provided with food and water ad libitum. All manipulations carried out within the framework of the study were carried out in accordance with the ethical principles established by the European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes (ETS 123, Strasbourg, March 18, 1986). The study protocol was approved by the local bioethics committee of the National Medical Research Centre for Oncology of the Ministry of Health of the Russian Federation.

Tumor samples. A total of eight patients receiving treatment at the National Medical Research Centre for Oncology of the Ministry of Health of the Russian Federation provided samples of uveal melanoma. Informed consent for the transfer of biological material was obtained from all patients.

Anesthesia. The surgical manipulations performed on the animals in this experiment were carried out using in-

tramuscular injection anesthesia of the veterinary drugs "Xyla" (20 mg/kg) and "Zolelil-100" (50 mg/kg).

Technique of creating a heterotopic (subcutaneous) model of uveal melanoma by transplanting a tumor fragment from a patient to the right side. We previously obtained samples of uveal melanoma tumor material from patients following enucleation of the eye or orbital exenteration. The tumor material was transported to the vivarium within 15 minutes of removal in DMEM nutrient medium containing 10% gentamicin. Tumor material obtained from one patient was implanted in a group of three mice. Upon reaching the requisite depth of anesthesia, the animals were incised along the right side of the skin, followed by the introduction of sterile closed blunt scissors into the subcutaneous space to separate the peritoneum from the skin, thereby creating a subcutaneous pocket. The isolated tumor material was then implanted without causing trauma to the abdomen. The surgical wound was sutured using an interrupted suture, and all surgical interventions were performed under sterile conditions. The second generation was created in a similar manner.

Assessment of tumor node growth. The linear dimensions of tumor nodes were measured using a caliper weekly, starting from the 14th day after implantation of tumor material into immunodeficient mice.

The volume of the tumor node was calculated using the formula:

$$V=LW^2/2,$$

where L, W are the linear dimensions of the tumor.

Observations and measurements of tumor nodes were performed for 4 months, starting from the date of implantation of tumor material.

Euthanasia. Euthanasia was performed by decapitation followed by tumor node collection at the end of the observation period.

Histological examination. The tumor node was fixed in a 10% formalin solution for 24 hours. After this procedure, the material was placed in paraffin for further preparation of histological microsections, which were stained with hematoxylin and eosin according to the standard technique. Using a ZEISS Axio light microscope, a histological study of the human donor tumor and tumor material of the PDX models was performed.

Research results and their discussion. Animal models are indispensable tools in the study of tumor growth and metastasis, as well as the evaluation of novel treatment methods. The reliability of research findings is contingent upon the selection of an appropriate model that accurately reflects the pathogenesis of the disease [10].

In the 2023–2024 period, a total of

Results of heterotopic implantation of uveal melanoma in Balb/C Nude mice

Code of xenograft procedures	Age	Sex	Method of obtaining material	Tumor localization	Stage	TNM stage	Results of heterotopic implantation
PDX-UM-01	66	F	Enucleation of the left eye	At 5-6 o'clock Posterior lateral wall of the eyeball	II	T3aN0M0	-
PDX-UM-02	76	F	Orbital exenteration of the left eye	At 7-8 o'clock Posterior lateral wall of the eyeball	II	T2aN0M0	+
PDX-UM-03	64	M	Enucleation of the right eye	In all quadrants Diffuse spread	III	T4aN0M0	+
PDX-UM-04	47	F	Orbital exenteration of the right eye	At 2-4 o'clock Along the nasal surface	II	T3aN0M0	-
PDX-UM-05	67	M	Orbital exenteration of the right eye	At 8-9 o'clock Left temporal region	II	T2aN0M0	-
PDX-UM-06	50	F	Orbital exenteration of the left eye	At 7-9 o'clock Posterior lateral wall of the eyeball	II	T3aN0M0	+
PDX-UM-07	70	F	Orbital exenteration of the left eye	In all quadrants Diffuse spread	III	T3bN0M0	-
PDX-UM-08	62	M	Enucleation of the right eye	In all quadrants Diffuse spread	III	T4bN0M0	-

Note: "+" – successfully engrafted material; "-" - lack of successful engraftment of the material.

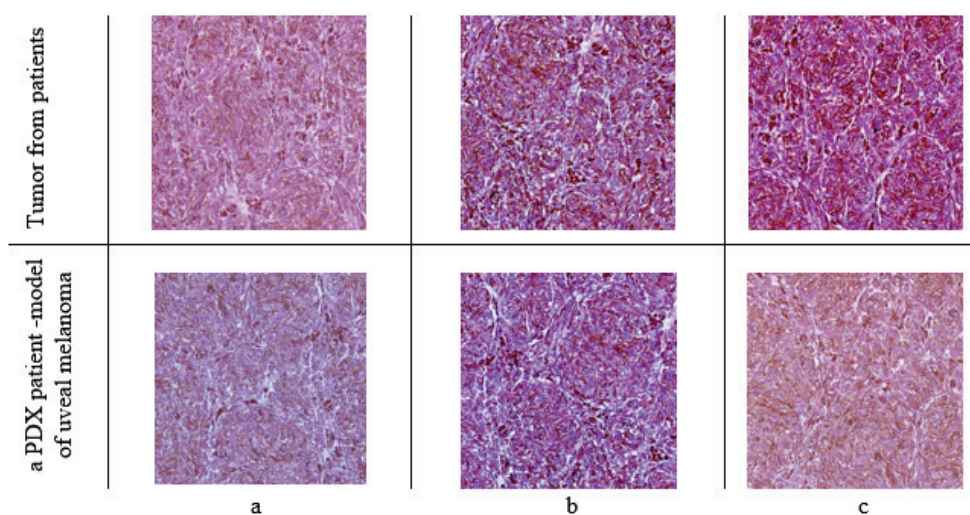


Fig. 1. Histological preparations of patient tumors and corresponding PDX models of uveal melanoma (a - PDX-UM-02; b - PDX-UM-03; c - PDX-UM-06)

eight patients diagnosed with uveal melanoma were enrolled in the study. These patients were treated at the National Medical Research Centre for Oncology of the Ministry of Health of the Russian Federation. The patients' tumor samples were then implanted subcutaneously into the right side of Balb/C Nude mice ($n = 3$). The success of the engraftment process was determined by the volume of the tumor node, which had to reach a minimum of 60 mm^3 for the tumor to be considered successfully engrafted. The observation period was four months, during which the absence of tumor growth was noted. In the event that no growth of tumor nodes was observed during this time, the xenotransplantation procedure was assessed as ineffective. It is noteworthy that all uveal melanoma samples were obtained during the surgical stage of treatment and implanted within 30 minutes post-surgery. The average age of the sample donor patients was 63 years (range, 47–76 years). The distribution of gender among the donor patients was as follows: 37.5% male and 62.5% female. The tumor donor patients did not receive neoadjuvant chemotherapy or radiotherapy. The majority of patients were diagnosed with stage II disease, and a total of 3 PDX models of human uveal melanoma were obtained from tumor samples from 8 patients. The detailed patient characteristics and tumor xenotransplantation results are presented in Table 1. The success rate of primary tumor engraftment obtained from patients was 37.5%, as shown in Table.

Next, samples of donor tumors and corresponding successfully engrafted xenogeneic (PDX) tumors were subjected to histological examination.

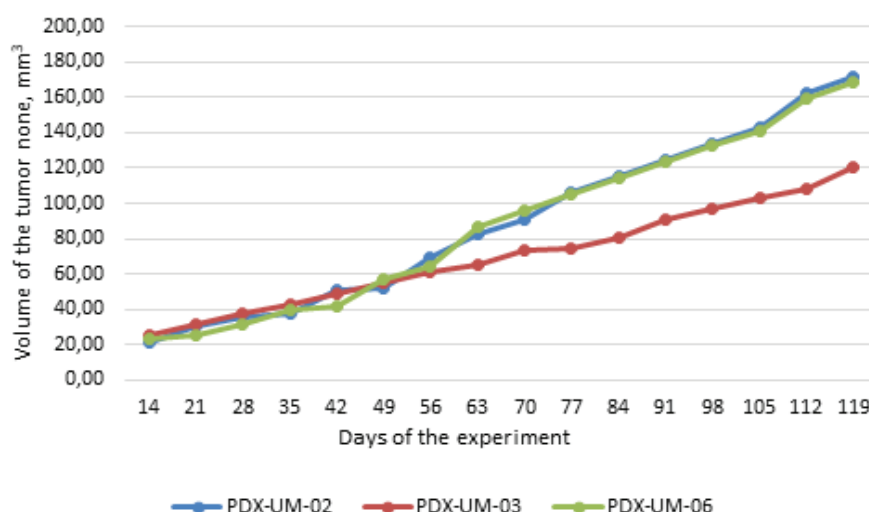


Fig. 2. Dynamics of tumor node growth in 1st generation (passage) PDX models of human uveal melanoma

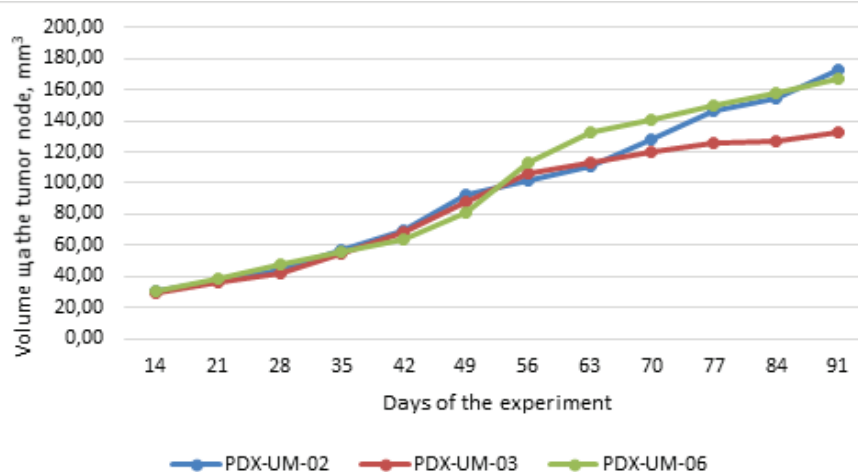


Fig. 3. Dynamics of tumor node growth in 2nd generation (passage) PDX models of human uveal melanoma

A histological examination revealed that xenotransplanted heterotopic (subcutaneous) tumors in mice exhibited a histotype consistent with the corresponding donor tumors. The models obtained consisted of epithelioid cells with a high content of pigment, melanin, and moderate cellular atypia. The preparations also contained mitotic division figures. It is noteworthy that the models retained the same degree of pigmentation as the original tumors from the patients.

The results of our study indicate that the rates of successful engraftment of the first generation tumor were 37.5% (3/8) when implanted into a heterotopic (subcutaneous) site, which is comparable to the results of the work of N émati F. et al., where the efficiency of implantation of human uveal melanoma samples was 28% (25 out of 90); in the work of Heegaard S. et al., the efficiency rates were slightly lower – 13%. Furthermore, histopathological analysis revealed that model samples obtained from these tumors also demonstrated characteristic features of uveal melanoma [7, 9].

In this study, we evaluated the growth dynamics of the obtained PDX models. According to the results presented in Figure 2, it can be concluded that the growth rate of the first-generation xenografts obtained from patients was quite low, with the doubling of the tumor volume occurring in 42 days.

In order to sustain the expansion of the PDX model, tumors obtained from the first generation mice were successively transplanted to the subsequent group of mice. The first generation models obtained were effectively established in the

second generation mice. Furthermore, it was observed that the tumor node growth rate was higher than in the first generation. A twofold increase in tumor volume was observed on day 35. (Figure 3).

A comprehensive analysis of tumor node growth dynamics revealed that the heterotopic PDX models of uveal melanoma, obtained by us, exhibited a relatively low growth rate. This finding suggests that the biological properties of xenotransplanted tumors, specifically their proliferative and tumorigenic potential, may be a contributing factor. Additionally, the probable influence of the microenvironment on the growth of xenogenic tumors must be considered, given that in heterotopic xenotransplantation, samples develop in the subcutaneous site in a microenvironment that is not conducive to uveal melanoma. The aforementioned facts underscore the necessity for further research in this area.

Conclusion. In the course of the research, PDX models of uveal melanoma were obtained. These models can be used in experimental studies in the field of oncology. They can be used to study the fundamental aspects of the pathogenesis of uveal melanoma. They can also be used to create a model of uveal melanoma metastasis. Furthermore, they can be used to evaluate effective tumor drugs.

Conflict of interest: The authors declare that they have no conflict of interest.

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