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Genetics and Biology of Glioblastoma Multiforme

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1. Introduction

Glioblastoma multiforme (GBM), the most common and biologically aggressive type of glioma is characterized by intra and intertumoral heterogeneity on the cytopathological, transcriptional, and genomic levels. GBMs account for 17.1% of brain and central nervous system (CNS) tumors and constitute 60-70% of all gliomas (The Central Brain Tumor Registry of the United States (CBTRUS) Statistical Report: NPCR/SEER, 2004-2006). Glial tumors can arise anywhere in the brain, but usually localize in the cerebral hemispheres and very rarely metastasize outside the CNS. In general, GBM affects individuals of both sexes and all age groups, but are usually more frequent in male patients (Bondy, et al., 2008) and the peak age at onset is between the fifth and the seventh decades of life (Wen, & Kesari, S., 2008).

Due to this complexity, and to the existence of a cancer stem cell (CSC) subpopulation, GBM presents an uncontrolled cellular proliferation, diffuse infiltration, propensity for necrosis, angiogenesis, resistance to apoptosis, and genomic instability (Louis, 2006) that makes this cancer one of the most difficult to treat. Despite multimodal aggressive treatment that includes surgical resection, followed by local radiotherapy and systemic chemotherapy (Furnari, et al., 2007), the median survival of GBM patients is 12-14 months. Unfortunately, only a small fraction of GBM patients (< 3%) survive for more than 36 months (Sonoda, et al., 2009). The existence of the blood-brain barrier and the diffuse infiltration into the surrounding brain limits the delivery of therapeutic agents, and increases the possibility of therapeutic toxicity. On the other hand, GBM tumors seem to overcome the effect of these therapies, through the ability to repair radiation-induced injury accomplished by aberrant or amplified growth and survival signaling pathways (Noda, et al., 2009), and ultimately becoming resistant to treatment.

Molecular therapeutic strategies targeting altered signaling pathways have successfully improved patient outcome, however, it is still not possible to distinguish, in advance, which patients are the most likely to effectively benefit from specific drugs. Favorable prognostic factors associated with longer survival include young age at the time of diagnosis, absent or minimal neurological damage signs and a good initial Karnofsky performance score (KPS) (Bussiere, et al., 2005). Recent data have reported that hypermethylation of the O6-methylguanine-DNA methyltransferase (*MGMT*) gene promoter is associated with prolonged progression-free survival in GBM patients treated with alkylating agents (Hegi,

et al., 2005), and the extent of tumor resection is also found to enhance the chances for a favorable outcome (Stummer, et al., 2006). Additionally, recent large-scale studies integrating genetic and gene expression datasets, have identified molecular subtypes within GBM that seem to have relevance to predict patient outcome and response to treatment (Freije, et al., 2004; Phillips, et al., 2006).

2. Histological characterization of GBM

Histological classification and grading criteria for glial neoplasms are well established, basing on morphologic evidences of cellular differentiation and on the presence or absence of histopathological features, such as cellularity, nuclear pleomorphism, mitotic activity, endothelial cell proliferation and necrosis (Furnari, et al., 2007).

Although the specific cell type of glioma origin is currently unknown, these brain tumors are probably originated from glial cells (or their precursors), the generic name of the non-neuronal cells that provide nutrients to neuronal cells, regulate metabolism and synaptic transmission. There are three main types of glial cells: astrocytes, oligodendrocytes and ependymal cells. Gliomas are classified as astrocytomas, oligodendrogliomas, oligoastocytomas (tumors presenting morphological features of both astrocytes and oligodendrocytes), or ependymomas, according to their presumable line of differentiation, that is, whether they resemble to these three types of glial cells. In the most widely accepted histological classification system of the CNS tumors, proposed by the World Health Organization (WHO) and recently revised (Louis, et al., 2007), each glioma type is further subdivided based on an increasing malignancy scale from grade I to IV, using a combination of criteria with predictive value in determining clinical outcome. Grade I tumors are biologically benign, displaying low proliferative potential and the possibility of cure following surgical resection alone. Grade II (diffuse) tumors are low-grade malignancies that may follow long clinical courses, with 5 to 8-years median survival, and some of these neoplasms tend to progress to higher grades of malignancy. Despite low-level proliferative activity, early diffuse infiltration of the surrounding brain renders them incurable by surgery. Grade III (anaplastic) tumors exhibit histological evidence of malignancy, including increased focal or dispersed anaplasia, cell proliferation, nuclear atypia and mitotic activity, and are more rapidly fatal, with survival times often less than 3 years. Grade IV designation is assigned to tumors exhibiting advanced cytologically malignant features, such as microvascular proliferation and necrosis, and as they are resistant to radio/chemotherapy they are generally lethal. Therefore, the type and grade of the tumor, as well as its localization, are currently recognized as the major factors determining patient prognosis.

GBM is classified as WHO grade IV astrocytomas due to the resemblance of the tumor cells to astrocytes, to the existence of microvascular proliferation and areas of necrosis (Brat, & Van Meir, 2004). This is a highly cellular tumor with pleomorphic, basophilic nuclei and either indistinct cytoplasmic borders or plump, pink cytoplasm with fibrillary backgrounds. Yet, even within a single tumor, the cellular composition can vary widely and mixed histologic features are typical. Most frequently, these tumors arise *de novo* as an aggressive highly invasive tumor, usually with no evidence of prior symptoms, antecedent or lower grade pathology - primary GBMs. Two-thirds of patients with this GBM subtype have a clinical history of <3 months (Ohgaki, et al., 2004), presenting a very rapid development of clinical symptoms. In contrast, secondary GBMs derive from the progressive transformation

of lower grade astrocytomas, with ~70% of grade II gliomas transforming into grade III/IV within 5–10 years of diagnosis (Maher, et al., 2001; Ohgaki, & Kleihues, 2009). True secondary GBMs are uncommon, accounting for ≤ 10% of all GBM cases (Ohgaki, et al., 2004). These two GBM types also affect patients at different ages: primary GBM constitute the great majority of GBM cases in older patients, while secondary GBM are quite rare and tend to occur in patients below the age of 45 years (Ohgaki, & Kleihues, 2007). Regardless of their distinct clinical histories or likely differences in prognosis and response to therapy, primary and secondary GBMs are usually histologically indistinguishable. Though both GBM subtypes achieve a common phenotypic endpoint, recent genomic profiles have revealed different transcriptional patterns and different DNA alterations, suggesting that these tumors arise through two different genetic pathways (Ohgaki, & Kleihues, 2007).

In addition to those subtypes, there are two other uncommon GBM variants, recognized as distinct clinico-pathological entities in the current WHO classification: i) gliosarcoma (GS) represents about 2% of GBMs and contains a biphasic growth pattern with clearly identifiable of both glial and sarcomatous components, the latter of which is associated with aberrant mesenchymal differentiation and is absent in all other glioma subtypes; ii) giant cell GBM (GC-GBM) constitutes approximately 5% of GBMs and is characterized by well-circumscribed masses of enlarged and bizarre tumor cells, often appearing multinucleated. This histological variant has been associated with longer survival (Shinojima, et al., 2004). In addition, other GBM subtypes with divergent patterns of differentiation have been described: i) small-cell GBM (SC-GBM), which can morphologically resemble anaplastic oligodendroglioma (AO), displays a monomorphic cell population characterized by small, round to slightly elongated, densely packed cells with mildly hyperchromatic nuclei and minimal discernible cytoplasm; ii) GBM with oligodendroglioma component (GBM-O), containing variable oligodendroglial foci, have a significantly better prognosis than other variants of GBMs (Klink, et al., 2010).

As these GBM subtypes/variants share similar morphologic features, precise GBMs subclassification is becoming increasingly important. Conventional histological analysis is still the primary methodology to determine diagnosis and treatment. Despite its current utility, it still relies on a subjective interpretation which has an impact on its reproducibility between different observers (Figarella-Branger, & Bouvier, 2005). Moreover, tumors sharing a common morphology can behave differently, even within the same tumor type and grading subgroup, which renders the histological classification of limited value in determining optimal therapy or response to treatment. Taken together, these evidences suggest that underlying genetic and molecular abnormalities, other than the histological parameters alone, may account for tumoral variability and could be predictive of clinical behavior and survival (Gravendeel, et al., 2009).

3. Cells of origin of GBM

GBMs display a rather heterogeneous cellular composition and, to date, the specific cells of origin for the formation of these malignant gliomas remain unknown. Traditional neuro-oncology postulated that tumors with an astrocytic phenotype arise from astrocytes. However, to undergo oncogenic events, mature glial cells would have to be proliferative and it is currently accepted that most brain cells do not undergo division, during adult life. Additionally, no epidemiological evidence indubitably relates processes likely to evoke

reactive proliferation or astrogliosis, such as trauma, with the development of glial tumors (Bondy, et al., 2008; Fisher, et al., 2007). On the other hand, gliomas mostly occur in adults, suggesting that their cells of origin must be present in the adult brain. It is now known that in addition to glial progenitor cells, neural stem cells (NSCs) also reside in the subventricular zone (SVZ) of adult human brain (Gil-Perotin, et al., 2009; Sanai, et al., 2005). During human development, the one important source of NSCs is the SVZ, and this region is widely viewed as the source of cells that can initiate primary brain tumors (Sanai, et al., 2005). Indeed, many gliomas are either periventricular or contiguous with the SVZ (Lim, et al., 2007).

The "neural-stem cell hypothesis" proposes that a rare subset of cells within GBM tumors may have significant expansion capacity and the ability to generate new tumors. The remainder of tumor cells, which predominantly constitutes GBM tumors, may represent partially differentiated cells with limited progenitor capacity or terminally differentiated cells that cannot form new tumors. Evidences sustain the observation that transformation of NSCs and their progenitors, through a sequence of genetic and/or epigenetic alterations, may lead to the formation of tumor-initiating cells (TICs) also in gliomas (Dietrich, et al., 2008; Singh, et al., 2004), rather than from a differentiated cell type. In fact, TICs are identified in the early stages of tumor development and may not yet have acquired the full tumorigenic potential of other premalignant cells with additional genetic mutations, referred to as cancer stem cells (CSCs). It was demonstrated that GBM-CSCs share some characteristics with normal NSCs, including the ability to self-renew and proliferate, as well as the capacity to engraft and migrate (Galli, et al., 2004; Sanai, et al., 2005; Singh, et al., 2003). The self-renewal ability of both NSCs and CSCs is provided by either symmetric cell division, which produces two daughter stem cells, or by asymmetric cell division, which produces a stem cell and a progenitor cell. The progenitor cell will then differentiate and migrate, while the stem cell retains the capacity of self-renewal and remains in the germinal zone (Berger, et al., 2004), maintaining intact the core population of stem cells. CSCs can also express stem cell markers typical of normal NSCs such as the cluster of differentiation 133 (CD133), also known as prominin-1 (originally described in mouse neuroepithelium and hematopoietic stem and progenitor cells), and the intermediate filament protein nestin (NES) (Galli, et al., 2004; Sanai, et al., 2005; Singh, et al., 2003). Several studies reported that CD133+ cells isolated from human brain tumor, when implanted into the brain of immunodeficient mice, were capable to generate a new and highly invasive tumor at low inoculation numbers, whereas CD133- GBM cells failed to have a tumor-initiating activity (Marumoto, et al., 2009; Singh, et al., 2004). However, recent studies indicate that CD133- cell subpopulation can also form orthotopic tumors, thus acting as brain tumor stem cells (Chen, et al., 2010). These evidences may partially reflect the presence of other types of GBM-CSCs devoid of CD133, which are not entirely defined by the expression of typical NSCs markers. Thus, the CD133 marker used to identify GBM-CSCs is likely only one of a variety of cell specific markers, still unknown to this point. Although the clinical value of CD133-expression in GBM tumors remains unclear, some groups have reported that the increased expression of CD133 was correlated with the poor prognosis and the decreased survival of glioma patient (Pallini, et al., 2008; Zeppernick, et al., 2008). Moreover, the frequency of CD133+ cells was shown to increase with tumor grade, suggesting that CD133 expression could serve as a prognostic factor for malignant progression and tumor regrowth. In this sense, several studies have provided evidence that GMB-CSCs may contribute to resistance to radiotherapy and chemotherapy in GBM (Eramo, et al., 2006;

Kang, & Kang, 2007). In CD133+ cells, the overexpression of drug resistance genes such as *BCRP1*, DNA-mismatch repair genes, such as *MGMT*, as well as genes related to the inhibition of the apoptosis (Juillerat-Jeanneret, et al., 2008), seems to contribute to the tumor's resistance to chemotherapy.

Like normal NSCs, brain CSCs are thought to reside in the perivascular area of the tumor, called "the vascular niche", where they are sheltered from apoptotic stimuli and maintain a proper balance between self-renewal and differentiation (Calabrese, et al., 2007; Gilbertson, & Rich, 2007). Evidences of reciprocal relationship between GBM-CSCs and their microenvironment demonstrated that CSCs not only receive the signals from surrounding niche, but are also capable of modulating it. It was also demonstrated that CSCs stimulated angiogenesis by secreting vascular endothelial growth factor (VEGF) but these cells also depended on the factors brought by vasculature (Calabrese, et al., 2007). Thus, GMB-CSCs and vascular niche may give positive feed-back to each other in order to promote tumor maintenance and expansion. Moreover, it was also hinted that vascular microenvironment might protect CSCs from chemo- and radiotherapies, enabling these cells to reform an initial clinical response (Dick, & Lapidot, 2005). The influence of the tumor microenvironment may also be reflected on the phenotypic endpoint of gliomas. In this way, the occurrence of specific genetic events on precursor cells or mature cells, in combination with the local environment, possibly alters the activity of particular cellular control pathways. Accordingly, progenitor cells that undergo *TP53* mutations generally activate pathways that induce astrocytic differentiation, whereas similar cells that undergo chromosomal loss of 1p and 19q most often become committed to an oligodendroglial phenotype (Alcantara Llaguno, et al., 2009). Several lines of evidence in the literature indicate that loss of *TP53* affects the properties of adult neural stem cells by providing a proliferative advantage (Armesilla-Diaz, et al., 2009; Gil-Perotin, et al., 2006). Regarding the effect of different tumorigenic events, more evidences come from targeting oncogenic stimuli to specific cell types. For instance, whereas overexpressing oncogenic Ras and Akt in progenitor cells results in mouse brain tumors that are histologically similar to human GBMs (Holland, et al., 2000), overexpression of platelet-derived growth factor-B (PDGF-B) in the same cells produces tumors histologically similar to oligodendrogliomas (Dai, et al., 2001). Taken together, these evidences point at the involvement of immature precursor cells in the development of tumor malignant phenotype. On the contrary, the existence of cells in the adult brain capable to revert to a less mature state, in response to certain stimuli (Canoll, & Goldman, 2008), supports the hypothesis that mature glial cells may be the target of transformation in gliomagenesis. In fact, these cells may be induced to dedifferentiate into transformed glia with stem cell–like properties, as observed in mice models, through retroviral transfection of EGF receptor (EGFR) in association with Ink4a/Arf (Bachoo, et al., 2002), or by generating pluripotent stem cells from mouse embryonic and adult differentiated fibroblast (Takahashi, & Yamanaka, 2006). However, the concept of dedifferentiation of mature glia is questionable and fails to explain adequately the origin of some gliomas, such as mixed oligoastrocytomas and gliosarcomas. These types are composed by a biphasic tissue pattern with areas of astrocytic and oligodendroglial differentiation or gliomatous and mesenchymal differentiation, respectively (Louis, et al., 2007). The presence of two morphologically distinct cell types within a tumor suggests either independent transformation events in two terminally differentiated cells or, more likely, the transformation of a single, bipotential progenitor cell (Chekenya, & Pilkington, 2002) that retains the capacity to differentiate into both cell types. If malignant

transformation occurs in the progenitor cell, one would expect a similar genetic profile in both cell types, especially on the level of chromosomal changes. Indeed, loss of heterozygosity (LOH) on chromosomes 1p and 19q was observed in both the astrocytic and oligodendrocytic components of mixed oligoastrocytomas, strongly suggesting a common cell of origin for both cells types (Kraus, et al., 1995).

Despite all the evidences already mentioned, GBM may develop from cells with different origins or via different molecular steps, which accounts for their heterogeneity. In this sense, the availability of more reliable and precise approaches for the classification of gliomas based on tumorigenic mechanisms would be extremely useful. Recently, integrated molecular genetic analysis has assumed an increasing part in the improvement of classification and therapeutic strategies for GBM patients.

4. Molecular characterization of GBM

It is recognized that morphologic changes observed during the process of malignant transformation, reflect the sequential acquisition of genetic alterations. So far, several studies have identified DNA copy number (CN) alterations, such as chromosomal and gene deletions, amplifications and gains, LOH and mutations as recurrent events in gliomagenesis, suggesting the involvement of tumor suppressor genes and oncogenes in tumor initiation or progression (Dahlback, et al., 2009; Furnari, et al., 2007a; Kotliarov, et al., 2006; Ohgaki, & Kleihues, 2009; Parsons, et al., 2008). CN changes are thought to affect genes expression either through overexpression of large amplification regions or inactivation of genes in deleted regions (Bralten, et al., 2010; Rao, et al., 2010). Ultimately, these genetic alterations seem to modify crucial cellular processes and often result in deregulation of metabolic key pathways affecting growth signaling and cell cycle control (Kanu, et al., 2009), as will be further discussed in the following section.

Over the past years, specific alterations associated with abnormal GBM features were identified, using classical cytogenetics and molecular methodologies, including interphase fluorescence *in situ* hybridization (iFISH) and comparative genomic hybridization (CGH). Given the large heterogeneity within GBMs, particular genetic changes may occur in some tumors and not in others, pointing out to distinct molecular subtypes of histologically defined GBM (Liang, et al., 2005). In fact, GBM profiling has identified differences in the molecular abnormalities observed between primary and secondary GBM. Specifically, primary GBMs typically harbor amplification and/or a high rate of *EGFR* mutation, cyclin-dependent kinase inhibitor 2A (*CDKN2A/p16*), deletion in chromosome 9p, and phosphatase and tensin homologue (*PTEN*) deletion in chromosome 10. The most common EGFR mutant type -variant 3 (EGFRvIII)- is due to an in-frame deletion of exons 2–7, and it is constitutively active in EGFR-mediated signal-transduction pathway, what confers cell proliferation and survival advantages (Ohgaki, & Kleihues, 2007; Van Meir, et al., 2010). The deletion in *PTEN* results in increased protein kinase B /mammalian target of rapamycin (AKT/mTOR) activity, which promotes cell survival, proliferation, and invasion (Louis, 2006; Sathornsumetee, & Rich, 2008). Recently, *ERBB2* mutations have also been identified as a recurrent event in primary GBMs. In contrast, secondary GBM are characterized by the occurrence of early detected mutations in the tumor suppressor gene *TP53* on chromosome 17p, especially those involving codons 248 and 273 or G:C→A:T mutations at CpG sites (Ohgaki, et al., 2004), abnormalities in the p16 and retinoblastoma (RB) pathways, and loss of heterozygosity of chromosome 10q. Isocitrate dehydrogenase 1 (*IDH1*) mutations have been recently identified as a very early and frequent

genetic alteration in the pathway to secondary GBMs, but rarely occurring in primary GBMs (Ichimura, et al., 2009).

Similarly, primary and secondary GBMs show significantly different mRNA and protein expression profiles, reflecting their significant differences in genetic alterations (Ohgaki, & Kleihues, 2007). Genes typically expressed in primary GBMs include *Fas* (Tohma, et al., 1998), *VEGF* (Karcher, et al., 2006), and *VEGF* fms-related tyrosine kinase 1, probably reflecting large ischemic necrosis (Tohma, et al., 1998). The insulin-like growth factor binding protein-2 (IGFBP-2), a modulator of the action of insulin-like growth factors, is also typically expressed at a high level in primary GBMs (Godard, et al., 2003). Primary GBMs also preferentially express genes characteristic of a stromal response, suggesting the importance of extracellular signaling (Tso, et al., 2006). The adipocyte lipid binding protein 1 (AEBP1), a transcriptional repressor that has been shown to interact with PTEN inhibiting its function (Reddy, S. P. et al., 2008), is up-regulated in the majority of primary GBMs. Proteins expressed uniquely in primary GBMs include tenascin-X precursor (TN-X), enolase 1 (ENO1), centrosome-associated protein 350 (CAP350), and EGFR.

On the other hand, achaete-scute complex homolog 1 (ASCL1), a protein that activates transcription and plays a role in the neuronal commitment and differentiation, is found to be highly overexpressed in secondary GBMs, which is accompanied by inhibition of Notch signaling (Somasundaram, et al., 2005). Other proteins typically expressed in secondary GBMs include excision repair cross-complementing 6 (ERCC6), dual oxidase 2 (DUOX2), heterogeneous nuclear ribonucleoprotein A3 (HNRPA3), WNT-11 protein precursor, cadherin-related tumor suppressor homolog precursor, ADAMTS-19 (Furuta, et al., 2004), platelet–derived growth factor-AB (PDGF-AB) (Karcher, et al., 2006), and PDGF receptor (PDGFR).

From the different genetic profiles recognized within GBM, it became clear that the underlying molecular characteristics of the tumor are essential to understand its variability and to identify potential therapeutic targets, however, do not allow by themselves to predict patient's outcome or response to treatment. Thus, in the past few years, efforts have been made to further characterize GBM into specific molecular subclasses, defined by combinations of over- or underexpressed genes with potential impact on survival. Recently, the availability of large-scale microarray-based genomic/expression profiling, together with advances in bioinformatics methods provided a comprehensive view of the genome-wide aberrations in malignant gliomas. Emerging studies integrating DNA CN alterations and gene expression data allowed refining GBM's classification (Liang, et al., 2005; Marko, et al., 2008; Mischel, et al., 2003; Tso, et al., 2006), and revealed a significant correlation between tumor subsets and survival (Freije, et al., 2004; Nutt, et al., 2003; Phillips, et al., 2006). Promising findings from whole-genome analysis came also from The Cancer Genome Atlas (TCGA) Research Network (The Cancer Genome Atlas (TCGA) Research Network, 2008; Verhaak, et al., 2010). In a recent multidimensional study expanding previous GBM classification, patterns of somatic mutations, DNA CN, and gene expression were assessed in a large cohort, to perform an integrated molecular classification of GBM (Verhaak, et al., 2010). Consequently, on the basis of prior naming and considering the expression profiles observed, four subtypes of tumors with a common morphologic diagnosis of GBMs were identified into Classical, Mesenchymal, Proneural and Neural. The first GBM subtype was labeled "Classical" since tumors of this subtype have a characteristic profile of highly proliferative cells and common gains on chromosome 7, paired with losses on chromosome

10 and frequent focal losses on chromosome 9p21.3. High-level amplification of the *EGFR* gene or gene rearrangements (point or vIII EGFR mutation) are frequently observed in this subtype and uncommon in other subtypes. Homozygous 9p21.3 deletion, targeting *CDKN2A*, is almost mutually exclusive with aberrations of other RB pathway components, such as *Rb1*, *CDK4*, and *CCDN2*, suggesting that the RB pathway is almost exclusively affected through *CDKN2A* deletion, in samples with focal *EGFR* amplification. Alterations in the *TP53*, neurofibromin 1*(NF1)*, *PDGFRA*, or *IDH1* genes are nearly absent. Classical GBMs are responsiveness to the radiation and chemotherapies. At the gene expression level, the Classical subtype demonstrates elevated expression of the neural precursor and stem cell marker *NES*, and both Notch (*NOTCH3*, *JAG1*, and *LFNG*) and Sonic hedgehog (*SMO*, *GAS1*, and *GLI2*) signaling pathways.

The second GBM subtype was labeled as "Mesenchymal" since tumors display overexpression of mesenchymal markers, such as *CHI3L1/YKL40* and *MET* genes, as well as the astrocytic markers *CD44* and *MERTK*. Mesenchymal GBMs have frequent inactivation of the *NF1*, *TP53*, and *PTEN* genes. Comutations of *NF1* and *PTEN*, both intersecting with the AKT pathway, are also observed in the Mesenchymal subtype. Genes in the tumor necrosis factor (TNF) super family and NF-kB pathways are highly expressed in this subtype, potentially as a consequence of higher overall necrosis and associated inflammatory infiltrates. These tumors are responsive to aggressive chemotherapy and radiation therapies and might in addition be responsive to Ras, phosphoinositide 3-kinase (PI3K), and angiogenesis inhibitors.

The third GBM subtype was called "Proneural," and these tumors are characterized by an expression profile reminiscent of gene activation in neuronal development. Thus, they present activation of oligodendrocytic (*PDGFRA*, *OLIG2*, *TCF3*, and *NKX2-2*) and proneural (*SOX*, *DCX*, *DLL3*, *ASCL1*, and *TCF4*) development genes. These tumors are also characterized by *TP53* gene mutations and LOH, and mutations in *PDGFRA*, *IDH1*, and *PIK3CA/PIK3R1* genes are also observed. Focal amplification of the *PDGFRA* locus, associated with high levels of *PDGFRA* expression, is seen almost exclusively in this tumor type. Interestingly, *PIK3CA/PIK3R1* mutations in the Proneural subtype is mostly observed in samples with no *PDGFRA* abnormalities. Amplification of chromosome 7 and loss on chromosome 10 are significant, but less frequent findings than in the Classical subtype. This GBM subtype may be responsive to inhibitors of the hypoxia-inducible factor (HIF), PI3K, and PDGFRA pathways. In this group, patients are younger and survival is slightly better than in the other 3 tumor subtypes.

The fourth GBM subtype called "Neural" is less well defined and has gene expression signatures similar to those found in normal brain tissue, with activation of neuron markers such as *NEFL*, *GABRA1*, *SYT1*, and *SLC12A5*. These tumors demonstrate a low degree of infiltration by normal cells; nevertheless, their expression signature is suggestive of cells with a differentiated phenotype.

Despite the existence of well defined differences in the genetic profile of the four subtypes, all tumor subgroups present inactivation of the p53 and RB tumor suppressor pathways, and activation of the receptor tyrosine kinase pathway. These observations suggest rather strongly that although molecular genetic profiling may eventually guide therapeutic decisions for patients with specific GBM subtypes, further studies linking the genetic alterations and the consequent affected signaling pathways, may be the key to better understand the occurrence and progression of these tumors.

5. Signaling pathways altered in GBM

5.1 EGFR/Akt/PTEN pathway

EGFR is a member of the erbB family receptor tyrosine kinase proteins, which comprises EGFR itself (ErbB1) (EGFR/HER1), ErbB2 (HER2/neu), ErbB3 (HER3), and ErbB4 (HER4) (Mellinghoff, et al., 2005; Ranson, 2004). These receptors are composed of an extracellular ligand-binding domain, a transmembrane lipophilic domain, and an intracellular tyrosine kinase domain. The binding of a specific set of ligands [EGF; transforming growth factor-α (TGF-α)] to the receptor promotes EGFR dimerization and the autophosphorylation of the receptors on tyrosine residues (Arora, & Scholar, 2005; Baselga, 2006). Upon autophosphorylation, several signal transduction pathways downstream of EGFR become activated such as the Ras/Raf/mitogen-activated protein kinase pathway, the PI3K/Akt pathway, protein kinase C (PKC), the signal transduction and activator of transcription (STAT), an the VEGF among others (Arora, & Scholar, 2005; Baselga, 2006). Taking in consideration that these signaling pathways control cell growth, regulate cell survival and apoptosis and that it was reported and overexpression of EGFR in several epithelial tumors, the EGFR/Akt/PTEN pathway is considered a potential target for cancer therapy (Arora, & Scholar, 2005; Baselga, 2006).

Regarding the activation of PI3K by EGFR it is known that there is a recruitment of PI3K to the cell membrane and phosphorylation of phosphatidynositol-4,5-bisphosphate to the respective 3-phosphate (PIP3), which in turns activates downstream effectors molecules such as Akt (Hirose, et al., 2005; Furnari, et al., 2007). Activated Akt regulates the function of downstream signaling proteins involved in cell cycle, proliferation, apoptosis and invasion, therefore, once deregulated, Akt may contribute to tumorigenesis. Activated Akt deregulates cell growth by stabilization of cyclin D and promotion of the nuclear entry of the mouse double minute protein 2 (MDM2), leading to the degradation of p53 (Han, et al., 2010). Akt might also inhibit p21 expression through its phosphorylation and activation of MDM2 (Zhou, et al., 2001). On the other hand, activated Akt exerts anti-apoptotic activity by phosphorylating and inactivating pro-apoptotic signaling proteins, such as BAD and caspase 9 (Dasari, et al., 2010). The involvement of Akt in diverse tumorigenic activities suggests that Akt activation alone might be sufficient to induce cancer (Testa, & Bellacosa, 2001). Moreover, Akt activation may contribute to tumor invasion/metastasis by stimulating secretion of matrix metalloproteinases (Dasari, et al., 2010). The action of PI3K enzyme is directly antagonized by a phosphatase encoded by the *PTEN* gene located at 10q23.3.

PTEN is a tumor suppressor gene that encodes a central domain with homology to the catalytic region of protein tyrosine phosphatases. For that reason, PTEN is a negative regulator of the PI3K pathway by removing the third phosphate from the inositol ring of the second messenger PIP3 (Mellinghoff, et al., 2005). Therefore, PTEN inactivation results in accumulation of PIP3 levels and persistent signaling through the serine/threonine kinase Akt. In addition, the amino terminal domain of PTEN, with homology to tensin and auxilin, is important in regulating cell migration and invasion by directly dephosphorylating focal adhesion kinase (FAK) (Furnari, et al., 2007; Ohgaki, & Kleihues, 2007). There are also some reports indicating that PTEN plays a significant role in inducing G1 cell cycle arrest and apoptosis, along with regulating cell differentiation (Di Cristofano, & Pandolfi, 2000).

As it is referred, *EGFR* mutation was detected in several tumor types. In GBMs, amplification of the *EGFR* gene occurs in 40% of primary GBMs but rarely in secondary

GBMs. EGFR overexpression is also more common in primary GBMs (60%) than in secondary GBMs (10%). All primary GBMs with amplification of the *EGFR* gene, also show EGFR overexpression (Ohgaki, & Kleihues, 2007; Van Meir, et al., 2010). EGFR amplicons are often mutated, being the most frequent type the variant 3 (EGFRvIII) with an in-frame deletion of exons 2 to 7 within the extracellular ligand-binding domain. This in-frame deletion is associated with constitutive activation of the receptor and failure to attenuate signaling by receptor down-regulation (Ohgaki, & Kleihues, 2007; Van Meir, et al., 2010). The constitutively active EGFRvIII can enhance cell proliferation through activation of the PI3K/Akt pathway and therefore may play a very important role in gliomagenesis (Mellinghoff, et al., 2005; Mizoguchi, et al., 2006).

For this reason, EGFR has been a prime target for therapeutic intervention in GBM with small molecule kinase inhibitors, antibody-based immunotherapy and, more recently, with small interfering RNA (siRNA)-directed neutralization of either wild-type EGFR or the EGFRvIII allele (Van Meir, et al., 2010). In fact, several small-molecule EGFR-inhibitors such as gefitinib and erlotinib have been used in clinical trials but progression-free survival was not prolonged (van den Bent, et al., 2009). The inability to increase survival of GBM patients was also observed with lapatanib (EGFR/HER-2 inhibitor) (Neyns, et al., 2009; Thiessen, et al., 2009), and with the cetuximab, the monoclonal antibody against EGFR (Neyns, et al., 2009).

The unsuccess of EGFR inhibitors in GBM treatment seems to be associated to the inactivation of PTEN and to the activation of the PI3K/Akt. Mutations in *PTEN* were detected in 15 to 40% of GBMs and almost exclusively in primary GBMs (Ohgaki, & Kleihues, P., 2007; Zhou, et al., 1999). PTEN loss could thus promote resistance to EGFR kinase inhibitors by dissociating EGFR/EGFRvIII inhibition from downstream inhibition of the PI3K signaling pathway.

The activation of PI3K/Akt pathway could also be due to the occurrence of mutations. In fact, it was reported that in up to 10% of GBMs, Akt activation is due to mutations that amplify or activate the catalytic and regulatory subunits of PI3K (Stommel, et al., 2007; The Cancer Genome Atlas (TCGA) Research Network, 2008). It was also reported that PI3K could be activated by c-MET and PDGFR alpha, which could be co-activated in EGFR-amplified tumors (Furnari, et al., 2007).

Taken together, these results indicate that PI3K activation plays a central role in the resistance of GBM to EGFR inhibitors, since there are several mechanisms that may provide alternative routes for maintaining PI3K activation. Haas-Kogan et al. demonstrated *in vitro* and in glioma patients that high levels of EGFR coupled with low levels of activated Akt were associated with a favorable response to EGFR inhibitors (Haas-Kogan, et al., 2005). Therefore, a possible therapeutic strategy in GBM should control the activation of EGFR and the activation of PI3K or key mediators of PI3K. However, since PI3K upon activation may potentially phosphorylate several proteins involved in cell proliferation, apoptosis and migration, it remains to be determined which downstream effectors of Akt are most critical for gliomagenesis. One of those downstream effectors, associated with increased cell proliferation, is the serine/threonine kinase mTOR which forms the catalytic core of at least two functionally distinct complexes (mTORC1 and mTORC2). mTOR integrates growth-inhibitory signals such as deprivation of glucose and amino acids, ATP depletion, hypoxia and lack of growth factors, in order to generate adaptive cellular responses (Wullschleger, et al., 2006). Deregulated mTOR signaling sustains proliferation of malignant cells by

antagonizing these physiological starvation signals (Wullschleger, et al., 2006). Therefore, it seems plausible that the commonly observed activation of mTOR signaling in GBM contributes to the typical pattern of GBM pathology, with necrotic cores and aggressive growth at the tumor margins (Masri, et al., 2007).

Once activated, the mechanism of mTOR action could occur through different mechanisms depending on the activated complex: mTOR complex 1 or complex 2. The mTORC2 is composed by rictor (rapamycin-insensitive regulator of mTOR), mSIN1, protor and mLST8. This complex is activated by PI3K, phosphorylates Akt on serine 473 (Ser473) of its C-terminal hydrophobic motif, promoting maximal Akt activity and acting as an upstream activator of Akt (Foster, & Fingar, 2010). mTORC2 also activates additional kinases, including serum glucocorticoid-induced protein kinase and PKCα, all of which may play important roles in regulating cellular proliferation and growth (Foster, & Fingar, 2010). mTORC2 activity is elevated in glioma cell lines and seems to enhance motility and to promote glioma cell proliferation *in vitro* (Akhavan, et al., 2010). Further, in a recent drosophila model of gliomagenesis promoted by constitutive coactivation of EGFR-Ras-Akt, mTORC2 activity was required for glial proliferation suggesting that mTORC2 signaling is essential for growth in the context of enhanced signal flux through the PI3K pathway (Akhavan, et al., 2010; Read, et al., 2009). However, the mechanism of mTORC2 is not clearly understood and future studies will be needed to determine the role of mTORC2 in gliomagenesis.

The mTOR complex 1 (mTORC1 or the mTOR-raptor complex which is rapamycin-sensitive) is regulated by both nutrients and growth factor signaling. mTORC1 acts as a downstream effector of PI3K/Akt signaling, regulates the translation initiation and ribosome biogenesis and plays a role in cell growth, proliferation and survival (Akhavan, et al., 2010; Foster, & Fingar, 2010; Manning, & Cantley, 2007). The translational effects of mTOR1 signaling are mediated by phosphorylation of its two direct target molecules, eukaryotic translation initiation factor 4E binding protein 1 (4E-BP1) and ribosomal protein S6 kinase 1 (S6K1), at specific sites. In a serial cell line transformation model, mTORC1 signaling through S6K1 was required for glioma formation, highlighting its functional importance (Akhavan, et al., 2010; Foster, & Fingar, 2010; Manning, & Cantley, 2007).

In the preclinical studies, mTOR inhibitors, specially rapamycin, were very promising since it was reported an increase in apoptotic and non-apoptotic cell death (Rao, et al., 2005), cell cycle arrest (Tanaka, et al., 2007) and decreased angiogenesis (Del Bufalo, et al., 2006). However, the results from phase I and II single-agent clinical trials of rapamycin conducted in glioma were disappointing (Heimberger, et al., 2005). The unsuccess could be related to the fact that mTORC1 plays a dual role as a positive and negative regulator of PI3K/Akt signaling and to the inability of rapamycin and its derivatives to target mTORC2. Recently, it was suggested a different interpretation of the clinical failure of rapamycin in GBM patients, namely, that the lack of efficacy is a consequence of incomplete inhibition rather than an injudicious choice of molecular target (Cloughesy, & Mischel, 2011). Cloughesy and Mischel hypothesize that since mTORC1 is both a positive and a negative regulator of the PI3K/Akt signaling pathway, the treatment with rapamycin causes derepression of mTORC1-mediated feedback and may paradoxically induce a more rapid clinical progression by promoting PI3K activity in GBM patients treated with rapamycin. To test this possibility, rapamycin treatment was reinstituted in patients after surgery and patients were monitored for progression. The results showed that rapamycin treatment led to Akt activation in seven of 14 patients, presumably due to loss of negative feedback, which was

associated with a significantly shorter time to progression, during postsurgical maintenance of rapamycin therapy (Guertin, & Sabatini, 2009). These results highlight the importance of pathway cross-talk in determining response to targeted therapy and put in evidence the importance of combined therapy (Cloughesy, & Mischel, 2011).

In fact, in the last years, several clinical trials used dual PI3K/mTOR inhibitors. Some of those inhibitors showed efficacy against a panel of GBM cell lines but, surprisingly, in preclinical GBM models, dual PI3K/mTOR inhibitors have failed to promote tumor cell apoptosis (Maira, et al., 2008). The remarkable plasticity of tumor cells was recently demonstrated in studies showing that mTORC1 inhibition leads to MAPK pathway activation through a PI3K-dependent mechanism (Carracedo, et al., 2008; Cloughesy, et al., 2008).

Mitogen-activated protein kinases (MAPKs) are serine/threonine protein kinases that transmit signals from the membrane to the nucleus in response to growth factors and cellular stress. Three principal isoforms of MAPKs exist: extracellular signal regulated kinase (ERK), c-jun NH2-terminal kinase (JNK) and p38 MAPK (p38). Activation of MAPKs occurs through phosphorylation of threonine and tyrosine residues of the Thr-X-Tyr motif by MAPK kinases (MEKs). In GBM, the existence of mutations affecting the expression or the activity of these serine/threonine protein kinases was not reported. However, since MAPKs may contribute to cellular proliferation and could be activated in response to the inhibition of PI3K/mTOR, its role in gliomagnesisis is under investigation.

In spite of the unsuccess of the mTOR inhibitors, the mTOR signaling pathway remains one of the most important therapeutic targets and several studies are being carried on, in order to evaluate the role of mTORC2 and of mTORC1, the interaction between PI3K/mTOR, and the interaction between mTOR, EGFR and MAPK.

5.2 p53 and cell cycle progression

The tumor suppressor p53 encoded by *TP53* gene was discovered in 1979 (DeLeo, et al., 1979; Lane, & Crawford, 1979; Linzer, & Levine, 1979). At the beginning, p53 was considered a tumor antigen but today it is known that the wild-type gene product functions as a tumor suppressor that regulates cell cycle progression and apoptosis in response to a wide variety of stress signals, including DNA damage (Baker, et al., 1989). When cells are exposed to various genotoxic agents, p53 is stabilized, accumulates in the nucleus, binds and transcriptionally regulates the promoters of >2500 potential effectors genes (Levine, et al., 2006). This leads to the expression of cell cycle checkpoint proteins and prevents the propagation of cells with unstable genomes, predominantly by halting the cell cycle in the G1 phase, or by initiating a program of apoptosis or proliferative arrest (Van Meir, et al., 2010). The best characterized of these effectors is the *p21* gene, which once transcriptionally activated blocks cell cycle progression in the G1 phase by inhibiting the function of the cyclin D family of proteins (Van Meir, et al., 2010). The cyclin D family is constituted by the regulatory subunits of the cyclin/cyclin–dependent kinase complexes that regulate cell cycle entry and progression by inducing the phosphorylation and inactivation of RB (Van Meir, et al., 2010). Due to the ability to coordinate the cellular responses to cellular stress factors, p53 was called "cellular gatekeeper" (Levine, 1997) or "the guardian of the genome" (Lane, 1992).

Since p53 function is critical for normal cell growth and development, its activity is tightly regulated by phosphorylation, which is the first step to induce stabilization of p53, and also through its interaction with MDM2, the endogenous E3-ligase that targets p53 for

proteasome degradation (Michael, & Oren, 2003). MDM2 itself is transcriptionally activated by p53, thus creating a negative feedback loop that maintains p53 at very low levels in the absence of cellular damage. Upon stress, particularly DNA damage, Ser15 and Ser20 within the p53 N-terminal are phosphorylated by a broad range of kinases, including ATM, ATR, DNA-PK, Chk1, and Chk2 in a manner that prevents MDM2 from interacting with p53, thus resulting in p53 stabilization (Soussi, & Wiman, 2007; Wade, et al., 2010). Another mechanism to stabilize p53 is by direct inhibition of the MDM2 ubiquitin ligase activity through its association with Arf, the alternative transcript of the Ink4a tumor suppressor locus, which may induce G1- and G2-phase arrest (Matheu, et al., 2008).

In 50% of the human tumors, the p53 pathway is inactivated via indirect mechanisms such as MDM2 amplification, viral infection or loss of Arf, leading to p53 destabilization (Brooks, & Gu, 2006). The other 50% of the tumors present missense mutations that originate a subset of p53 mutant proteins with oncogenic properties that may contribute to the neoplastic transformation (Soussi, & Wiman, 2007; Wade, et al., 2010).

In GBM, *TP53* is the most frequently mutated gene, and the majority of its mutations occur in "Proneural" subtype of GBM samples (The Cancer Genome Atlas (TCGA) Research Network, 2008; Verhaak, et al., 2010). In addition, the TCGA reported that mutations are clustered in the DNA binding domain, a hotspot for *p53* mutations in human cancers (The Cancer Genome Atlas (TCGA) Research Network, 2008). In secondary GBM, loss of p53 is detectable in two thirds of the tumors and may occur through either point mutations that prevent DNA binding, or loss of chromosome 17p. In primary GBM, *TP53* inactivation is now considered a common event which may occur via amplification or overexpression of the p53 negative regulators MDM2 and MDM4, which suppresses p53's transcriptional activity (Foster, & Fingar, 2010; Ohgaki, et al., 2004; Zheng, et al., 2008).

According to the TCGA, in GBM the inactivation of the p53 pathway occurs in the form of *Arf* deletions (55%), amplifications of *MDM2* (11%) and *MDM4* (4%), in addition to mutations of p53 itself (The Cancer Genome Atlas (TCGA) Research Network, 2008). Among the 91 samples sequenced by this Network, genetic lesions in *TP53* were mutually exclusive of those in *MDM2* or *MDM4* but not of those in ARF (encoded by *CDKN2A*).

Since MDM2 is considered the main regulator of p53, the inhibition of p53-MDM2 interaction is regarded as having therapeutic potential. A number of different strategies have been employed to develop small molecules that bind specifically to the N-terminal region of MDM2, that interacts with p53 (Shangary, & Wang, 2009). The most promising candidate described so far has been nutlin-3A (Vassilev, et al., 2004). In fact, glioma C6 cells were quite sensitive to nutlin-3A treatment but further studies will be needed to understand the real potential of this strategy (Merkel, et al., 2010).

5.3 Retinoblastoma signaling pathway

The RB pathway consists of several proteins: the cyclin-dependent kinase inhibitor (CDKN), the cyclin D-dependent protein kinases (cdk4, cdk6), the E2F-family of transcription factors (heterodimers of E2F1-8 with DP1-2) and the RB-family (RB, p107, p130) (Knudsen, & Wang, 2010; Scambia, et al., 2006; Van Meir, et al., 2010). The *Rb* gene is localized on the 13q14 chromosome and was the first known human suppressor of tumors (Huang, et al., 1988). The RB family consists of pRb/p105, p107 and pRb2/p130 which have an homologous functional domain known as the pocket region. This region allows the interaction of RB proteins with multiple peptides and the assembly of nuclear protein-complexes that regulate transcription factor activities, G1-phase cell cycle arrest, cell proliferation and differentiation

(Weinberg, 1995). Since RB is phosphorylated and dephosphorylated during the cell cycle, its activity depends on the phosphorylation status: the hyperphosphorylated (inactive) form predominates in proliferating cells, whereas the hypophosphorylated (active) form is generally more abundant in quiescent or differentiating cells (Harbour, & Dean, 2000). In quiescent cells, hypophosphorylated RB blocks proliferation by binding and sequestering the E2F family of transcription factors, which prevents the activation of genes essential for cell cycle progression, ensuring that transcription factors remain inactive during M and G0 phases (Furnari, et al., 2007). In proliferating cells, growth factors lead to the induction of cyclin D1, as well as to the degradation of the Cdk2/cyclin E inhibitor, p27Kip1 (White, et al., 2005). These activated Cdk complexes trigger the phosphorylation of RB in late G1-phase, which is maintained in S-, G2-, and M-phases. The pRB enables E2F release leading to transcriptional activation of growth promoting genes which are required for transcription of cellular genes that participate in growth control and DNA synthesis (Furnari, et al., 2007; Harbour, & Dean, 2000; Malumbres, & Barbacid, 2001). Among the E2F-regulated genes are cyclin E, cyclin A2 and Emi1, which are key effectors of the G1/S transition in normal cells (Furnari, et al., 2007; Knudsen, & Wang, 2010).

One of the negative regulators of the RB signaling pathway is the Ink4-family of proteins, p16Ink4a, p15Ink4b, p18Ink4c, and p19Ink4d which are small heat-stable proteins containing the AKN (ankyrin repeat) domain (Knudsen, & Wang, 2010). Each of the Ink4 proteins can bind to and inhibit the activity of Cdk4 and Cdk6. The Ink4 proteins compete with the D-cyclins for Cdk4/6 to prevent the formation of the active kinase complex that phosphorylates pRB (Knudsen, & Wang, 2010).

In the comprehensive analysis of the genome and transcriptome of 206 primary GBM tumors performed by the TCGA project, the sequence of 601 genes in 91 of the 206 tumor samples was analyzed. The results have shown that the RB pathway was altered in 78% of the primary GBM tumor samples, directly by mutations, deletions or promoter methylations at *Rb* loci, as well as indirectly by alterations on the positive or negative regulators (The Cancer Genome Atlas (TCGA) Research Network, 2008). *Rb* promoter methylation and gene silencing were more frequently found in secondary GBMs (43%) than in primary GBMs (14%) (Grzmil, & Hemmings, 2010; Xiao, et al., 2002). The alterations in the RB-pathway included homozygous deletion and mutation of *CDKN2A* (p16Ink4a) and *Rb1* (RB) in 52% and 11% of the samples, respectively, and homozygous deletion of *CDKN2B* (p15Ink4b) and *CDKN2C* (p18Ink4c) in 47% and 2% of the tumor samples, respectively. On the other hand, the *CDK4*, *CDK6*, and *CCND2* (cyclin D2) genes were amplified in 18%, 1%, and 2% of the GBM tumors examined (The Cancer Genome Atlas (TCGA) Research Network, 2008).

In spite of the importance of RB pathway, several *in vitro* and *in vivo* studies demonstrated that the neutralization of this pathway alone is insufficient to abrogate cell cycle control, suggesting that cell cycle is also regulated by other signaling pathways (Furnari, et al., 2007).

In the last years, several studies have been performed in order to establish a therapeutic strategy based on the manipulation of the cyclin–RB–E2F pathway. The development of small molecules that specifically inhibit certain cyclin/CDK complexes, or that could affect E2F-dependent transcription, as well as peptides that can mimic the functional regions of RB proteins inactivated in specific tumors, is currently under investigation (Scambia, et al., 2006).

5.4 Vascular endothelial growth factors

Vascular endothelial growth factors (VEGFs) were originally described as vascular permeability factors (VPF), since they were released by tumor cells that promote vascular leakage (Keck, et al., 1989). VEGFs are predominantly produced by endothelial, hematopoietic and stromal cells in response to hypoxia, and upon stimulation with growth factors such as transforming growth factors, interleukins or PDGF.

VEGFs are dimeric cysteine-linked secreted glycoproteins with a molecular weight of approximately 40 kDa. In mammals, VEGFs are encoded by a family of genes that includes *VEGF-A, -B, -C, -D* and *PlGF* (Cebe-Suarez, et al., 2006; Van Meir, et al., 2010).

VEGFs bind to three variants of type III receptor tyrosine kinases, VEGF receptor 1, 2 and 3 (Cebe-Suarez, et al., 2006). The binding of VEGF to its cognate receptors induces dimerization and subsequent phosphorylation of the receptors, leading to the activation of several intracellular signaling molecules such as PI3K, phospholipase Cg (PLCg), PKC, nitric oxide synthase (NOS), MAPKs and focal adhesion kinases (FAKs) (Karkkainen, & Petrova, 2000).

VEGF has been demonstrated to be one of the dominant drivers of angiogenesis contributing to tumor growth and progression (Criscuolo, G. R. et al., 1990). Due to this role, inhibitors of VEGF are widely used in cancer therapy in order to reduce angiogenesis (Ferrara, & Kerbel, 2005; Huang, et al., 2003), to cause regression of tumor vessels, and to reduce tumor growth (Huang, et al., 2003).

Extensive experimental data support the concept that angiogenesis is required for GBM growth (Jain, et al., 2007; Schmidt, et al., 1999; Van Meir, et al., 2010). The process is driven primarily by tumor secreted VEGF-A, but there are a large number of alternative secreted proangiogenic factors, including basic FGF (bFGF), angiopoietins, PDGF, interleukin-8 (IL-8), and hepatocyte growth factor/scatter factor (HGF/SF). Endothelial cells in the vicinity of the tumor express VEGFR2, which establishes a paracrine signaling loop that stimulates endothelial cell growth and proliferation. The level of VEGF production in a tumor increases with the degree of malignancy. In a study of surgical glioma specimens, high-grade tumors produced greater than 10-fold more VEGF compared with low-grade tumors (Schmidt, et al., 1999). It was also demonstrated that EGFR activation transcriptionally upregulates VEGF expression in GBM cells (Mischel, et al., 2003).

A targeted molecular therapy for GBM included agents that interfere with angiogenesis. The early trials with the first generation of anti-angiogenic agents (for example, thalidomide), conducted in GBM patients, had disappointing results (Jain, et al., 2007). The new generation of antiangiogenic drugs evaluated in clinical trials uses the anti-VEGF antibody bevacizumab, which neutralizes VEGF, and the VEGF-receptor tyrosine kinase inhibitors (TKIs) (sorafinib and sunitinib) (Jain, et al., 2007). Bevacizumab, a humanized anti-VEGF antibody, has shown promising results in exploratory phase II trials of recurrent GBM. However, these results are based on small patient cohorts and, because anti-angiogenic agents directly affect vessel permeability, the imaging response assessment based on contrast enhancement (CE) is highly ambiguous (Vredenburgh, et al., 2007). However, there is increasing interest in targeting proangiogenic molecules that function by alternative mechanisms (Van Meir, et al., 2010). For example, the neuropilins are nontyrosine kinase receptors that are activated by VEGF binding and potentiate VEGFR signaling. The angiopoietins (Ang-1 and Ang-2) are involved in the stability and maintenance of the tumor vasculature. Binding of Ang-2 to its cognate receptor, Tie-2 destabilizes vessels, which is a requirement for angiogenesis to proceed (Holash, et al., 1999). Inhibitors of specific chemotactic signaling may also have therapeutic value, since tumor cells secrete chemokines

that serve to recruit proangiogenic myeloid cells to the tumor (Van Meir, et al., 2010). However, it must be noted that antibodies and many of these low-molecular weight compounds may not cross the blood-brain barrier.

6. Conclusion

Despite all the evidences already mentioned, the mechanism of gliomagenesis and the cells of origin for GBM remain unknown. Several studies suggest that different cells of origin give rise to distinct types of GBM-CSC. Thus, GBM may also develop differently from different cells of origin or via different molecular steps, which accounts for their heterogeneity. From the therapeutic point of view, the inter- and intratumoral heterogeneity presents a unique challenge for GBM treatment.

In the last years, many progresses were done in order to identify the molecular signature of GBM, and these recent studies confirmed that each GBM is characterized by alterations in several signaling pathways that control cellular proliferation and apoptosis such as EGFR/mTOR/Akt/PTEN, p53, RB, VEGF, which contribute to the unsuccess of monotherapies and put in evidence the need to perform combined therapy, adjusted to the genetic and molecular alterations of each patient.

In addition, these studies reinforced the remarkable plasticity of GBM cells that allow cells to switch from one signaling pathway to another, according to the difficulties imposed by the therapeutic agents.

Thus, combined therapeutic strategies, targeting both the brain tumor stem cells and the molecular alterations that characterize the brain tumor of each patient, could turn out to be the most effective approach to increase the survival of GBM patients.

7. References

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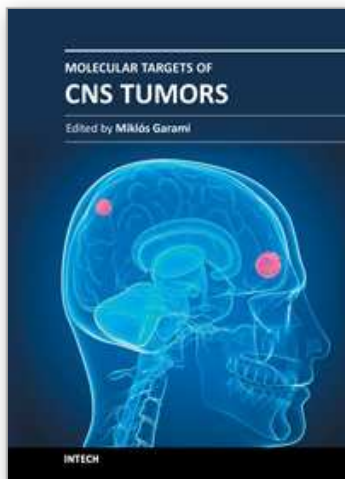
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