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CELLULAR AND MOLECULAR MECHANISMS OF ANGIOGENESIS IN TUMORS OF THE NERVOUS AND SENSORY SYSTEMS

Research article

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Abstract

Relevance. Despite the known angiogenic receptors and factors activated in tumors, stimulating angiogenesis signalling pathways by suppressing tumor suppressor genes in various organs, this issue remains the least studied in malignant structures of the human eye [3], [10]. Expression of VEGF and CD34 can serve as an important prognostic biomarker in the outcome of brain and sensory system tumors, as well as in the development of an angiogenic targeted treatment strategy [10], [16]. High mortality in patients with tumors of the nervous and sensory systems dictates the direction of research on the study of key targets to increase the effectiveness of targeted antiangiogenic therapy that reduces tumor growth [4], [5], which served as our choice of the direction of research.

Objective: to establish the features of localization and the level of expression of VEGFA and CD34-positive cells in human brain and choroid tumors to develop targeted conservative treatment with a controlled outcome prognosis.

Materials and methods: using the immunohistochemical method to identify the level of expression of VEGFA and CD34, 41 biopsies of brain tumors and 15 biopsies of human eye tumors were studied.

Results of the study: it was found that against the background of angiogenesis in brain and ciliary body tumors of the human eye, some vessels are subject to calcification and obliteration of the lumen with the formation of psammoma bodies. Identification of psammomas in the stroma of the ciliary body and in the vitreous body indicates the long history of the malignancy process.

VEGFA positivity is highly expressed in the non-pigmented epithelium of the ciliary body processes and brain tumors. CD34 marker assessed angiogenesis against the background of developing malignant tumors.

Conclusion: the absence of pigment in the epithelium of the ciliary body processes and the localization of high expression of angiogenic factors in the vitreous body indicate that the malignancy process in the choroid corresponds to embryonic histogenesis. Targeted antiangiogenic therapy using angiogenesis inhibitors can be directed to the stroma of the ciliary processes and to the vitreous body in accordance with the localization of the tumor. The level of expression of VEGFA and CD34 positive cells correlates with destructive processes in tumors and surrounding tissues.

Keywords: organ of vision, ciliary body, brain, malignancy, psammoma bodies, calcification, apoptosis, angiogenesis, hypoxia, blood vessels, ischemia.

КЛЕТОЧНЫЕ И МОЛЕКУЛЯРНЫЕ МЕХАНИЗМЫ АНГИОГЕНЕЗА В ОПУХОЛЯХ НЕРВНОЙ И СЕНСОРНОЙ СИСТЕМ

Научная статья

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Аннотация

Актуальность. Несмотря на известные ангиогенные рецепторы и факторы, активирующиеся в опухолях, стимулирующие сигнальные пути ангиогенеза подавлением генов-супрессоров опухолей в различных органах, этот вопрос остается наименее изученным в малигнизирующихся структурах глаза человека [3], [10]. Экспрессия VEGF и CD34 может служить важным прогностическим биомаркером в исходе опухолей мозга и сенсорных систем, а также при разработке стратегии ангиогенного таргетного лечения [10], [16]. Высокая смертность пациентов с опухолями нервной и сенсорных систем диктуют направление исследований на изучение ключевых мишеней для повышения эффективности таргетной антиангиогенной терапии, снижающей рост опухоли [4], [5], что послужило для выбора нами направления исследований.

Цель исследования: установить особенности локализации и уровень экспрессии VEGFA и CD34 – позитивных клеток в опухолях мозга и сосудистой оболочки глаза человека для разработки таргетного консервативного лечения с контролируемым прогнозом исхода.

Материалы и методы: с помощью иммуногистохимического метода на выявление уровня экспрессии VEGFA и CD34 изучены 41 биоптат опухолей мозга и 15 биоптатов опухолей глаза человека.

Результаты исследования: установлено, что на фоне ангиогенеза в опухолях мозга и цилиарного тела глаза человека часть сосудов подвержена кальцификации и облитерации просвета с образованием псаммозных телец. Идентификация псаммом в строме цилиарного тела и в стекловидном теле свидетельствует о давности развития процесса малигнизации.

VEGFA позитивность экспрессируется в высокой степени в беспигментном эпителии отростков цилиарного тела и опухоли мозга. CD34 маркером оценён ангиогенез на фоне развивающихся злокачественных опухолей.

Заключение: отсутствие пигмента в эпителии отростков цилиарного тела и локализация высокой экспрессии ангиогенных факторов в стекловидном теле свидетельствуют о соответствии процесса малигнизации в сосудистой оболочке глаза эмбриональному гистогенезу. Таргетная антиангиогенная терапия с помощью ингибиторов ангиогенеза может быть направлена на строму цилиарных отростков и в стекловидное тело в соответствии с локализацией опухоли. Уровень экспрессии VEGFA и CD34 позитивных клеток коррелирует с деструктивными процессами в опухолях и окружающих опухоли тканях.

Ключевые слова: орган зрения, цилиарное тело, мозг, малигнизация, псаммозные тельца, кальцификация, апоптоз, ангиогенез, гипоксия, кровеносные сосуды, ишемия.

Introduction

In tumor histogenesis, the level of aggressiveness and the tendency to metastasize are affected by angiogenesis, which develops under the influence of increasing hypoxia [2], [12], which has been known for more than a century since Rudolf Virchow obtained evidence of the proliferation of blood vessels in a developing tumor and its dependence on angiogenesis, which is induced by the tumor cells themselves [9], [13]. Angiogenesis is a complex process, accompanied, according to Ahir B.K., Engelhard H.H., Lakka S.S. (2020), by proliferation, migration and differentiation of endothelial cells under the influence of specific signalling molecules with the control of inducers and inhibitors [1], which has been studied to a greater extent in experimental animals and requires in-depth study on human material. This fact served as a basis for our choice of research direction.

Material and methods

A total of 41 biopsies of primary brain tumors and postoperative material from the removal of 15 melanomas of the ciliary body of the eye of patients of different ages and sexes were studied. Sections of 5 µm thickness were made from paraffin blocks, followed by staining with hematoxylin and eosin and examined using the immunohistochemical method to identify the localization and level of expression of VEGFA and CD34.

The relationship between the microvessel density (MVD), the expression of the vascular endothelial growth factor (VEGF) and histopathology in primary tumors of the brain and ciliary body was compared. The removed tumors were examined for MVD using immunostaining with CD34 antibodies, as well as VEGF expression, assessed using immunostaining with VEGF antibodies.

Microscopic analysis of the preparations was performed on an Olympus Bx52 microscope. Histology slide images were acquired using CellSens proprietary imaging software (Olympus Life Science, Tokyo, Japan), and digital slides were prepared using Aperio Scan Scope (Leica Biosystems Inc., Buffalo Grove, IL).

Research results and their discussion

A study of sections of human eye tumor biopsies showed the presence of psammosis bodies of various shapes and sizes in the stromal tissue of the ciliary body of the human eye, identical to calcifications in brain tumors (Fig. 1).

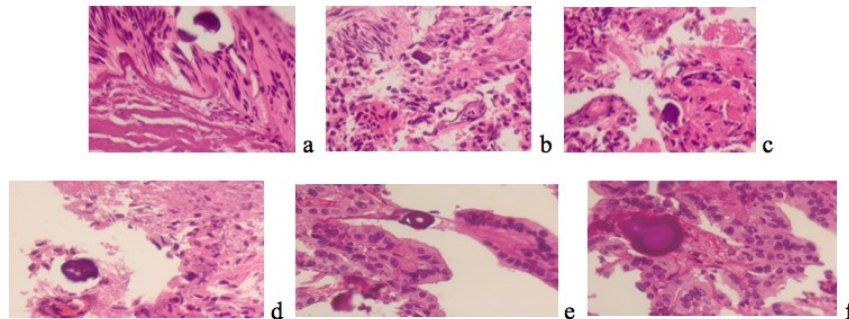


Figure 1 - Processes of the ciliary body of the human eye. Psammosis bodies. Hematoxylin and eosin staining
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Note: microphoto. Magnification X400

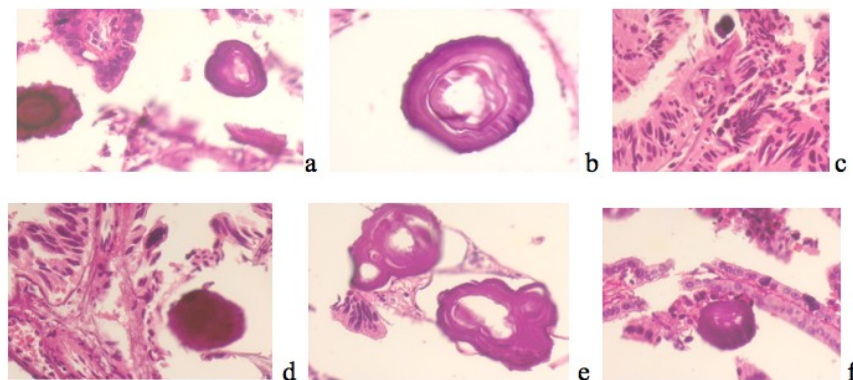


Figure 2 - Psammosis bodies identified in the ciliary body, vitreous body of patients with ocular melanoma. Psammosis bodies. Hematoxylin and eosin staining
DOI: <https://doi.org/10.60797/IRJ.2025.153.106.2>

Note: microphoto. Magnification X400

The psammosis bodies identified in the ciliary body structure have different sizes, shapes and densities when stained on cross-sections. In addition, psammosis bodies were identified in the vitreous body near the ciliary body processes (Fig. 2).

We have established that some bodies had unstained lumens in the central part, a round or oval shape, a layered structure, compacted areas in the wall of psammous bodies and sizes from 5 to 15 μm . Analysis of the obtained material allowed us to note both complete obliteration of blood vessels and the preservation of a small lumen inside the vessel, as a result of incomplete calcification. The process of vascular calcification and the absence of vascular endothelium on the inner surface were previously called vasculogenic mimicry – the ability of tumor cells to form vascular-like channels limited by the basal membrane without the participation of endothelial cells and fibroblasts. Vasculogenic mimicry (VM) was first discovered by R. Folberg et al. (1992) in the pathohistological material of patients with choroidal melanoma and was subsequently confirmed in vitro during the cultivation of tumor cells on extracellular matrices [6]. The development of vasculogenic mimicry is a process of vascular calcification with the formation of psammous bodies (PB), a complex biological process involving several signalling pathways. The fact that VM-PB is found in various types of aggressive tumors (in melanoma of the ciliary body and brain tumors) indicates that this process reflects the characteristics of tumor aggressiveness. More and more experimental data

are accumulating indicating a high statistical correlation between the presence of PB and the ability of the tumor to metastasize [8]. The presence of unchanged blood vessels with a typical structure for arterioles located near PB suggests that calcification primarily affects the vessels of the ciliary body outflow system (Fig. 3).

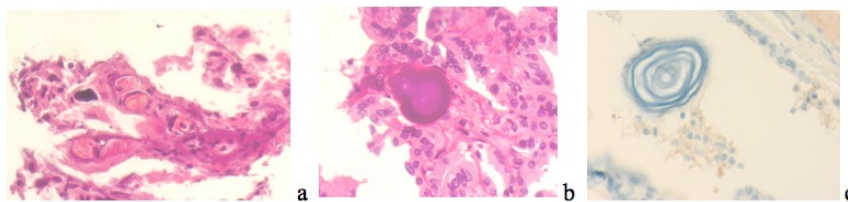


Figure 3 - Psammoma bodies in the ciliary body processes of the human eye

DOI: <https://doi.org/10.60797/IRJ.2025.153.106.3>

Note: a, b – hematoxylin and eosin staining; c – immunohistochemistry to detect VEGFA positive expression.

Microphoto. Magnification X400

The appearance of blood vessels in the vitreous body of the human eye additionally confirms the development of malignancy with a return to histogenesis according to the embryonic type, in accordance with the stage of development of the vascular vitreous body of the human eye. This corresponds not only to the timing of the formation of the hyaloid pool, but also to its involution with metabolic disorders. Identification of psammoma bodies in the vitreous body of the human eye at the stage of partial and complete calcification of the wall with closure of the lumen of the vessel indicates the duration of the disease and the high degree of aggressiveness of the developing tumor, prone to distant metastasis. Vascularization of the tumor and its environment occurs through several different biological processes that not only vary depending on the tumor type and anatomical location, but also occur simultaneously in the same malignant tissue. These processes are organized by a number of secreted factors and signalling pathways and may include the participation of non-endothelial cells, such as progenitor cells or cancer stem cells. Antiangiogenic therapy using antibodies or tyrosine kinase inhibitors has been used to treat several types of cancer. However, the benefit of treatment has so far been modest, with low sensitivity to current drugs, and most patients developing resistance [11]. It is becoming increasingly clear that blocking tumor access to the bloodstream is not an easy task. Tumor vascular functionality and gene expression often vary greatly between different cancer subtypes, and vascular phenotype can be markedly heterogeneous within a single tumor. Understanding the cellular and molecular mechanisms involved in tumor angiogenesis requires addressing the issue of developing morphological criteria with targeted antiangiogenic action [3]. We found localization of high VEGFA positive expression in epithelial cells, predominantly on the surface of the apical part of the ciliary processes (Fig. 4).

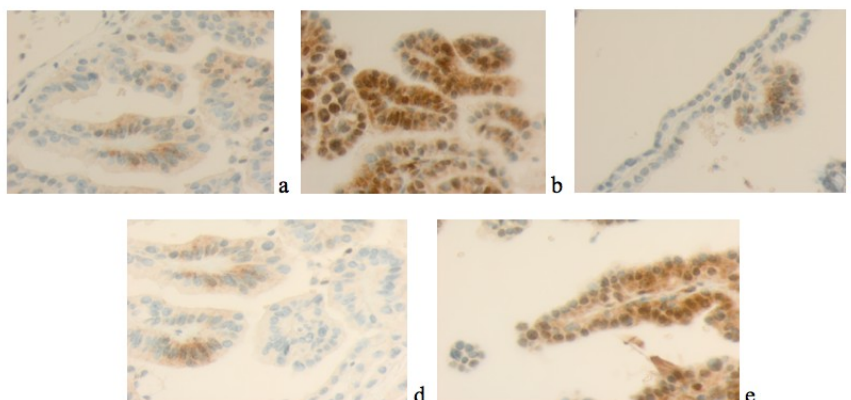


Figure 4 - Processes of the ciliary body of the human eye. Immunohistochemistry. Localization of VEGFA positive expression

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Note: microphoto. Magnification X400

We have noted that cells with VEGFA positive expression exhibit higher activity in the vitreous body surrounded by obliterated calcifying vessels, which indicates the participation of hyalocytes in the induction of tumor angiogenesis. Analysis

of the localization of CD34-positive expression showed that it is identified predominantly in the endothelial cells of the blood vessels of the stroma of the ciliary body processes (Fig. 5).

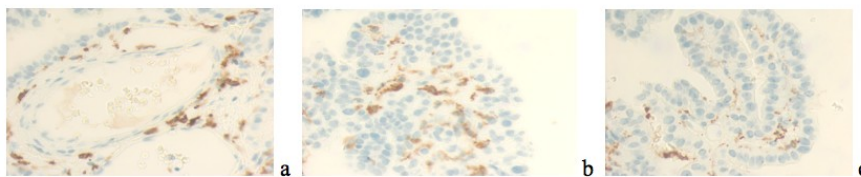


Figure 5 - Ciliary body processes of the human eye. Immunohistochemistry. Localization of CD34 positive expression
DOI: <https://doi.org/10.60797/IRJ.2025.153.106.5>

Note: microphoto. Magnification X400

In the samples we examined, a complete absence of pigment epithelium was noted, which also indicates not only its important role in the normal physiology of the human eye, but also its damage during malignancy in the structures of the ciliary body. A practical study by Mera M., Bárzu M. (1995) [14] of 20 cases of uveal malignant melanoma (UMM), with an analysis of the histological aspects and the shape of the tumor vessels in accordance with the classification of Callender, modified by MacLean et al. in 1983, as well as Folberg (1919, 2000) [7], [8], showed a correlation between the histopathological type of uveal melanoma and vascular aspects. The authors reported that there is a correlation between the histopathological type of UMM and the vascularization aspect. Folberg et al. (1992) reported that in spindle cell UMM parallel or normal vessels predominate; in epithelioid cell UMM arcuate or ring-shaped vessels predominate; and in mixed UMM parallel, ring-shaped and arcuate vessels and networks were noted. Our studies are consistent with the data of Mera M., Bárzu M. (1995) and Folberg et al. (1992), but add to these data the absence of pigment epithelium on the surface of the ciliary processes due to either its death or depigmentation.

Conclusion

The absence of pigment in the epithelium of the ciliary body processes and the localization of high expression of angiogenic factors in the vitreous body around the ciliary body processes, in the ciliary epithelium, and not only in the vascular endothelium, indicate that the malignancy process in the choroid of the human eye corresponds to embryonic histogenesis using signalling pathways of early human ontogenesis. Targeted antiangiogenic therapy using angiogenesis inhibitors can be directed not only to the stroma of the ciliary processes, but also directly to the vitreous body of the human eye in the anterior right and left sectors of the eye and in accordance with the localization of the tumor. The level of expression of VEGFA and CD34 positive cells correlates with destructive processes in tumors and surrounding tissues. Analysis of the identified features of localization of CD34 and VEGFA positive expression, molecular mediators that regulate angiogenesis in malignant tissues of the brain and ciliary body of the human eye, contributes to the disclosure of the mechanisms of tumor angiogenesis and dictates the direction of the vector of clinical studies for the development of a strategy for the conservative treatment of glioblastomas and ciliary body tumors using angiogenic pathways and corresponding signaling molecules.

Конфликт интересов

Не указан.

Рецензия

Все статьи проходят рецензирование. Но рецензент или автор статьи предпочли не публиковать рецензию к этой статье в открытом доступе. Рецензия может быть предоставлена компетентным органам по запросу.

Conflict of Interest

None declared.

Review

All articles are peer-reviewed. But the reviewer or the author of the article chose not to publish a review of this article in the public domain. The review can be provided to the competent authorities upon request.

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