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Features of extracellular matrix remodeling in human uveal melanoma

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Motivation and Aim: The tumor microenvironment (TME) is a specific heterogeneous environment surrounding the tumor, consisting of blood and lymphatic vessels, stromal and immune cells, progenitor cells, signaling molecules and extracellular matrix (ECM) [1]. The interaction of tumor cells with TME components promotes the activation of signaling pathways aimed at carcinogenesis, evasion of immune surveillance and the development of drug resistance [2].

A key element of the TME is the ECM, an organized three-dimensional network of macromolecules that maintains the integrity of tissues and organs. The architecture of the ECM includes the interstitial matrix and basement membrane. The interstitial matrix mainly consists of fibrillar collagens, fibronectin, elastin, hyaluronic acid and other components [3]. The main source of synthesis of ECM components are activated cancer-associated fibroblasts (CAFs) [4]. The ECM contributes to the formation of a mechanical framework for tumor cells, participates in the regulation of signaling cascades that influence the phenotype of stromal and immune cells, and modulates the processes of metastasis, angiogenesis and proliferation of tumor cells [4].

ECM remodeling is a complex process of degradation and renewal of a network of macromolecules [5], however, the factors secreted by tumor cells and matrix metalloproteinases (MMPs) produced by CAFs can affect the ECM to support the processes of carcinogenesis and the development of drug resistance [5].

Uveal melanoma (UM) is the most common intraocular malignancy arising from choroidal pigment cells. It is known that UM metastases are diagnosed in 50 % of patients, regardless of the type of treatment [6] and are the main cause of death in patients with UM. Despite advances in the development of new therapies for UM, the average survival rate for metastatic disease is approximately 5–10 months [6].

It has been shown that the TME, in particular the ECM, regulates signaling pathways associated with metastasis and the development of resistance to therapy [7]. It is assumed that tumors are capable of forming a unique tumor-specific architectural type of ECM: some types of cancer are characterized by the accumulation of ECM components to regulate the signaling pathways of angiogenesis, growth and proliferation; in other cases, the processes of degradation of ECM components are increased to facilitate invasion and metastasis [7]. For example, UM is characterized by the destruction of collagen types I

and IV under the influence of MMP2, MMP9 and MMP14, secreted by CAFs [8]. Despite advances in studies of UM carcinogenesis, currently the role of individual ECM components in the pathogenesis of UM is not well understood and requires detailed study [9]. Thus, the study of the structural features and processes of reorganization of the ECM can contribute to the development of new approaches to the treatment of UM. The aim of this study was to evaluate the expression of markers associated with ECM remodeling in UM.

Methods and Algorithms: The archived materials were received from 15 patients with uveal melanoma who had undergone curative enucleation in S. Fyodorov Eye Microsurgery Federal State Institution, Novosibirsk, Russia. The study was approved by the local ethics committee of the RICEL – branch of the Institute of Cytology and Genetics, Siberian Branch of the Russian Academy of Sciences (Protocol No. 180 of 04/28/2023).

The formalin-fixed, paraffin-embedded sections from post-equatorial zone of the choroid and intratumoral area of the tumor samples were dewaxed and rehydrated and submitted to heat-induced epitope retrieval (citrate buffer, pH 6.0). Sections were stained using anti-αSMA (Abcam, UK), Collagen I (Invitrogen, USA), FN1 (Elabscience, China), MMP2 (Abcam, UK), MMP14 (Elabscience, China), TIMP2 (Abcam, UK) primary antibodies. The images were captured with an Axio Scope microscope A1 (ZEISS, Germany) using an Axiocam 512 color CCD camera (ZEISS, Germany) and ZEN 2.3 software (ZEISS, Germany) at 40× magnification. Microscopic analysis was carried out at the Multiple-access Center for Microscopy of Biological Subjects (Institute of Cytology and Genetics, Novosibirsk, Russia).

The analysis of digital images of the choroid and tumor (10 fields per patient) was performed using Image J software (Wayne Rasband, USA). Stained choroid and tumor sections were assessed using an immunoreactive scale. Scores were established for the intensity and percentage of positive staining of the cytoplasm. The extent of staining (area) was scored as "0" (< 1 %), "1" (1–25 %), "2" (>25–50 %), "3" (> 50–75 %), or "4" (> 75 %). The staining intensity was scored as "0" (no staining), "1" (weak staining), "2" (moderate staining), or "3" (strongly staining). The weighted scores were calculated by the formula: area × intensity of staining.

The significance of differences between the studied parameters was determined using the Statistica 6.0 software (StatSoft, USA) and the Mann-Whitney U-test (non-parametric statistics) at a 95 % significance level ($p < 0.05$). Data are presented as mean and standard deviation (M±SD).

Results: An analysis of the expression of proteins associated with ECM remodeling and an assessment of the area of $\alpha SMA^{(+)}$ fibroblasts were performed. The area of $\alpha SMA^{(+)}$ staining zones indicating CAFs activity in the tumor was 4 times higher than in the choroid ($p < 0.0005$). Type I collagen expression levels were 76.5 % higher ($p < 0.005$) in the post-equatorial choroid compared to the UM. When assessing the expression of FN (FN1), it was revealed that the expression level of this protein was 4.6 times higher in UM samples ($p < 0.00005$) compared to the choroid. Tumor samples were also characterized by high expression of MMPs and tissue inhibitor of MMPs, compared with the choroid: MMP2 expression by 57 % ($p < 0.05$), MMP14 by 62 % ($p < 0.005$), and TIMP2 2 times ($p < 0.05$).

Conclusion: It is known, that αSMA (alpha smooth muscle actin) represents a key marker of CAFs activation [4]. Furthermore, αSMA can be expressed on the surface of vascular smooth muscle cells; however, in UM, the proliferation of the vascular network occurs

due to the formation of small blood vessels, which are characterized by the absence of smooth muscle cells [6]. In this study, when analyzing postequatorial choroidal samples, $\alpha SMA^{(+)}$ staining was observed predominantly along large blood vessels, while stromal cells were stained in the intratumoral zone of the tumor. Thus, it can be assumed that a high content of $\alpha SMA^{(+)}$ zones in the tumor may indicate high functional activity of CAFs. ECM remodeling occurs primarily through the regulation of the production of MMPs by CAFs. Tissue inhibitors of matrix metalloproteinases (TIMPs) regulate the activity of MMPs by forming non-covalent complexes, thereby limiting tissue destruction. It has been shown that the formation of the MMP14-MMP2-TIMP2 complex activates MMP2 and stimulates the degradation of collagen fibers and ECM remodeling to enhance the processes of angiogenesis and tumor metastasis [10]. The studied tumor samples were characterized by high levels of expression of proteins associated with ECM degradation – fibronectin, MMP14, MMP2 and TIMP2. Thus, the results obtained may indicate an active process of ECM remodeling, which may affect the invasive ability and metastasis of tumor cells and requires further study.

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