Chapter 69

Molecular profile of glioblastoma and its clinical correlations

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Correa-Silva, MH
Universidade Nove de Julho, Health Department III. Medicine Course, Osasco Campus, Brazil.
Laboratory for Industrial Development and Innovation. Butantan Institute, São Paulo, Brazil
E-mail: murilohenriquecorreadasilva@gmail.com

Cirelli-Moraes, A
Laboratory for Industrial Development and Innovation. Butantan Institute, São Paulo, Brazil
E-mail: angelinacirelli@gmail.com

Maria, DA
Laboratory for Industrial Development and Innovation. Butantan Institute, São Paulo, Brazil
E-mail: durvanei.maria@butantan.gov.br
Corresponding Author: Maria DA.
Durvanei Augusto Maria, PhD

ABSTRACT
Glioblastoma multiforme (GBM) is a malignant neoplasm of the central nervous system that is highly invasive and has a poor prognosis, with an average patient survival of 15 to 17 months. It is characterized by amplification of the epidermal growth factor receptor (EGFR) gene, overexpression of the EGFR protein, and loss of the tumor suppressor gene phosphatase and tensin homolog (PTEN). And the secondary form, which accounts for about 10% of cases, is associated with the younger age of the patient and arises from the progression of low-grade gliomas. It has a better prognosis and usually has mutations in the isocitrate dehydrogenase 1 (IDH1) and tumor protein 53 (TP53) genes. However, detailed knowledge about the heterogeneity of molecular alterations of the tumor may contribute to the prognosis of the patient and his response to treatment and to the improvement of current therapeutic strategies, besides contributing to the development of new strategies and treatment protocols for this type of tumor.

1 INTRODUCTION

Glioblastoma multiforme (GBM) is a malignant neoplasm of the central nervous system that is highly invasive in the adjacent parenchyma and has a poor prognosis; The histopathological features of this neoplasm are necrosis and endothelial proliferation, resulting in a grade IV classification, the highest grade in the World Health Organization (WHO) classification of brain tumors (Wirsching et al., 2016).The average age-adjusted incidence rate is 3.2 per 100,000 population (Ostrom et al., 2014). The incidence is relatively higher in males than in females (1.6:1) and in Caucasians compared to other ethnicities(Ellor et al., 2014) and the median survival of patients is 15 to 17 months (Wang et al., 2015).

The causes of glioblastoma, are not yet well identified. Environmental and occupational exposures are mostly inconclusive and insufficient. According to (Ellor et al., 2014) , 2014, ionizing radiation, is one of the few risk factors that can increase the development of glioma, and this is seen years after therapeutic radiation for another tumor (Johnson et al., 2015)

Other environmental exposures to vinyl chloride, pesticides, smoking, oil refining, and synthetic rubber manufacturing have been loosely associated with the development of gliomas. Electromagnetic fields, formaldehyde, and non-ionizing radiation from cell phones have not been proven to lead to GBM (Alifieris & Trafalis, 2015). According to (Ellor et al., 2014), less than 1% of glioma patients have a known inherited disease, little data exists in the literature

The size and location of the tumor and anatomical structures in the brain, are indicative of a newly diagnosed GBM patient (Young et al., 2015).

Increased intracranial pressure, headaches, and neurological deficits are frequent symptoms in patients with GBM. Seizures appear in up to 25% of them, and may later reach up to 50% of them (Perry et al., n.d.) (Schiff et al., 2015) . Antiepileptic drugs (AEDs) are used only in patients with seizures, and are prohibitive for those without seizures (Glantz et al., 2000) (Perry et al., n.d.) . To help control vasogenic edema and relieve signs and symptoms, corticosteroids may be prescribed at diagnosis.

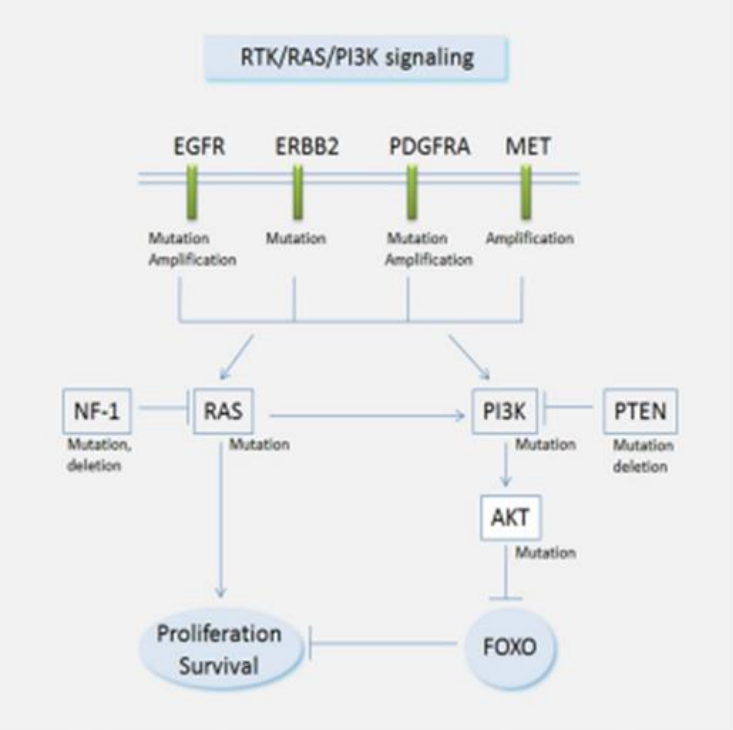
Initial diagnostic imaging may include a computed tomography (CT) scan or magnetic resonance imaging (MRI). On MRI, almost all GBMs enhance with gadolinium contrast and show an irregularly shaped mass with a dense ring of enhancement and hypointense center of necrosis. Necrosis is a hallmark feature of GBM, and the presence of necrosis is required for a brain tumor to be grade IV or to be classified as Glioblastoma in the World Health Organization classification system. Surrounding vasogenic edema, which may cause a mass effect, hemorrhage, and ventricular distortion or displacement may also be present on diagnostic imaging (Ellor et al., 2014)

Clinical Classification

Glioblastoma multiforme is generally classified into two different clinical forms: Primary GBM and Secondary GBM. Primary GBM is the most common form, about 95% of cases, and typically arises de novo within 3-6 months in older patients. Secondary GBM arises from previous low-grade (II-III) gliomas as a result of molecular progression and increased malignancy of a glioma over time and have a longer course of 10-15 years in younger patients. (Alifieris & Trafalis, 2015)

Histologically primary and secondary glioblastomas may be largely indistinguishable, however they differ in their genetic and epigenetic profile. Typical genetic alterations in primary GBM are mutations in the gene encoding tensin homologous phosphatase (PTEN), overexpression of epidermal growth factor receptor (EGFR), mutations in the promoter of the telomerase reverse transcriptase (TERT) gene, and loss of chromosome 10q. In secondary GBM the frequently altered pathways are due to mutations in the isocitrate dehydrogenase-1/2 gene (IDH1 and IDH2), mutations in p53, and loss of chromosome 19q. There are also signaling pathways that are shared by both clinical forms such as the p53 signaling pathway, the retinoblastoma pathway, and the receptor tyrosine kinase/ RAS/ phosphoinositide-3-kinase pathway. (Davis, 2016)

Fig. 1 Alterations in the RTK/RAS/PI3 K signaling pathway in GBM. Several genes that encode proteins involved in the RTK/RAS/ PI3 K signaling pathway are considerably altered in GBM. Genes that are most frequently amplified in this pathway are epidermal growth factor receptor (EGFR) and platelet-derived growth factor receptor α (PDGFRA), two transmembrane receptors with tyrosine kinase activity. The most commonly deleted gene in the RTK pathway is phosphatase and tensin homolog (PTEN), a tumor suppressor that inhibits phosphatidylinositol-3 kinase (PI3 K) signaling such as retinoblastoma (RB1), a cell cycle inhibitor of PARK2, a regulator of dopaminergic cell death, and neurofibromin 1 (NF1), a negative regulator of the RAS signal transduction pathway. The most commonly mutated genes in this pathway are PTEN, NF1, EGFR, and PIK3R1, and PIK3CA. (Aldape et al., 2015)



Treatment

The current standard treatment for glioblastoma involves surgical resection and radiation therapy (RT), in conjunction with the alkylating agent Temozolomide (TMZ), achieving a median survival of 14.6 months (Parker et al., 2015). These treatments can be combined with alternating electric fields of intermediate frequency (Stupp et al., n.d.) (Weller et al., 2009). After surgical resection it is necessary to wait up to 4 weeks for the surgical wound to heal. Until 2005 radiotherapy alone was the standard treatment, providing a survival of 12.1 months. In the same year (Stupp et al., n.d.) 2005 achieved a 14.6 month survival rate for GBM patients by offering radiation therapy plus concomitant Temozolomide chemotherapy.

RT + TMZ conjugate treatment is usually applied at a dose of 75mg/m², daily for 6 weeks. This is followed by a period of about 1 month for reapplication of TMZ at a dose of 150mg/m² daily for 5 days. If there is no adverse reaction, the dose can be increased to 200mg/m² for 5 days per month, until the end of therapy. Many physicians continue TMZ cycles for 12 to 18 months, however these data have not demonstrated superior survival (Johnson et al., 2015) .

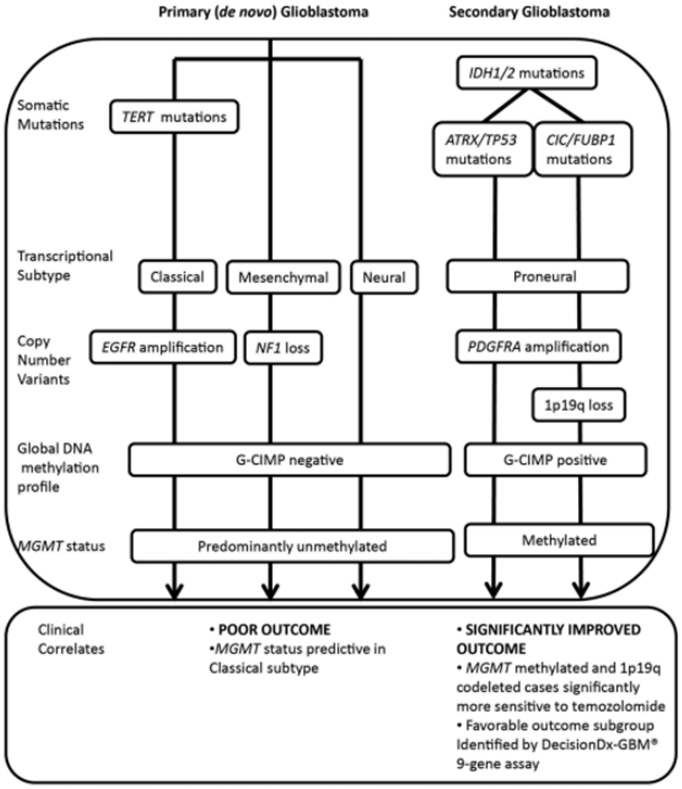
Unfortunately, due to the frequency of recurrence of Glioblastoma, the therapeutic options are limited. Much research is being done in order to define the molecular profiles of brain tumors, which may indicate new therapeutic procedures. The detailed knowledge about the heterogeneity of the molecular alterations of the tumor can contribute to the prognosis of the patient and his response to treatment and to the improvement of the current therapies, and also contribute to the development of new strategies and treatment protocols for this neoplasm.

Molecular classification

In order to better understand the molecular heterogeneity of glioblastoma, The Cancer Genome Atlas (TCGA) has divided the tumor into molecular subclasses based on its distinct genetic, epigenetic and transcriptional characteristics. The neoplasms can be classified based on somatic mutations in isocitrate dehydrogenase (IDH) 1/2 and TP53; their transcriptional signature which can be of classical, mesenchymal, neural or proneural type; based on copy number variation, including co-deletion of chromosomes 1p and 19q; and amplification or mutation of the epidermal growth factor receptor (EGFR) and increased DNA hypermethylation of promoter-associated CpG islands.

Classic GBM is defined by having aberrant EGFR amplification, characteristic astrocyte cell expression pattern, loss of chromosome 10 whereas mutations in IDH1, TP53 or the neurofibromatosis type-1 (NF1) gene are not common. The mesenchymal type is characterized by mutations in PTEN and NF1, mesenchymal expression profile and a lower amplification of the epidermal growth factor receptor (EGFR) than in other types of GBM. The proneural type is marked by focal amplification of platelet-derived growth factor receptor-A (PDGFRA), mutations in TP53 and IDH1 with a cellular expression profile characteristic of oligodendrocytes and presentation at a younger age. Finally, the neural type is characterized by gene expression profile of normal brain tissue and markers of astrocytic and oligodendrocytic cells. (Alifieris & Trafalis, 2015); (Parker et al., 2015).

FIGURE 2 |Molecular classification of major glioblastoma subtypes and correlation with treatment response and outcome. IDH1/2 mutations are major prognostic biomarkers, stratifying primary and secondary pathways of gliomagenesis. Primary glioblastoma features a high frequency of TERT mutations, whereas IDH1/2 mutated glioma (including secondary glioblastoma and low grade glioma) may be further subdivided on the basis of co-mutations in either ATRX and TP53 or CIC and FUBP1, occurring at high frequency in astrocytic or oligodendroglial tumor subtypes. Co-deletion of 1p and 19q, a marker of enhanced chemosensitivity, also clusters with mutations in CIC and FUBP1. Primary glioblastomas display classical, mesenchymal, and neural phenotypes , whereas secondary glioblastomas tend to display a proneural phenotype that shifts toward a mesenchymal phenotype with recurrence. The significantly improved outcome of the proneural subset is due to the G-CIMP phenotype, established by IDH1/2-mediated metabolic reprogramming of the epigenome. Primary glioblastomas and a subset of proneural tumors are glioma-CpG island hypermethylator phenotype (G-CIMP) negative, and a large proportion are MGMT unmethylated. (Parker et al., 2015)



Molecular biomarkers in glioblastoma and clinical **applications**

The current standard treatment for glioblastoma involves surgical resection and radiation therapy, in conjunction with the alkylating agent Temozolomide achieving a median survival of 14.6 months (Parker et al., 2015). However, detailed knowledge about the heterogeneity of molecular changes in the tumor can contribute to the patient's prognosis and response to treatment and to the improvement of current therapeutic strategies, and also contribute to the development of new treatment strategies and protocols for this neoplasm. A great example is the fact that the response to chemotherapy with the agent temozolomide is mediated by the O-6-methylguanine-DNA-Methyl-transferase (MGMT) gene, which encodes a DNA repair enzyme involved in the repair of cytotoxic adducts produced by this alkylating agent. Hypermethylation or epigenetic silencing of MGMT disables the ability of cells to promote DNA repair, making cells more sensitive to treatment. (Davis, 2016). Furthermore, Hypermethylation of the MGMT promoter is associated with longer progression-free survival and overall survival in glioblastoma patients treated with alkylating agents such as nitrosourea or temozolomide (Weller et al., 2009) .In addition to this fact, hypermethylation

of the MGMT promoter is a clinically important predictive marker to stratify and guide adjuvant therapy in elderly patients. In this scenario, the methylation status of the MGMT promoter emerges as a predictive marker to determine the best therapy and the inclusion of temozolomide, thus contributing to stratify patients into groups who lack methylation of the MGMT promoter and should be treated with radiotherapy only, and those who have methylation of the MGMT gene promoter and should be treated with either temozolomide or chemotherapy with temozolomide combined with radiotherapy. (Alifieris & Trafalis, 2015) (Hegi et al., n.d.; Olson et al., 2011). (Aldape et al., 2015). However, the methylation profile of the MGMT promoter in younger patients is still a matter of debate since these patients are treated with temozolomide regardless of their tumor methylation status. However, the methylation status of the MGMT gene promoter of the tumors of these patients may be useful to distinguish a pseudoprogression from a true progression.A pseudoprogression is often observed a few months after treatment in patients who have been treated with radiotherapy and chemotherapy with TMZ, it is characterized as an increase in tumor size observed on radiological images but is not accompanied by the worsening of neurological signs and symptoms that the patient would otherwise experience due to the increased tumor mass. Pseudoprogression was observed in 91% of patients who had hypermethylation status of the MGMT promoter and in 41% of patients who did not have methylation status. (Aldape et al., 2015) (Brandes et al., 2008).

Somatic mutations in IDH1/2 are definitive markers of secondary glioblastoma and pose a significantly better prognosis . IDH1 mutations occur with high frequency, more than 80% of cases, in WHO grade II and III astrocytomas, precursor lesions to secondary glioblastoma, while IDH2 mutations occur largely in oligodendroglial tumors, with much lower frequency. Both mutations are rare in primary glioblastoma occurring in less than 5% of cases. IDH mutated glioblastoma occurs predominantly in the frontal lobe, whereas the anatomical distribution of wild-type IDH mutated glioblastoma is more heterogeneous. Recently, the loss of the ATRX gene, alpha thalassemia/X-chromosome linked mental retardation syndrome, has been shown to further refine the classification of astrocytic tumors with mutations in IDH, it has been observed that tumors that have mutations in IDH/ATRX carry a more favorable prognosis.(Parker et al., 2015). In addition to its role as a biomarker, the mutation in IDH1 may provide the basis for targeted immunotherapy. A mutant peptide containing (R132H) IDH1 has been shown to be immunogenic, suggesting the potential for a glioma-specific vaccine with mutation in IDH1 (Schumacher et al., 2014)

Most IDH mutated glioblastomas have a proneural transcript profile, including a high frequency of TP53 mutations and PDGFRA amplification. (Verhaak et al., 2010) .In contrast, IDH mutation wild-type glioblastoma tends to be subclassified into mesenchymal, classical, and neural transcriptional subtypes. On recurrence, proneural tumors shift to a more aggressive mesenchymal phenotype, enriched for expression of mesenchymal markers, including chitinase 3 (CHI3L1 or YKL40), CD44, and signal transducer and activator of transcription 3 (STAT3). (Parker et al., 2015)

A robust 9-gene expression assay (now a proprietary test called DecisionDx-GBM®) has been developed with the ability to discriminate between glioblastoma patients with a more favorable prognosis, associated with a high level of proneural gene expression, and those with a poor prognosis, which is associated with enriched expression of mesenchymal genes and angiogenesis. (Colman et al., 2010)

Approximately 40% of primary GBMs carry amplification of the EGFR gene . In addition, about 50% of GBMs with EGFR amplification also harbor a mutation in this same gene that codes for a constitutively active variant, EGFRvIII, which promotes tumor proliferation and is potentially associated with a worse clinical prognosis. (Aldape et al., 2015); (Colman et al., 2010). Studies have shown that ectopic overexpression of EGFRvIII results in constitutive autophosphorylation and activation of the Shc-Grb2-Ras and PI3K classse I pathways, cell proliferation and resistance to apoptosis through modulation of Bcl XL gene expression (Aldape et al., 2015). It has also been observed that a small number of EGFRvIII positive cells are able not only to drive their own proliferation but also to enhance the proliferation of neighboring cells expressing wild-type EGFR, via paracrine signaling mediated by the EGFvIII expressing cells (Nishikawa et al., 2004). The mutation in EGFRvIII has become clinically relevant as this deletion-type mutation generates a novel peptide sequence that may serve as a tumor-specific immunogenic target that can be exploited in a peptide-based vaccination strategy. Initial results from single arm trials employing this EGFRvIII-specific vaccination strategy provided promising results when compared to controls.(Aldape et al., 2015; Sampson et al., 2010)Furthermore, it has been observed that the use of Temozolomide can result in EGFRvIII induction and EGFR expansion, which can be managed with the use of EGFR inhibitors such as erlotinib, gefitinib, afatinib, dacomitinib (Munoz et al., 2014).

In approximately 30% of human gliomas, there is expression of genes associated with platelet-derived growth factor receptor (PDGFR) signaling and genes involved in oligodendrocyte development (OLIG2, NKX2-2 and PDGF) and are considered hallmarks of the proneural type signature in glioblastoma. PDGFR activation triggers several intracellular signaling pathways, very similar with EGFR, such as PI3K, MAPK, Jak family kinase, Src family kinase and phospholipase C-gamma (PLC-γ) and is also similarly associated with the promotion of aggressive glioblastoma proliferation ((Joensuu et al., 2005). Thus, in addition to promoting tumor proliferation through autocrine stimulation, activation of the pathway is able to stimulate angiogenesis through paracrine effects on reciprocal endothelial cells and affects the regulation of tumor fibroblasts which in turn leads to transvascular drug transport through changes in intratumoral pressures (Östman, 2004). In addition, drugs that block the platelet-derived growth factor receptor include tyrosine kinase receptor inhibitors and monoclonal antibodies (MAbs) can be used in association with conventional therapy (Alifieris & Trafalis, 2015).

The search for a better understanding and management of GBM patients has directed research to look for a specific type of cells to focus studies on. The discovery of multipotent central nervous system stem cells capable of proliferation, renewal and differentiation has suggested that there are specific central

nervous system stem cells that possess the characteristics of a somatic stem cell and behave like a tumorigenic cell. Studies have shown that these stem cells are able to promote extensive angiogenesis through the expression of vascular endothelial growth factor (VEGF) and thereby create a tumor microenvironment and vascular support suitable for tumor survival and growth (Cheng et al., 2013) (Facchino et al., 2011).

Resistance to chemotherapy and radiotherapy

Many patients with glioblastoma have an intrinsic or acquired resistance to treatment with chemotherapy or radiation therapy. The mechanisms underlying resistance to chemotherapy treatment using alkylating agents is the methylation status of the MGMT gene promoter. One way to transpose this resistance mechanism is with dense doses of 1-21 days every 28 days or with intense dose regimens (Hegi et al., n.d.) (Anel et al., 2000) or by direct inhibition of the MGMT promoter with O6-Benzylguanine in combination with temozolomide. Another mechanism that confers the resistance profile to these tumors is that of Poly (ADP-ribose) polymerase-1 (PARP-1). PARP-1 is an extremely important gene for the base excision repair pathway of cells that when inactivated results in lesions in the N7- and N3-regions of the purines that are potentially fatal to cells and contributes to the cytotoxicity of temozolomide when O6-methylguanine adducts are repaired or tolerated by cells. PARP-1 is clinically important as it is possible to achieve its inhibition using inhibitors such as Iniparib and ABT-888 alone or in combination with temozolomide. (Alifieris & Trafalis, 2015). It has also been observed in patients who have resistance to chemotherapy the presence of tumor stem cells with high expression of multidrug resistance protein-1 (MRP1), a fact that favors the mechanism of resistance that these patients have to chemotherapeutic agents such as temozolomide, etoposide, paclitaxel and carboplatin, which also explains the reason for the high frequency of relapse that tumors present even with the presence of treatments.(Liu et al., 2005).

Amplifications of the epidermal growth factor receptor (EGFR) is one of the most commonly observed gene amplifications in malignant gliomas. Activating mutations of EGFR , such as mutated EGFRvIII are associated with the resistance to radiotherapy that certain tumors exhibit. Alterations in the EGFR signaling pathway are associated with a worse clinical prognosis and reduced overall survival (Ye et al., 2010).

Mutations in the Sonic Hedgehog (SHH) signaling pathway may be associated with the mechanism that confers resistance to chemotherapy and radiotherapy and also with the process of renewal of tumor stem cells. The SHH signaling pathway acts through activation of the glioma-associated oncogene transcription factor (Gli1) and thus participates in the maintenance and proliferation of tumor stem cells. (Clement et al., n.d.).

Molecular Targets of Drugs in Glioblastoma

The field of research to better understand the role played by molecular alterations in the pathogenesis of glioblastoma has allowed the more rational and strategic use of drugs that are molecular targets. The use of receptor tyrosine kinase inhibitors, EGF,PDGF,HGF and IGF receptors, and signal transduction inhibitors that target mTOR, AKT/PI3K and farnesyltransferase is being studied. However, to date monotherapy with these agents has not shown such satisfactory results with mild to moderate efficacy between 0-15% and are unable to prolong progression-free survival. (Sathornsumetee et al., 2007). For this reason, the current approach is to perform inhibition of multiple molecular targets within the same pathway, proximal or distal, or between separate parallel pathways; however this strategy increases toxicity in patients. (Wilson et al., 2014).

Activation of EGFR family receptors result in the activation of multiple downstream signaling pathways such as RAS/RAF/MEK/ERK 1-2-protein mitogen-activated kinase (MAPK), PI3K/AKT/mTOR and STAT3 and STAT5. These signaling pathways alter cell proliferation, migration, differentiation, inhibition of apoptosis, and up-regulate the expression of VEGF, which is involved in the angiogenesis process. For this reason EGFR inhibition must be performed and can be accomplished with the use of competitive antagonists such as orally administered Erlotinib and Gefitinib, irreversible antagonists such as Afatinib and Dacomitinib, monoclonal antibodies, peptide vaccines and conjugated immunotoxins.(Alifieris & Trafalis, 2015).

The use Small molecule inhibitors developed targeting the c-MET pathway have shown selectivity for the c-MET receptor. SGX523 is a high-selectivity oral c-MET inhibitor that has been shown to be effective in vitro and in vivo against glioblastoma cells. (Buchanan et al., 2009; Pearson & Regad, 2017)

Farnesyltransferase inhibitors such as Tipifarnib have been introduced in the treatment of malignant gliomas and have demonstrated modest efficacy as monotherapy in patients with recurrent gliomas. Toxicity was well tolerated by patients with the exception of patients taking antiepileptic drugs that are enzyme inducers these patients showed greater hematologic toxicity. (Cloughesy et al., 2006)

Glioblastoma is a highly vascularized malignant neoplasm with extensive angiogenic capacity and high expression of VEGF, thus one approach used is the inhibition of the angiogenic pathway with the use of molecular targeting drugs which have shown promising results in many studies. (Onishi et al., 2013). Old drugs such as thalidomide and new anti-VEGF agents that include small molecule tyrosine inhibitors and MAbs or anti-VEGFR can be used, as well as drugs that inhibit hepatocyte-derived growth factor (anti-HGF) and anti-alpha-V beta 5 integrin agents can also be included.(Alifieris & Trafalis, 2015).

2 CONCLUSION

Despite several advances in early diagnosis and treatment of tumors in recent decades, Glioblastoma Multiforme remains one of the cancers with the highest mortality rate and low life expectancy. The

molecular characteristics of glioblastoma multiforme are a subject of constant scientific investigation, which has advanced greatly, especially in the description of mutational profiles and molecular pathways that are altered in this malignant neoplasm. Continuous progress continues to be made to better characterize these molecular alterations and associate them with accurate diagnosis and provide relevant and strategic information about the prognosis and response to treatment that these patients may present, thus contributing to the clinical practice of patients with glioblastoma multiforme.

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