



Brain Tumor Heterogeneity

Heterogeneidade dos tumores cerebrais

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Abstract

Keywords

- brain tumor
- tumor heterogeneity
- glioma stem cell
- genetics
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- microenvironment

Tumor heterogeneity is the concept that different tumor cells provide distinct biomorphological lesions, gene expressions, proliferation, microenvironment and graduated capacity of metastatic lesions. Brain tumor heterogeneity has been recently discussed about the interesting interaction of chronic inflammation, microenvironment, epigenetics and glioma stem cells. Brain tumors remain a challenge with regards to medication and disease, due to the lack of treatment options and unsatisfactory results. These results might be the result of the brain tumor heterogeneity and its multiple resistance mechanisms to chemo and radiotherapy.

Resumo

Palavras-chave

- tumor cerebral
- heterogeneidade tumoral
- células-tronco glioma
- genética
- epigenética
- microambiente

Heterogeneidade tumoral significa que diferentes células tumorais levam a lesões morfológicas e fenotípicas distintas, com diferentes morfologias celulares, expressão gênica, metabolismo, microambiente, proliferação e possibilidade de lesões metastáticas. A heterogeneidade dos tumores cerebrais malignos tem sido o foco essencial de pesquisas recentes devido às interações notáveis entre genética, epigenética, microambiente e células-tronco glioma, todas mediadas por inflamação crônica. Tumores cerebrais ainda são um desafio no que tange a medicação e doença, podendo, com a carência de opções terapêuticas aliada a resultados insatisfatórios, ocorrer devido à heterogeneidade do tumor e seus múltiplos mecanismos de resistência à quimio e radioterapia. Foi realizada uma revisão da literatura na base de dados PubMed usando os termos: *brain tumor, heterogeneity, epigenetic, microenvironment, e glioma stem cells*.

Introduction

Tumor heterogeneity means that different tumor cells lead to distinct morphological and phenotypic lesions, with different cell morphology, gene expression, metabolism, microenvironment, proliferation and possibility of metastatic lesions.

Malignant brain tumors have unsatisfactory results, despite advanced multimodal treatments with neurosurgery, oncology and chemotherapy. In adults, glioblastoma multiforme (GBM) is the most aggressive and most common malignant brain tumor, with a global survival of patients of between 4 to 6 months without treatment, and of 14 months with multimodal therapy.¹

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In addition, brain tumors represent the leading cause of mortality in children, with medulloblastoma (MB) being the first cause.² Recently, transcriptional studies showed distinct molecular subgroups of MB, which differ among themselves in demographic data, transcriptomes, genetics, and prognosis. These studies not only established genetic subtypes, but also paved the way for the pathogenesis of MB and the possibility of cerebellar stem cells precursors.^{3,4}

The lack of adequate treatments for brain tumors can occur due to tumor heterogeneity, which is controlled by at least two mechanisms that can be integrated through clonal evolution and hierarchies, and by the hypothesis of carcinogenic stem cells.⁵

In the present study, we will analyze the possible integration of the genomics, epigenomics, stem cell, chronic inflammation and microenvironment hypotheses of these brain tumors.

Clonal Evolution and Stem Cell Model

The clonal evolution model indicates that all cancerous cells can proliferate, change, and regenerate due to random mutations, creating clonal subpopulations within the tumor.⁵

On the other hand, the stem cell hypothesis proposes that cancers are hierarchically organized, with cells with the same properties of stem cells at the apex of this organization.⁵

The two models can be integrative, and recent studies have defended the existence of cancerous stem cells (CTCs) and have shown, in the laboratory, that these cells have greater tumorigenic potential, and are potentially more resistant to radiation and chemotherapy.^{6,7}

Glioma Stem Cells

The GBM has several aspects, such as polymorphism and cellular heterogeneity, which makes it an essential lesion for the research. Glioblastoma multiforme CTCs (CTGs) have similar characteristics to normal neural progenitors, such as self-renewal capacity, long-term proliferation, and neurospheres formation. However, few studies describe its ability for multiple nervous system cells (neurons, astrocytes and oligodendrocytes).⁶

The molecular signs that control tumor formation and maintenance are slightly similar to normal progenitors, but differ in frequency, aberrant markers, and chromosomes. Glioblastoma multiforme CTC has already been shown with surface marker of the CD133 stem cells; However, other surface markers are emerging, such as A2B5, CD15, and CD171. There is evidence that not all CTGs present the classic marker CD133, but the genotypic profile of the brain tumor differs among the patients and the surface markers may also vary. In addition, the inflammation process during the course of the disease is multiple, and CTG plays a key role in the maintenance and promotion of microenvironments and niches.⁶

Microenvironment and Glioma Stem Cell Niches

Vascular Niche

The CTGs are in specific anatomical-functional sites with direct contact between specific cell types and extracellular

matrix, as well as with cytokines and important factors for renewal and proliferation. Interestingly, healthy neural cells, as well as the CTGs, are also regulated by adhesion and by the vascular niche. In addition, they promote angiogenesis through proangiogenic growth factors, such as vascular endothelial growth factor (FCEV), endothelial migration and tube formation. Tumors with CD133+ have more necrosis, hemorrhages and are highly vascularized when compared with tumors with CD133-.^{8,9}

Similarly, the complexity between CTGs and endothelial cells is far from trivial. Recent studies have shown that between 20 and 90% of endothelial cells within the tumor present the same mutations present in GBMs, such as the epidermal growth factor receptor (RFCE) and the alteration in chromosomes.^{7,9}

Moreover, it was demonstrated that the CTGs cause a differentiation in the pericytes, thus maintaining the function of the vessel and the development of the tumor. They also express several biological markers of pericytes, such as the actin smooth muscle α , NG2, CD248 and CD146, and are also endothelial cells recruiters via SDF-1/CXCR4. In general, we see the integration of CTGs with the vascular niche in a dual-hand pathway.^{8,9}

Hypoxia Signaling

Gliomas promote a recruitment of vessels, mediated by tumor, and also neovascularization. However, these vessels are disorganized, and the oxygen supply is limited in specific areas, with irregular blood flow and hypoxic oxygen stress level, < 5%. These hypoxic regions often express MGMT, and are linked to tumor resistance and poor prognosis, since the cells produce more CD133.^{10,11}

In normal homeostasis, the cells hydroxylate the hypoxia-inducible factor (HIF), responsible for promoting genes and activating and modulating responses that involve cell survival, motility, metabolism and angiogenesis.^{10,11}

The HIF-1 α is expressed in several tissues; however, the HIF-2 α is not restricted only to the CTGs: it is overexpressed by them in gliomas, and is practically not expressed in cells that are not CTGs. Moreover, its overexpression is crucial for the reprogramming of cancer, by increasing the CD133 cells, and by positive regulation of OCT4, Nanog and C-MYC mRNA.^{12,13} There are also other hypoxia inducing genes, which are more expressed when in hypoxia state: GLUT1, SerpinB9 and FCEV.^{12,13}

Sathornsumetee et al showed, in a study with 60 recurrent malignant gliomas, that carbonic anhydrase 9 (AC9) and HIF-2 α , expressed in acidotic and hypoxic niches, were associated with a poor prognosis and a survival rate of < 1 year with the use of bevacizumab.¹⁴

Glioma Stem Cells Pathway

Notch proteins (1, 2, 3, 4) are essential during the development of the central nervous system, as they promote renewal and contribute to stem cell survival, and are also crucial for adult neuronal plasticity. Pathologically, notch signaling modulates the progression of the brain tumor and the differentiation of stem cells. In addition, the γ -secrease cascade releases the Notch intracellular domain, and its inhibition is an improved

response to temozolomide, decreasing the radioresistance, cell growth, and the differentiation of CTGs.¹⁵⁻¹⁷

Tyrosine Kinase Receptor Signaling

Tyrosine kinase receptors (TKRs) are pathways promoted by several cytokines and growth factors, such as epidermal growth factor and fibroblasts growth factor. One of these paths is the PI3K/Akt/mTOR, found in GBMs and overexpressed by CTGs. This pathway is activated by the FCEV, which increases tumor growth and transduces several stem cell markers, such as CD133, which have increased Akt pathway and are directly correlated with the degree of the tumor.⁵

Hedgehog

The Sonic hedgehog protein is crucial for the embryological formation and differentiation of the structures of the dorsal brain; In adults, it regulates neural stem cells. In GBM, this protein is overactivated, and is related to the expression of genes and stem cell markers, such as CD133, promoting growth and contributing to tumor survival. In rats, the inhibition of the hedgehog pathway leads to apoptosis induction, reduction of self-renewal, and also to a better response of temozolomide.^{18,19}

Glioma Stem Cell Transcription Factors

Several signaling pathways lead to extracellular signals to the regulating factors of CTGs transcription, such as OCT4, Sox2, C-Myc and Olig2.

OCT4 and Sox2 factors interact in the regulation and differentiation of embryonic stem cells, as well as in the increase of the CTGs, and in the promotion of the tumorigenic activity.²⁰ c-Myc leads to cell reprogramming in the fibroblast to induce a pluripotency. In addition, it is correlated with the degree of the tumor, and is further expressed in CTGs, which can reach ~ 50% of the CD133 positive cells.²¹ The Olig² is a transcription factor restricted to the central nervous system, specifically to the oligodendrocyte and multipotent progenitors. It is overexpressed in diffuse astrocytomas, oligodendrogliomas and oligoastrocytomas.^{22,23} In fresh human GBMs, it is positive in 85% of the gliomas cells that are positive for Ki67, and in ~ 98% of All CTGS CD133.

Olig² can also control CTG proliferation in the different forms of adhesion and cell cycle.²³

Epigenetic Regulation of Glioma Stem Cells

Epigenetic is the occurrence of a hereditary DNA change, which regulates gene expression, without changing the actual DNA sequence. Recently, DNA methylation in high-grade gliomas is one of the most significant progressions, with the identification of mutations of the enzyme isocitrate dehydrogenase 1 (IDH-1).⁵ Isocitrate dehydrogenases are multiple mutations that lead to the specific change of the Krebs cycle enzymes. Isocitrate dehydrogenase mutant enzymes generate an oncometabolite known as D-2-hydroxyglutarate (D-2-HG), instead of the α -ketoglutarate (α -CG), in the citric acid cycle. This protein

promotes gliomagenesis through the activation of HIF-1 nuclear translocation, which leads to an increase in cell proliferation and angiogenesis, as well as to the hypermethylation of histones, which restructure the cellular epigenetic state.²³

In addition, the histones methylation process can control proteins transcription. It opens the chromatin by means of methylation of H3K4 to promote its transcription. The closure occurs by the H3K27, thereby interrupting the process. Histone methyltransferase is stimulated in hypoxic CTGs, supporting the HIF-2 expression pathway and the tumorigenic pathway.²⁴

Another epigenetic factor is the microRNAs (miRNAs), which are noncodifier regulatory RNAs, with an essential role in neural development/biological process, and in the tumorigenesis of the GBM, composed approximately by 22 noncodifier nucleotides with regulator gene expression ability downwards and translation inhibition. Therefore, they have an essential role in CTG pluripotency, reprogramming, and pathway. MicroRNA-124, miRNA-146a and miRNA-34a contribute to gliomagenesis, while miRNA-125b and miRNA-9 regulate the process of resistance to chemotherapy and radiotherapy.^{25,26}

Chronic Inflammation Process

As already discussed, the development of brain cancer is an interaction of multiple processes, from genetic alterations to inflammation. Several genetic mutations have already been related to cerebral tumorigenesis, such as: tumor protein p53 (PT-53), homologous to tensine phosphatase (FTEN), neurofibromatosis type 1 (NF-1), RFCE, retinoblastoma (RB) and regulatory subunit 1 of phosphoinositide-3-kinase (SR1FI3Q). Most of these genes code proteins related to tumor suppression. Their mutations may lead to alterations in the metabolic circuits, such as: tyrosine kinase receptor (RTC)/RAS (rat sarcoma)/FI3Q, via p53, via RB, and via of the IDH-1 or IDH-2.²³

Brain cancer development occurs with the integration between genetics, epigenetic and inflammation. In cancer, inflammation has two pathways: the intrinsic pathway, which is the integration between genetic events that lead to the chronic inflammatory microenvironment, and the extrinsic pathway, which leads to a constant inflammation and facilitates the development of cancer. Due to its persistent inflammation, immunosuppressive and inhibitory cytokines are secreted, and the cells that infiltrate the tumor secrete inflammation mediators instead of a cytotoxic response. Therefore, the microglia and macrophages associated with tumors (MATs) secrete cytokines and growth factors that create a propitious microenvironment for tumor growth and invasion.²³

In addition, cyclooxygenase (COX), in particular COX2, has an essential role in chronic inflammation due to the increase of prostaglandins, prostacyclin and thromboxane. Cyclooxygenase -2 is increased in the premalignant lesions, and is overexpressed in malignant tumors, with the existence of a correlation between its levels and the tumor aggressiveness.²⁴

Similarly, changes in the signal transductor protein and transcription activator (TSAT) can be a crucial point in cancer immune deregulation. Transcription activator proteins are cytoplasmic transcription factors that mediate the signaling of tyrosine kinase/growth factors and cytoplasmic enzymes.

The TSAT-3 is overactivated in several brain tumors, and increases the inflammatory process by means of IL-6 and IL-10, also inducing immunosuppression, and decreasing neutrophils activity and natural exterminating cells.^{23,24}

IL-10, Similarly, inflammatory cytokines activate and release free NF- κ B, which translocates into core genes and transcribes genes that code antiapoptotic proteins and proinflammatory cytokines, chemokines, adhesion molecules, proteases and DNA repair proteins, such as MGMT.²⁴ Temozolomide and other chemotherapies add an alkyl group to the tumoral DN to stop the cell cycle and provoke tumor death. On the other hand, MGMT has the function of repairing the DNA and removing the alkyl groups, which results in resistance to temozolomide.²³

Thus, chronic inflammation causes oxidative stress, with the release of reactive oxygen and nitrogen specimens, which deregulate the repair of wrong pairings (RPE) of DNA, the base excision repair (REB), the nucleotide excision repair (REN), and the cell cycle and homologous recombination (RH). This oxidative stress creates a vicious circle for genetic instability and epigenetic silencing, called microsatellite instability (MSI).^{23,24}

Conclusion

Brain tumors are one of the most aggressive lesions in existence, although they are one of the less understood. Future perspectives point to the interruption of the cell cycle in the stem cell pathways, for the differentiation of phenotypes/genotypes and the hierarchy of stem cells in the brain tumor. Finally, the chronic inflammation can be a bridge between the genetic and epigenetic disorder, creating a complex tumor microenvironment and, because of this, more studies are necessary to provide better forms of treatment to our patients.

Conflicts of Interest

We declare that the authors have not received payments or allowances from any institution, we have no ties with any company that could relate to the work developed, and we do not hold any patent that may be involved with the scientific production of this work.

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