THE INFLUENCE OF GLIOBLASTOMA STEM CELLS AND MICROENVIRONMENT ON TUMOR GROWTH AND MALIGNANCY: A LITERATURE REVIEW

Ana Clara P. Ribeiro,[1] Victor Andrade Batistella,[2] Fernando Arfux Maluf,[3] Carlos Eduardo Coral de Oliveira[4]

ABSTRACT
Glioblastoma is the most malignant and common primary brain tumor, with a median survival rate of less than 15 months. Researchers think the key to understanding its complexity involves the glioblastoma stem cells. Important characteristics of them are plasticity and the quiescent state, which enables them to evade chemotherapy and to hide from the immune system. Besides that, they are notable for their interaction with the microenvironment and for modulating the expression of genes and substances around it. Possible therapeutic targets being explored involve: monoclonal antibodies, normoxia inducing mechanisms, modified natural killer cells, immuno-viral therapy. There is an intimate relationship between glioblastoma stem cells, microenvironment and the tumorigenesis and resistance processes, we have explored the main development axes of this tumor and some novel therapies. Finally, our findings led us to think it would be necessary to have a multifactorial approach, aiming for more than one therapeutic target.

Keywords: Glioblastoma; Tumor microenvironment; Stem cells.

A INFLUÊNCIA DAS CÉLULAS TRONCO DE GLIOBLASTOMA E DO MICROEAMBIENTE SOBRE O CRESCIMENTO TUMORAL E MALIGNIDADE: UMA REVISÃO DE LITERATURA

RESUMO
O glioblastoma é o tumor cerebral primário mais comum e maligno, com uma sobrevida média de menos de 15 meses. Pesquisadores acreditam que a chave para entender sua complexidade envolve as células-tronco do glioblastoma. Características importantes são a plasticidade e o estado de repouso, o que permite o escape da quimioterapia e camuflagem do sistema imunológico. Além disso, eles se destacam por sua interação com o microambiente e por modular a expressão de genes e substâncias ao seu redor. Os possíveis alvos terapêuticos a serem explorados envolvem: anticorpos monoclonais, mecanismos de indução de normóxia, células *natural killer* modificadas e terapia imuno-viral. Existe uma íntima relação entre as células-tronco do glioblastoma, o microambiente e os processos de tumorigênese e resistência, exploramos os principais eixos de desenvolvimento desse tumor e algumas novas terapias. Por fim, nossos achados nos levaram a pensar que seria necessária uma abordagem multifatorial, visando mais de um alvo terapêutico.

Palavras-chave: Glioblastoma. Microambiente tumoral. Células-tronco.

INTRODUCTION

Glioblastoma is the most malignant and common of all primary malignant central nervous system (CNS) tumors[1, 2, 3]. Counting up to 45,2% of malignant primary brain and central nervous system tumors and 54% of all glioma types[4]. A median survival of 15 months and a relative survival of only 13.7% at 2 years[4].

[1] Acadêmica de medicina no sexto período na Pontifícia Universidade Católica do Paraná (PUCPR), anaclara.pinesso@gmail.com
[2] Acadêmico de medicina do oitavo período na Pontifícia Universidade Católica do Paraná (PUCPR), vicbatistella@gmail.com
[3] Acadêmico de medicina do oitavo período na Pontifícia Universidade Católica do Paraná (PUCPR), fernandoarfuxmaluf@gmail.com
[4] Professor Adjunto da Pontifícia Universidade Católica do Paraná (PUCPR) no curso de medicina, : carlos.coral@pucpr.br

Like other tumors, the excessive proliferation, necrosis and hypoxia generate inflammatory signals which activate an immune response[5]. Although most of the mechanisms related to cancer cells survival are still unknown, cancer stem cells could be one of the reasons[5].

Moreover, glioblastoma tends to be treatment resistant, which is noted especially in cancer cells with stem cell like properties, suggesting an importance in the understanding of those properties in the mechanisms related to the proliferation and maintenance of glioblastoma[6]. The glioblastoma stem cells (GSCs) play an important role in the standard chemotherapy resistance as well as in radiotherapy, since they are related to the start, progression and remission of the disease[7].

The clinical relevance of the glioblastoma is attributed to its high incidence and poor survival rate - only 5% of those diagnosed live for more than 5 years, and the median survival is less than 15 months[2, 8]. The aim of this review is to clarify features of the glioblastoma, including its complexity, and the influence of the tumor stem cells in the genesis and modulation of the tumor malignancy, as well as to explore new therapy targets and possible treatments.

MATERIALS AND METHODS

This review was conducted through database search at PubMed, using the keywords: "glioblastoma"; "tumor microenvironment"; "stem cells". Inclusion criteria were articles published in English from 2014 to 2019, with humans or human tumor cells, excluding those with obvious irrelevance after reading of the abstracts and those without “glioma” nor “glioblastoma” on the title, coming to twenty six (26) articles.

RESULTS AND DISCUSSION

GLIOBLASTOMA BIOLOGICAL FEATURES

Glioblastoma is characterized by extensive hypoxia, angiogenesis, proliferation and invasion[9]. It features a complex cell heterogeneity, and one of the aspects that makes it difficult to be studied is the constant change in its composition[9]. The tumour cells have their own dynamic functional properties, and express different phenotypes according to the conditions of its microenvironment[10]. The microenvironment on its own also changes constantly[10].

In this review, the most important cellular component found are the GSCs, which are thought to be capable of self-renewal, high proliferation and tumorigenesis[7]. Also, these cells are considered to be responsible for disease remission, since they can remain quiescent (a static state of non-proliferation that protects them from radio and chemotherapy) for long periods of time. The information about how these cells enter in this state is limited, however, it is known that they are influenced by intrinsic and extrinsic factors. Both plasticity and quiescence of the GCSs' are pointed as good answers for the low efficacy of therapies, even though some characteristics of these processes remain a mystery[6,11].

STEM CELLS IN GLIOBLASTOMA

Stem cells are able to self-renew and differentiate, cancer stem cells have the same characteristics plus deleterious behaviour and the ability to originate a population of oncogenic cells[12]. Some characteristics of glioblastoma can be attributed to its stem cells, which first prospective isolation of human GSCs was performed using CD133[7].

Plasticity allows the GSCs to adopt different phenotypes, according to the microenvironment they are located. Therefore, discovering key factors that play an important role on the different microenvironments would represent a great discovery to the understanding of the tumorigenesis process and about how the resistance to conventional therapies work[8].

The characteristic resistance to treatment in Glioblastoma, along with its metastatic tendency could be, in part, a consequence of the interaction with the Stem Cells composing it's microenvironment[13]. That is also the case with tumor growth and recurrence[8]. Accordingly, these types of cells are found in highly proliferating tumor areas, such as perivascular or peri-hypoxic niches[1].

An example of the relevance of the stem cells in treatment resistance is the increase in the chemotherapy and radiotherapy resistance after initial treatment, plausibly being a consequence of the increase in the stem cells population[12]. The presence of the cells is thought to lead to resistance through activation of cell cycle checkpoint pathways, enhancement of DNA repair and aberrant cell survival mechanisms[12].

Besides the intimate relation between GSCs and the microenvironment, it is possible to identify six main mechanism that act on the regulation of these cells, which include intrinsic factors such as genetics, epigenetics, and metabolism, as well as extrinsic qualities of niche factors, cellular microenvironment, and the host immune system[7]. Regarding the genetic

mechanism, there are mutations and deletions in many areas, such as: EGFR, IDHI, PDGFRA, HDM2, PIK3CA. Altered tumor promoter and suppressor genes are also observed: promoters TERT and PI3KRI; suppressors PTEN, TP53, CDKN2A, NF1, ATRX and RB1[7]. Regarding metabolism, it is of high importance the different oxygen levels in which the cells from the niche are found[7]. The niche is believed to play an important role in tumorigenesis, since the initial tumor cells are originated there and from that point they get established and start to regulate things around it, acquiring characteristics of a cancer cell, like apoptosis evasion, unlimited replication and others[7]. Tumors in general can cause immunosuppression, this way they are able to evade human body defenses, as well as they can "recruit" immune cells to work in their favor[7].

Moreover, cells expressing the CD133 biomarker, one of the key biomarkers of stem cells, are associated with 10-20 fold higher levels of vascular endothelial growth factor (VEGF) in comparison to cells not expressing CD133[12]. The effect is that VEGF increases endothelial cell migration, proliferation and tube formation, contributing to angiogenesis similarly to hypoxia, which stimulates VEGF and stromal cell-derived factor 1 (SDF-1) production[12].

GLIOBLASTOMA MICROENVIRONMENT

GSCs reside in niches, which are specific microenvironments that protect them from radiotherapy and chemotherapy[14]. Besides GSCs, other cells are found there, like endothelial cells, microglia, pericytes and astrocytes[6]. Recent studies have shown an intimate relation between the glioblastoma cells - specially stem cells - and its microenvironment as a cross-talk, in which both parts contribute positively to their survival and, in consequence, to the tumor development[11, 12]. The influence, sometimes through autocrine or paracrine stimulation, varies according to the different niches of the tumor, which can be divided in perivascular niche, periarteriolar niche, peri-hypoxic niche, peri-immune niche and extracellular matrix niches[14, 15].

The perivascular niche's most important aspect is the interaction between the endothelial cells (ECs) and the glioblastoma stem cells[15]. This relation can enhance its malignancy and promote GSC's survival, through EC's secretion of transforming growth factor-β (TGF-β) and platelet derived growth factor (PDGF), which stimulate the expression of stemness-related genes[14]. This interaction is a crosstalk, and the GSCs also stimulate the ECs, promoting angiogenesis, through the expression of VEGF and other pro angiogenic

substances[15]. Another important aspect is the influence of this process in the pericytes proliferation, since some studies show that tumor vessels covered with less pericytes are more sensitive to radiation and chemotherapy[14, 15].

The periarteriolar niche seems strange at first, because hypoxia is a hallmark of glioblastoma and arterioles are rich in oxygen, however this can be in behalf of the fact that the arterioles are transport vessels, not exchange vessels like capillaries, which makes the existence of hypoxic areas near the arterioles possible[17, 16]. More studies are needed to clarify the role of this niche in the tumour, but it's suggested that substances of this niche like stromal-derived factor-1α (SDF-1α) and osteopontin (OPN) are associated with homing of GSC's in niches *via* interactions with the GSCs receptors C-X-C receptor type 4 (CXCR4) and CD44, and that cathepsin K (CatK) plays a significant role in migration of GSCs out of niches[14, 15].

The peri-hypoxic niche is perhaps the most important of them. Hypoxia is an essential factor to the development of glioblastoma, studies have shown that the exposure of glioblastoma cells to a hypoxic environment induces the expression of SOX2, OCT4 and CD133, which are markers of GSCs and indicate dedifferentiation of tumour cells to GSCs[15]. This niche not only increased the expression of stem cell markers, but also the expression of hypoxia inducible factors (HIFs), which induce the production of proangiogenic growth factors, justifying the angiogenesis[15, 17, 18, 19]. HIF-1α and HIF-2α help in the maintenance of the stemness on GSCs, expression of phenotypes, tumour survival and activates a multidrug resistance-1 (MDR-1) gene[14, 19]. HIF-1α is present in non-cancerous cells, but HIF-2α is specifically expressed by GSCs, so it can be an important biomarker for future treatments[14]. The low levels of oxygen attenuate the expression of mismatched DNA repair genes and inhibits the free radicals produced by radiation treatment, which increases resistance[14, 18].

Another aspect of this niche is the acidosis - a result of the altered metabolism of glioblastoma cells -, which upregulates the production of HIFs and may help the activity of cysteine cathepsins (e.g., catK and catL)[14]. The Hypoxia stress-induced chaperone protein (HSP90) expressed in the acidic microenvironment has an important role on HIF production, and the pharmacological or genetic inactivation of this protein could inhibit HIF function, therefore reducing tumorigenic and self-renewal characteristics of the tumor[15].

The peri-immune niche major cell is CD11b-positive tumor-associated macrophages (TAMs), they're divided in M1-type TAMs and M2-type TAMs. The M1-type TAMs are pro-inflammatory, therefore anti-cancer, whereas the M2-type TAMs are anti-inflammatory, thus

pro-cancer[15]. GSCs are able to differentiate to M2-type TAMs and TAMs can differentiate into ECs, which secretes interleukin-6 (IL-6)[15]. IL-6 attracts and activates TAM via HIF-2α-mediated arginase-1 expression and contributes to glioblastoma progression[15]. TAMs are also responsible for GSC invasion through high levels of TGF-β1[15]. The amount of TAMs present on the peri-immune niche is correlated to tumour progression, and inversely correlated to patient survival[15].

The extracellular matrix (ECM) niche is related to perivascular and periarteriolar niches[15]. It is constantly changing due to GSCs secretion of its own ECM, such as fibronectin and type 1 collagen[15]. Some proteins from the ECM, like laminins and tenascin-C can affect angiogenesis in other niches[15, 20]. The importance of this niche in glioblastoma progression is not totally understood, but its suggested that its properties regulate the migratory response in GSC[21].

The GSCs interact with the microenvironment through the expression of cell adhesion molecules (CAMs), which participate on the intra and extracellular communication, and also communicate with the extracellular matrix[6]. Although some evidence says CAMs would contribute to tumor suppression, an article found different results that suggest CAMs could even accelerate tumoral development due to its contribution to the interaction between microenvironment and GSCs[6]. Therefore, it is said that these molecules could be therapeutic targets, even though this proposal goes against the classic paradigm that says CAMs are immunosuppressants[6].

Monoamine neurotransmitters (dopamine, serotonin, norepinephrine) were identified as contributors to the composition of the microenvironment, once they can influence the behavior of the GSCs. The study that made this statement suggests that tumoral cells would be the responsible to produce monoamines as a way to produce an autocrine stimulation to control the GSCs[8].

PERSPECTIVES IN GLIOBLASTOMA TREATMENT

Since stem cells play such important roles in the malignancy of the Glioblastoma, new therapy possibilities have arised as alternatives to the currently ineffective therapy, which is multimodal, including surgery, postoperative radiotherapy (RT) and temozolomide (TMZ)-based concomitant and adjuvant chemotherapy (CT)[1].

Many factors contribute to the therapeutic resistance in this type of tumor, some of them are: intratumoral heterogeneity, tumor invasiveness, microenvironment, blood–brain

tumor barrier and limited drug delivery, and immune suppression. Besides that, the heterogeneous characteristic of the GSCs allows them to easily avoid hostile environments and chemotherapy drugs. The quiescent state of these cells makes them able to hide, not being targeted by the therapeutic medications. As a consequence, the remission of the disease can happen at any moment without any advice or remaining trace[22].

Monoclonal antibodies targeting GSCs directly are not yet available, but the already existing ones can have its efficacy attributed to the repercussions in the stem cells[23]. That's the case of bevacizumab, which targets VEGF, inhibiting the tumor growth. One possibility is that this inhibition occurs through the disruption of the perivascular niche, where GSC are found[23].

Moreover, using the CD133 as a target might represent a compelling form of treatment, since it is the main biomarker in GSC[12]. Carbon nanotubes conjugated to a monoclonal antibody anti-CD133 could select the stem cells, and through irradiation with near-infrared laser light, kill the cells by photothermolysis[12].

A group of researchers noticed that GSCs exposed to normoxia would be more vulnerable to the neoadjuvant therapy with TMZ and cisplatin than to the same treatment but with hypoxia[1]. This fact is directly related to the action of this microenvironment over the GSCs, once they noticed a larger expression of CD133 while in hypoxia, as commented earlier[1]. However, normoxia induction mechanisms that can be used as therapeutic measures are still a hard obstacle for medicine.

Tumors have a variety of mechanisms for apoptosis evasion, also, they can escape from the immune system defenses, with this in mind, researchers proposed a therapy with natural killer (NK) cells modified to express TGF-β-dominant-negative receptor II (DNRII), since the TGF-β is constantly produced by the tumor cells to slow down and sabotage the NK cells function[4].

It was discovered that the family of proteins DHHC (Asp-His-His-Cys) is intimately related to the plasticity regulation on the conversion of the cellular state of the GSCs[24]. Therefore, this enzyme is pointed as a possible molecular target for the treatment of patients with glioma[24].

Disulfiram and Carbenoxolone are two substances presented in a study with high expectations for the treatment of patients with glioma, since both interfere in important pathways used by the GSCs[25]. Disulfiram prevents the formation of communicating junctions (gap junction), which are important for the apoptosis evasion[25]. Furthermore, this substance

might target mainly in invasive cells, since they are very dependent on this type of communication. Whereas Carbenoxolone acts on the modulation of NF-κB signaling, which interferes on the cell-substrate adhesion[25].

An interesting novelty to reverse the immunosuppression caused by the tumor would be the use of oncolytic viruses, so that it is possible to induce an effective immune response on the body[22]. This strategy uses the introduction of the herpes simplex virus on the person to induce the recruitment of TCD4+ and TCD8+ lymphocytes, and macrophages[22]. This immuno-viral therapy generates a natural inflammatory response, causing a safe antitumor immunity, the virus can be molecularly modified to produce a controlled and effective immunomodulation[22]. At the moment, nine new types of modified herpes virus are being tested on clinical trials and five of those are for glioblastoma treatment[22]. Recently, the treatment with a type of herpes virus, *talimogene laherparepvec*, was approved for melanoma in the United States of America (USA)[22].

Considering a diagnosed cancer, it is possible to apply vaccines as a therapy. Here, the objective is not prophylaxis, but inducing an effective immune response on the host, which is likely immunosuppressed[26]. Many researchers tested the possibilities, as follows, multipeptide vaccines, personalized peptide vaccines, dendritic cell-based and immuno-viral therapy[26].

CONCLUSION

Analyzing the available literature allowed us to confirm the intimate relationship between GSCs, microenvironment and the tumorigenesis and resistance processes, as well as their interaction with the metabolic, genetic, epigenetic and niche factors, and with the immune system. These are the main axes involved in the development of this tumor, that work together in a very complex correlation to generate high heterogeneity and resistance.

The knowledge of these key factors of glioblastoma, brings to light multiple pathways that could be the target for future therapeutic approaches. Facing the heterogeneity of the tumor, we believe it is necessary to have a multifactorial approach, aiming for more than one tumoral development axis - since they are all connected and could possibly get balanced to avoid unimodal therapeutic approaches.

ACKNOWLEDGEMENTS

The authors thank Prof. Dr. Carlos Eduardo Coral de Oliveira for his support and dazzling advices, which were crucial to the writing of this article. Furthermore, to acknowledge all the

authors who contributed to the current knowledge of the Glioblastoma characteristics, without whom this article wouldn't have been possible.

DECLARATION OF INTEREST STATEMENT

The authors declare no conflicts of interest.

REFERENCES

1. Ahmed EM, Bandopadhyay G, Coyle B, Grabdowska A. A HIF-independent, CD133-mediated mechanism of cisplatin resistance in glioblastoma cells. Cellular Oncology. 2018; 41(3): 319-328. doi: 10.1007/s13402-018-0374-8

2. Gimple RC, Bhargava S, Dixit D, Rich JN. Glioblastoma stem cells: lessons from the tumor hierarchy in a lethal cancer. Genes & Development. 2019;33(11-12):591–609. doi: 10.1101/gad.324301.119.

3. Iu YC, Lee IC, Chen PY. Biomimetic brain tumor niche regulates glioblastoma cells towards a cancer stem cell phenotype. Journal Of Neuro-Oncology. 2018; 137(3):511-522. doi: 10.1007/s11060-018-2763-2.

4. Yvon ES, et al. Cord blood natural killer cells expressing a dominant negative TGF-β receptor: Implications for adoptive immunotherapy for glioblastoma. Cytotherapy. 2017;19(3):408-418. doi: 10.1016/j.jcyt.2016.12.005.

5. Alvarado AG, Thiagarajan PS, Mulkearns-Hubert EE, Silver DJ, Hale JS, Alban TJ, et al. Glioblastoma Cancer Stem Cells Evade Innate Immune Suppression of Self-Renewal through Reduced TLR4 Expression. Cell Stem Cell. 2017;20(4). doi: 10.1016/j.stem.2016.12.001.

6. Turaga SM, Lathia JD. Adhering towards tumorigenicity: altered adhesion mechanisms in glioblastoma cancer stem cells. Cns Oncology. 2016;5(4):251-259. doi: 10.2217/cns-2016-0015.

7. Lathia JD, Mack SC, Mulkearns-Hubert EE, Valentim CLL, Rich JN. Cancer stem cells in glioblastoma. Genes & Development.2015;29(12):1203-1217. doi: 10.1101/gad.261982.115

8. Caragher SP, *et al.* Activation of Dopamine Receptor 2 Prompts Transcriptomic and Metabolic Plasticity in Glioblastoma. J Neurosci. 2019;39(11): 1982–1993. doi: 10.1523/JNEUROSCI.1589-18.2018

9. Dirkse A, Golebiewska A, Buder T, Nazarov PV, Muller A, Poovathingal S, et al. Stem cell-associated heterogeneity in Glioblastoma results from intrinsic tumor plasticity shaped by the microenvironment. Nature Communications. 2019;10(1). doi: 10.1038/s41467-019-09853-z

10. Hubert CG, Rivera M, Spangler LC, Wu Q, Mack SC, Prager BC, et al. A Three-Dimensional Organoid Culture System Derived from Human Glioblastomas Recapitulates the Hypoxic Gradients and Cancer Stem Cell Heterogeneity of Tumors Found In Vivo. Cancer Research. 2016;76(8):2465–77. doi: 10.1158/0008-5472.CAN-15-2402

11. Tejero R, et al. Gene signatures of quiescent glioblastoma cells reveal mesenchymal shift and interactions with niche microenvironment. Ebiomedicine. 2019; 42 :252-269. doi: 10.1016/j.ebiom.2019.03.064

12. Liebelt BD, Shingu T, Zhou X, Ren J, Shin SA, Hu J. Glioma Stem Cells: Signaling, Microenvironment, and Therapy. Stem Cells International. 2016;2016:1–10. doi: 10.1155/2016/7849890

13. Ros MD, Gregorio VD, Iorio A, Giunti L, Guidi M, Martino MD, et al. Glioblastoma Chemoresistance: The Double Play by Microenvironment and Blood-Brain Barrier. International Journal of Molecular Sciences. 2018;19(10):2879. doi: 10.3390/ijms19102879

14. Ho IAW, Shim WSN. Contribution of the Microenvironmental Niche to Glioblastoma Heterogeneity. BioMed Research International. 2017;2017:1–13. Doi: 10.1155/2017/9634172

15. Aderetti DA, Hira VV, Molenaar RJ, Noorden CJV. The hypoxic peri-arteriolar glioma stem cell niche, an integrated concept of five types of niches in human glioblastoma. Biochimica et Biophysica Acta (BBA) - Reviews on Cancer. 2018;1869(2):346–54. Doi: 10.1016/j.bbcan.2018.04.008

16. Hira VV, Aderetti DA, Noorden CJV. Glioma Stem Cell Niches in Human Glioblastoma Are Periarteriolar. Journal of Histochemistry & Cytochemistry. 2018Dec;66(5):349–58. Doi: 10.1369/0022155417752676

17. Karsy M, Guan J, Jensen R, Huang LE, Colman H. The Impact of Hypoxia and Mesenchymal Transition on Glioblastoma Pathogenesis and Cancer Stem Cells Regulation. World Neurosurgery. 2016;88:222–36. doi:10.1016/j.wneu.2015.12.032

18. Musah-Eroje A, Watson S. Adaptive Changes of Glioblastoma Cells Following Exposure to Hypoxic (1% Oxygen) Tumour Microenvironment. International Journal of Molecular Sciences. 2019;20(9):2091. doi: 10.3390/ijms20092091

19. Kalkan R. Hypoxia Is the Driving Force Behind GBM and Could Be a New Tool in GBM Treatment. Critical Reviews in Eukaryotic Gene Expression. 2015;25(4):363–9.

20. Reinhard J, Brösicke N, Theocharidis U, Faissner A. The extracellular matrix niche microenvironment of neural and cancer stem cells in the brain. The International Journal of Biochemistry & Cell Biology. 2016;81:174–83. doi: 10.1615/critreveukaryotgeneexpr.2015015601

21. Herrera-Perez M, Voytik-Harbin SL, Rickus JL. Extracellular Matrix Properties Regulate the Migratory Response of Glioblastoma Stem Cells in Three-Dimensional Culture. Tissue Engineering Part A. 2015;21(19-20):2572–82. Doi: 10.1089/ten.TEA.2014.0504

22. Saha D, Martuza RL, Rabkin SD. Oncolytic herpes simplex virus immunovirotherapy in combination with immune checkpoint blockade to treat glioblastoma. Immunotherapy. 2018; 10(9):779-786. Doi: 10.2217/imt-2018-0009

23. Esparza R, Azad TD, Feroze AH, Mitra SS, Cheshier SH. Glioblastoma stem cells and stem cell-targeting immunotherapies. Journal of Neuro-Oncology. 2015;123(3):449–57. Doi: 10.1007/s11060-015-1729-x

24. Chen X, *et al.* DHHC protein family targets different subsets of glioma stem cells in specific niches. Journal of Experimental & Clinical Cancer Research. 2019; 38 (1). Doi: 10.1186/s13046-019-1033-2

25. Mettang M, *et al.* Blocking distinct interactions between Glioblastoma cells and their tissue microenvironment: a novel multi-targeted therapeutic approach. Scientific Reports.2018; 8(1). Doi: 10.1038/s41598-018-23592-z

26. Cuoco JA, Benko MJ, Busch CM, Rogers CM, Prickkett JT, Marvin EA.Vaccine-Based Immunotherapeutics for the Treatment of Glioblastoma: advances, challenges, and future perspectives. World Neurosurgery. 2018; 120: 302-315. Doi: 10.1016/j.wneu.2018.08.202