Chapter 11
Glioblastoma Cancer Stem Cells

Luca Persano, Alessandro Della Puppa, Elena Porcù, Francesca Maule, and Giampietro Viola

Abstract Many types of cancer, including Glioblastoma (GBM), contain functionally subsets of cells with stem-like properties named cancer stem cells (CSCs). These are characterized by chemotherapy resistance and considered one of the key determinants driving tumor relapse. Many studies demonstrated that glioma stem cells (GSCs) reside in particular tumor niches that are necessary to support their behaviour. Indeed, the microenvironment is essential for GBM tumorigenesis and progression, particularly for the continuous signal communications between GSCs and cells belonging to the GBM niches, like endothelium or pericytes, which give rise to a complex plasticity of the tumor. This signal integration originates numerous mechanisms which lead to resistance to therapy. Understanding the mechanism of action of the microenvironmental signals and the interplay between different cell types within the tumor mass, open new questions on how GSCs modulate GBM aggressiveness and response to therapy. The definition of these tumor features will allow to setup innovative multimodal therapies able to target GBM cells at multiple levels. In this chapter, we will discuss the major advances in the study of GSCs role in GBM and the therapeutic implications resulting from them, thus reporting the development of new targeted-therapies applied to counteract and overcome GBM intrinsic resistance to therapy which could improve the overall therapeutic ratio of conventional treatments.

Keywords Glioblastoma multiforme • Cancer stem cells • Glioblastoma cancer stem cells • Hypoxia • Vascular niche

L. Persano
Corso Stati Uniti 4, Pedriatric Research Institute-IRP, 35127 Padova, Italy

Department of Woman's and Child's Health, Oncohematology Laboratory, University of Padova, Via Giustiniani 3, 35128 Padova, Italy

A. Della Puppa
Division of Neurosurgery, University-Hospital of Padova, Via Giustiniani 2, 35128 Padova, Italy

E. Porcù • F. Maule • G. Viola (✉)
Department of Woman's and Child's Health, Oncohematology Laboratory, University of Padova, Via Giustiniani 3, 35128 Padova, Italy
e-mail: giampietro.viola.1@unipd.it

S. Babashah (ed.), *Cancer Stem Cells: Emerging Concepts and Future Perspectives in Translational Oncology*, DOI 10.1007/978-3-319-21030-8_11

1 Introduction

The term 'glioma' is referred to all tumors that are thought to be of glial cell origin. As described by the World Health Organization (WHO) classification (Louis et al. 2007), malignant diffuse gliomas are comprised of astrocytic, oligodengroglial, and mixed oligoastrocytic neoplasms based solely on morphology and are further subdivided by tumor grade based on additional histological features in the tumor. Nuclear atypias and mitotic activity are required criteria for grade III lesions, and the presence of necrosis or microvascular proliferation is required for the diagnosis of grade IV astrocytomas, named glioblastoma. Glioblastoma (GBM) is the most common and lethal primary malignant brain tumor. Together with grade III anaplastic astrocytoma, these tumors embrace the clinical entity termed "malignant glioma."

Extensive genomic characterization has recently provided a high resolution picture of the molecular alterations underlying this tumor providing the emerging view that "GBM" represents several histologically similar but molecularly heterogeneous diseases, thus influencing classification systems, prognosis, and therapeutic decisions. GBM represents the most common primary intrinsic malignant brain tumor diagnosed each year in the United States; there are ~10,000 new diagnoses annually, and >50,000 patients are currently living with the disease (Dolecek et al. 2012). All gliomas are more common in men than in women. GBM is associated with the highest median age at diagnosis. Examination of brain tumor incidence data from CBTRUS for the 10-year period from 1985 to 1994 revealed a slight but statistically significant average annual percentage increase in incidence (0.9 %). It is likely, however, that most, if not all, of this increase is attributable to improvements in diagnostic imaging and increased availability of medical care and neurosurgeons. While 90–95 % of GBM arise de novo and are considered "primary," about 5–10 % arise from lower-grade gliomas in younger patients and are termed "secondary" (Ohgaki and Kleihues 2005). Although many risk factors for developing GBM have remained unidentified, risk factors such as exposure to ionizing radiation have proven to be detrimental for disease development in some cases. Other risk factors including cell phone use, head trauma, and pesticide exposure have yet to be confirmed as increasing risk for gliomagenesis. Symptoms of disease depend on the specific location of the tumor, and diagnosis is most commonly made following surgical resection. The prognosis for patients with GBM is often very poor (only 2 % of patients aged 65 years or older, and only 30 % of those under the age of 45 years at diagnosis, survive for 2 years or more), and treatments to cure this cancer have yet to be devised.

The clinical hallmarks of GBM are its aggressive growth and inexorable recurrence despite multimodal therapy with surgery followed by radiation and temozolomide (TMZ) therapy. Unfortunately, current standard-of-care therapy results in a median survival of only 12–15 months (Stupp et al. 2005). Consequently, our present strategy is to identify genetic, behavioral, environmental and developmental contributors to glioma risk through epidemiological studies, with the ultimate goal of reducing the disease burden.

2 Emerging Role of Glioblastoma Stem Cells and the Therapeutic Challenges

GBM is a highly heterogeneous tumor with individual histologic hallmarks including high cell density, intratumoral necrosis, vascular hyperplasia and invasion through brain parenchyma (Westphal and Lamszus 2011). This heterogeneity is also displayed at the microscopic level, where the cellular hierarchy has been demonstrated to be governed by the presence of GSCs (Dirks 2008; Ignatova et al. 2002). The clinical implications of CSC targeting to improve treatment of GBM could be remarkable. Since GBM presents different phenotypic patterns and molecular signaling activation in distinct regions (layers) of the tumor mass, the pathological characterization can be influenced by the site of sample collected by the surgeon throughout the tumor (Pistollato et al. 2010). Indeed, O(6)-methylguanine-DNA methyltransferase (MGMT) has been found differentially expressed among the three layers, and both MGMT protein expression and promoter methylation status are considered important prognostic factors (Della Puppa et al. 2012; Stupp et al. 2005). This issue is crucial because in the modern neuro-oncological setting, several diagnostic and prognostic markers are commonly analyzed to predict tumor grade and the consequential therapeutic approach. In addition, biomarkers are pivotal in the selection of glioma patients for their recruitment into clinical trials following surgery. In this sense, site of the tumor sample collection could represent a remarkable bias for both selection and stratification of patients.

Current treatment of GBM is based on surgery, followed by radio and chemotherapy. In GBM surgery, intra-operative targeting of CSCs should be a main purpose. Indeed, being putative CSCs considered the major responsible of resistance requiring supplementary treatments, surgeon should achieve the complete removal of CSC population (Rampazzo et al. 2014). Currently, no techniques aiming at this purpose are available.

A further consideration can be done about loco-regional therapies, which are treatments that surgeons can carry out directly in the surgical cave after tumor removal. This is the case of carmustine (bis-chloroethylnitrosourea, BCNU or BiCNU), an alkylating agent, wafers that are a worldwide approved treatment for both newly diagnosed and recurrent high-grade gliomas. They are constituted by degradable biopolymer wafers impregnated of BCNU that is released over few weeks in the surgical cave. Wafers are implanted in the surgical cave after tumor removal, and positioned in tight contact with the brain surface infiltrated by tumor. When a complete removal of central core of tumor has been achieved, loco-regional therapy such as BCNU wafers could be more effective against a limited CSC population. However, the residual GSCs might be targeted by using pro-differentiating treatments together with conventional therapies, thus affecting CSC phenotype and aggressiveness (Persano et al. 2012). During GBM management, surgery is followed by radiotherapy and concomitant alkylating agents based chemotherapy that could be virtually more effective against a tumoral residue possibly depleted of CSCs (Pistollato et al. 2010).

3 Glioblastoma Stem Cells

In the adult brain, neural stem cells (NSCs) were observed at any stage of the development, from the embryo to the adult organism. NSCs are located primarily in the subventricular zone (Altman 1965), in the subgranular zone and the dentate gyrus of the hippocampus (Altman and Das 1965). In particular NSC have been described to reside in their specific niches around the blood vessels where they are in communication with other cells and the extracellular matrix. Different cellular types are present in these niches, such as neuroblasts, and transitory amplifying progenitors and all these cells are surrounded by ependymal cells (Facchino et al. 2011; McLendon and Rich 2011). NSCs are pluripotent cells capable of differentiation as a result of which they lose their stem properties (Schiffer et al. 2010). Moreover, their proliferative capacity and the association with blood vessels stimulate NSCs to migrate and invade surrounded tissues. While NSCs are necessary for a correct neurological development and activity, cells with aberrant NSC characteristics have been often correlated to brain tumors. Indeed, increasing evidences suggest the existence of a population of CSCs or tumor initiating cells (TICs) with high self-renewal ability, promoting brain tumor growth, in contrast to the other cancer cells (Persano et al. 2011).

In the light of the "CSC hypothesis", the transformation of NSCs or progenitors in CSCs follows the rules of the normal physiology but with aberrant order, timing and intensity of the underlying mechanisms. CSCs may originate from normal NSCs undergoing tumorigenic alterations. Differently, they can derive from more differentiated or terminally differentiated transit-amplifying neural cells being affected by multiple mutations, thus reverting to a stem phenotype. Moreover, an arrest of the normal maturation process of the NSC has been also reported, thus leading to intensive cell division and lack of differentiation. CSCs originating through these different processes are generally described as a small sub-population of dividing cells with stem cell-like properties, huge self-renewal ability, peculiar genetic alterations, tumorigenic potential, and the ability to differentiate into all different bulk tumor cells (Vescovi et al. 2006).

The first evidence of the existence of cells with stem-like characteristics in GBM was reported by Steindler and colleagues, who isolated clonogenic, neurosphere-forming precursors from post-surgery specimens of human GBM (Ignatova et al. 2002). At a later stage, two independent groups demonstrated that GBM and medulloblastoma contain neurosphere-forming cells that are able to give rise to neuronal and astroglial-like cells (Lee et al. 2006; Singh et al. 2003, 2004). GBM cells need specific criteria to be classified as GSCs. In particular, they should be able to self-renew, differentiate into distinct lineages and initiate tumors in immunodeficient animal models, recapitulating the original phenotype and heterogeneity of the parental tumor (Singh et al. 2003, 2004). The presence of these cells in GBM specimens was observed by culturing GBM tissues in serum-free media supplemented with EGF and bFGF growth factors, which formed non-adherent spheroids with an enhanced GSCs population. Neurosphere cultures are currently the most common

method used to propagate GSCs in vitro. It has been demonstrated that these neurosphere cultures maintain genetic profiles similar to the original GBM patients and form invasive tumors in intracranial xenografts (Ernst et al. 2009; Lee et al. 2006; Singh et al. 2004). Each neurosphere arises from an individual GSC or transit-amplifying cell and despite their clonal origin, neurospheres are heterogeneous aggregates that consist of GSCs, transit-amplifying cells and more differentiated GBM cells. When these neurosphere cultures are dissociated to single cells, a small proportion of them can give rise to secondary neurospheres (Chen et al. 2010; Reynolds and Weiss 1996). In contrast when they are exposed to fetal bovine serum, neurosphere originating cells differentiate into the different cell lineages of the parent tumor (Singh et al. 2003). Thus, GSCs show high capacity to proliferate, self-renewal properties and the ability to form secondary neurospheres. Moreover, GSCs significantly differ from NSCs for their ability to differentiate and then revert to the original stem/immature phenotype. Indeed, differentiation induced by serum of normal NSCs is permanent (Lee et al. 2006), while glioma lines established by serum cultures are reversible and they can be converted to neurospheres when cultured in serum-free media (Gilbert et al. 2010; Qiang et al. 2009).

GBM tumor mass consists of different cell phenotypes, requiring the individuation of specific markers to more precisely identify GSCs. GSCs are expected to share common markers with their normal counterparts showing usually elevated expression of Nestin, an intermediate filament expressed in NSCs, located in neurogenic niches (Reynolds and Weiss 1992; Uhrbom et al. 2002) and correlated with 'stemness' and cytoskeleton organization, cellular signaling, organogenesis and metabolism. During the differentiation process NSCs lose the expression of Nestin and start to express βIII-tubulin and glial fibrillary acidic protein (GFAP) (Jackson and Alvarez-Buylla 2008; Sequerra et al. 2013). GSCs have been reported to show increased GFAP expression, a marker of astrocyte differentiation that can be co-expressed similarly to Nestin by NSCs. GSCs are also enriched for Sox2, a transcription factor expressed by NSCs with cytoplasmic localization which is connected to the differentiation process and associated with multipotency and pluripotency (Ikushima et al. 2009, 2011). Comparative gene expression analysis led to identification of more GSC markers, including Oct4, SSEA-1/ CD15, Bmi-1, Musashi-1, Nanog, integrin-α6, L1CAM, A2B5 and ABC-type transporters (Gonzalez-Gomez et al. 2011; Ikushima et al. 2011; Son et al. 2009). However, the marker which is commonly used to identify and isolate GSCs is CD133 (also known as Prominin-1), a 5-TM glycoprotein expressed by human hematopoietic cells and neural progenitor cells (Pfenninger et al. 2007; Wang et al. 2008). In the human fetal brain, CD133 is a marker for NSCs (Uchida et al. 2000) and its expression has also been observed in intermediate radial glial cells in the early postnatal brain, and in ependymal cells in the adult brain (Coskun et al. 2008; Pfenninger et al. 2007). $CD133^{+}$ cells from GBM are capable of multi-lineage differentiation and have a high capacity to form neurospheres, unlike the corresponding $CD133^{-}$ cells which did not proliferate in neurosphere cultures. In addition, $CD133^{+}$ cells from GBM have an increased capacity of tumor initiation after serial transplantations in immunodeficient mice (Singh et al. 2004).

The GSCs biology is influenced by various signaling pathways that maintain self-renewal or regulate differentiation in the appropriate context. The group of Fine started culturing tumor cells in serum-free conditions (Lee et al. 2006). By using EGF and FGF, we can reduce cell differentiation and promote GSC self-renewal. These mitogens act through their receptor tyrosine kinases (RTKs) inducing activation of downstream pathways such as the phosphoinositide 3-kinase/Akt (PI3K/Akt) and Mitogen-Activated Protein Kinase (MAPK), leading to cell proliferation, survival and tumorigenicity (Hambardzumyan et al. 2008a, b; Lee et al. 2006).

Originally identified as a regulator of neurogenesis, Notch signaling plays a central role in nervous system development, including maintenance of self-renewal ability and regulation of fate decisions into neural and glial lineages (Artavanis-Tsakonas and Simpson 1991; Yoon and Gaiano 2005). Upon binding to its ligands (Delta-like and Jagged), heterodimeric Notch receptors (Notch1–4) get cleaved by γ-secretase in the cytoplasm, releasing the Notch intracellular domain (NICD). NICD translocates into the nucleus where it acts as co-activator for the transcriptional repressors of neurogenic genes, such as Hes and Hey, sustaining stemness in activated cells (Mizutani et al. 2007). In GBM, Notch signaling is involved in several distinct mechanism in tumorigenesis, through the regulation of both self-renewal and differentiation of GSCs (Hovinga et al. 2010; Lino et al. 2010; Wang et al. 2010). Furthermore, Numb, which prevents NICD from traveling to the nucleus and thus inhibits downstream signaling upon Notch activation, was shown to be asymmetrically distributed within GSCs and to promote asymmetric division, giving rise to a stem cell and a more restricted and differentiated cell (Jiang et al. 2012).

Transforming growth factor-β (TGF-β) signaling promotes GSC self-renewal through regulation of distinct mechanisms. In particular, it was shown to act through SRY-Related HMG-Box transcription factors Sox2 and Sox4, to induce self-renewal (Ikushima et al. 2009).

Sonic Hedgehog (Shh)-Gli signaling is highly important for brain and spinal cord patterning during embryonic development and plays crucial functions in GSC maintenance (Cayuso et al. 2006; Shahi et al. 2008). It has been shown to promote GSC self-renewal and expression of stem cell genes, whereas its blockage leads to apoptosis, delay in tumorigenesis and inhibition of GSC self-renewal and migration (Bar et al. 2007; Rossi et al. 2011; Ulasov et al. 2013).

The Wnt/β-catenin pathway induces proliferation and/or differentiation of progenitor cells within gliomas and it is important for GSC self-renewal. Moreover, overexpression of Wnt ligands, Wnt3a and Wnt1, has been observed in GSCs (Kim et al. 2012; Rampazzo et al. 2013).

Bone morphogenetic protein (BMP), a member of TGF-β superfamily, functions as a differentiation signal within GBM, as opposed to the previously discussed roles of other members of the TGF-β family in maintenance of self-renewal (Ikushima et al. 2009). The difference between BMP and TGF-β effects on GSC biology can be owed to distinct signaling cascades, even though they belong to the same superfamily of ligands. Recent evidences suggested that Notch signaling is also important for transdifferentiation of GSCs into tumor-derived endothelial cells (Wang

et al. 2010). Similarly, TGF-β was shown to induce GSCs differentiation into vascular pericytes, supporting vessel formation and leading to further tumor growth (Cheng et al. 2013; Wang et al. 2010).

4 Glioblastoma Microenvironment

GBM complexity is driven by numerous stimuli which originate from the microenvironment, important for pathogenesis and resistance to therapy. It has been described that GBMs display high cellular heterogeneity, and Pistollato et al. (2010) described a model which integrates the plethora of signals which regulate GBM plasticity.

GBM cells communicate with the perivascular niche and with the hypoxic niche, by originating a "teamwork", withstanding to hierarchic rules and complex networks. The three-layers concentric model represents a clear explanation to elucidate the complexity of signals integration in GBMs, particularly deriving from microenvironment (Fig. 11.1). According to the hierarchical theory for tumor progression, the "tumor-initiating cells" GSCs should originate from the sub-ventricular zone (SVZ) and the sub-granular zone (SGZ), which include progenitor cells able to originate multilineage differentiated cells. These specific niches are essential for maintaining stemness and self-renewal properties of GBM precursors, which are secondly instructed to proliferate and differentiate.

The central area of the tumor mass consists of a necrotic core, highly hypoxic and enriched in GSCs, and as going to the periphery, the tumor mass includes an intermediate layer, hypoxic and rich in GSCs too. The surrounding peri-tumor zone corresponds to the peripheral layer of the "three-layer model", and it is highly vascularized and presents few GSCs and more differentiated cells (Fig. 11.1). A hypoxic gradient is arranged from the core to the periphery, associated to a progressive change in the expression of specific markers, from stemness markers, like CD133 and Nestin in the necrotic area, to differentiation markers, such as GFAP and β-III-tubulin, in the more oxygenated periphery.

Two main niches are detected in GBM microenvironment, the hypoxic and the perivascular ones. They finely regulate cellular fate by releasing numerous stimuli, which promote cell differentiation or stemness maintenance. GBMs are highly vascularized tumors, characterized by strong angiogenesis, but the blood flow is not the only determinant factor to have a pivotal role to contribute to the complexity of vascular microenvironment, since many cell types infiltrate the tumor mass. Precisely, the perivascular niche consists of the surrounded area of angiogenic and tumor microvascular structures, characterized by the presence of several mature and differentiated cells (endothelial cells, fibroblasts, astrocytes, macrophages or microglia) which orchestrate intercellular crosstalk. Endothelial cells are the principal component of the vascular niche, and they differ from endothelial cells which constitute vessel walls. Blood flow is necessary to provide oxygen and nutrients to GBM cells, particularly to CSCs, nevertheless many non-structural endothelial cells

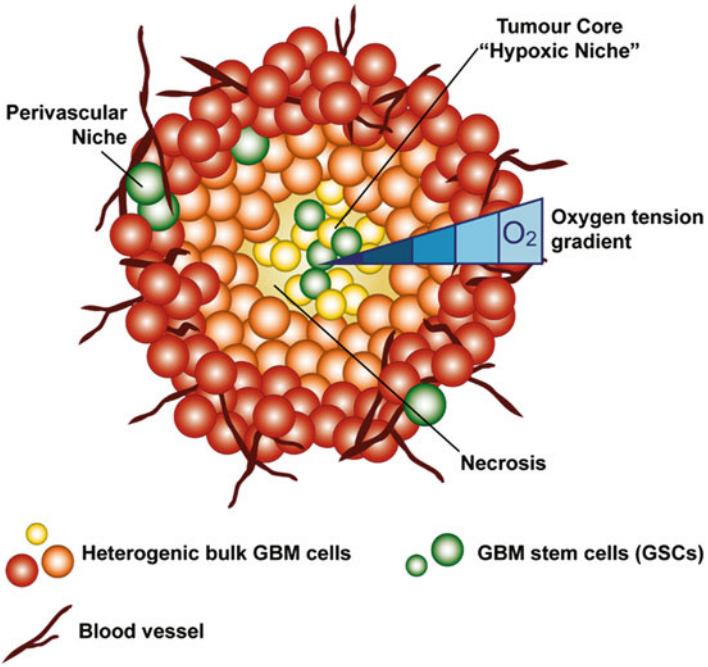

Fig. 11.1 *The three layer model of glioblastoma.* In this model GSCs are located along the hypoxic gradient in the tumor mass, mostly residing in the inner portions of the mass and in the so called perivascular niche. The GBM cells derived from the inner areas of the mass are resistant to chemotherapy in vitro. Accounting for the heterogenic landscape of genetic and genomic aberration characterizing GBM cells isolated GSC from the tumor core and the perivascular niche of the GBM mass are characterized by a different phenotype and tumorigenic potential. Cytogenetic analysis demonstrated that the two types of GSCs bear quite different genetic abnormalities, nevertheless deriving at least in part from common precursor cells. A hypoxic gradient is present from the tumor core to the periphery, associated to a progressive change in the expression of specific markers such as stemness markers, like CD133 and Nestin in the necrotic area, to differentiation markers, such as GFAP and β-III-tubulin, in the more oxygenated periphery

exist, and they remain separate from tumor capillaries, without increasing the tumor microvascular density. They have the task of releasing a lot of diffusible factors to maintain the self-renewal ability of neural stem cells and neurogenesis. On the other hand, GBM cells release pro-angiogenic stimuli like VEGF to recruit endothelial cells which proliferate and give rise to new capillaries. Moreover other pro-angiogenic mechanisms were described for GBM angiogenesis, such as the transdifferentiation of cancer stem cells into tumor-derived endothelial cells (TDECs), to continuously preserve the vascular microenvironment (Calabrese et al. 2007; Soda et al. 2011; Charles and Holland 2010).

Pericytes are contractile cells which are tightly associated to endothelial cells, to stabilize and maintain the integrity of the newly formed tumor vessels. They has been described to be involved in the regulation of the angio-architecture structural shape of the tumor vascular niche, and they intimately depend on endothelial cells

along the vessel walls. Analogously, astrocytes are closely associated to the endothelial cells forming blood vessels, and they both maintain the integrity of the blood brain barrier, and produce neurotrophic factors which promote GBM proliferation (Hoelzinger et al. 2007).

Fibroblasts reside in the perivascular niche, and they are responsible of GBM invasion, as reported for other cancer types. They express critical markers associated to tumor progression and malignancy, such as metalloproteases (pro-MMP2).

The presence of tumor induces a physiological immune response, and GSCs showed the expression of pro-inflammatory genes, which stimulate the enrichment of microglia at the tumor perivascular site. Microglia are the macrophages which lie in brain tissue, and they are the principal cytokine stimulators important for tumor proliferation, migration and progression. They are located in many sites, depending on their role. They promote metastasis when arranged in the perivascular space, cell motility and invasion when sited in the advanced tip of tumor, and their localization in the perinecrotic area increases angiogenesis, explaining the positive correlation between macrophages infiltration and vascular density in gliomas (Nishie et al. 1999; Roggendorf et al. 1996).

The combination of all these cell types results in a complex system of crosstalk between cells, which culminates in a fine balance of a plethora stimuli for GBM cells. Particularly GSCs are strictly connected to endothelial cells, as well as other stromal cells, defining the entirely plasticity, typical of the tumor microenvironment. It has been observed that GSCs arrange themselves along the capillaries, in order to be prone to respond to signaling cues deriving from endothelium, by direct cell-to-cell contact and soluble factors. They stimulate GSCs to proliferate and self-renew, and the increase of the number of endothelial cells has been associated to an accelerated brain tumor initiation and growth. On the other hand, GSCs express elevated levels of VEGF or other pro-angiogenic factors, which in turn stimulate endothelial cells to proliferate and undergo angiogenesis. This evidence shows a bidirectional signaling and cross-talk between stem cells and vascular niche (Charles and Holland 2010).

A peculiar aspect of GBM microenvironment is the hypoxic niche. GBM mass is characterized by low oxygen concentrations, ranging between 0.1 % and 2.5 %, unlike in healthy brain which physiologically range between 12.5 % and 2.5 % of oxygen. GBMs are marked out by hypoxic gradients, which present areas with moderate or severe hypoxia, and necrotic zones in the tumor core. The inner layer shows a considerable expression of hypoxic markers, associated to tumor aggressiveness and GSCs maintenance. The milestone of hypoxia are HIFs, a family of transcription factors which response to oxygen tension and regulate hypoxia responsive genes, playing a pivotal role in cancer progression, metastasis and resistance to therapy. HIFs consist of two subunits, HIF-α and HIF-β, which form a functional heterodimer acting as nuclear transcription factor in hypoxic conditions. Normoxia induces HIF-α hydroxylation providing its proteasomal degradation. Human HIF-α consists of three oxygen-sensitive subunits, HIF-1α, HIF-2α, HIF-3α. HIF-1α is the most ubiquitously expressed, and the mostly studied. HIF-2α is predominant in GSCs niche, unlike HIF-3α which does not work as transcription factor as lacking

the transcriptional activation domain, but it acts as dominant negative by sequestrating HIF-β. This subunit is not responsive to oxygen concentration, and it is constitutively expressed in all cell types (Yang et al. 2012). HIF-1α and HIF-2α are important to determine a switch to an acute response to hypoxia, mediated by HIF-1α, and a chronic reaction principally regulated by HIF-2α (Koh and Powis 2012). HIFs are involved in several processes since they regulate both normal tissue homeostasis and disease progression. HIF controls metabolism, induces angiogenesis and stemness maintenance, it is involved in tumor initiation and progression and stimulates tumor invasion (Majmundar et al. 2010).

The putative CSCs are preserved in the hypoxic niche, since HIF promotes an undifferentiate state in populations of progenitors and stem cells. It has been shown both in vitro and in vivo that HIF depletion in $CD133^+$ GSCs impairs their ability to induce angiogenesis and tumorigenesis (Li et al. 2009).

HIFs can transcribe for more than 40 target genes (Semenza 2002), among which the carbonic anhydrase isoform 9 (CAIX), involved in increasing the metastatic potential of GBM by acidification of the tumor microenvironment, and Notch1, which leads to NFAT activation and cell proliferation and tumor growth. Thus hypoxia sustains GBM cells proliferation, particularly preserving the stem population in the perivascular and hypoxic niches, by up-regulating other transcription factors like Notch and Oct4, which control self-renewal and multipotency of stem cells. Moreover, it has been described that HIF counteracts the differentiating stimuli induced by BMPs (Pistollato et al. 2009). In vitro hypoxia stimulates both the expression of the stem markers CD133, Nestin, Sox2, and the formation of neurospheres, characterized by elevated stem potential (Bar et al. 2010; Harris 2002; McCord et al. 2009). HIF is directly engaged in angiogenesis and tumor invasion, by activating several factors such as VEGF, metalloproteases, TGF factors and CXCR4 (Kaur et al. 2005).

GBM microenvironment is essential for GBM tumorigenesis and progression, particularly for the continuous signal communications between GSCs and cells belonging to the GBM niches, like endothelium or perycites, which give rise to a complex plasticity of the tumor. This signal integration originates numerous mechanisms which lead to resistance to therapy. HIF expression is associated to drug resistance and poor patient prognosis in multiple tumor types, and in GBM hypoxia has been shown to mediate both radiotherapy and chemotherapy resistance. Indeed, in hypoxic conditions the radiation dose required to have the same biological effect as in normoxic conditions is three times higher (Spence et al. 2008). Moreover, traditional chemotherapy for GBM and specifically treatment with TMZ is impaired by signals induced by the hypoxic niche, resulting ineffective (Persano et al. 2012; Pistollato et al. 2010). This phenomena are explained by the observations that low oxygenation induces significant changes in the expression pattern of genes and proteins which are related to the regulation of DNA-damage response, apoptosis and proliferation.

Since the strict connection of GSCs and GBMs niches, several therapeutic targets have been found among the signaling molecules deriving from the microenvironment stimuli. Clearly HIF-1α has been identified as the principal target for

improving GBM therapy, with the strategy to reduce its mRNA/protein levels, to impair the interaction with HIF-β or with DNA at the transcription sites, or to increase the protein degradation. Secondly, GBM vascular compartment and endothelial cells are other important targets with the purpose to reduce the nutrients supply of tumor. Nevertheless, in order to design combination treatment, recent evidences demonstrated the importance of the correct timing of treatment, to facilitate the delivery of chemotherapeutics into the tumor mass, and successively deplete the tumor vasculature. The principal molecular targets of the vascular niche are VEGFs and PDGFs signaling pathways. Moreover, other signal cues may be arrested, such as chemokines associated to tumor migration and invasion of surrounded tissues, among which the more relevant is CXCR4.

In conclusion, the complex integration of signals deriving from GBM niches is necessary for GBM tumorigenesis and aggressiveness, and to regulate the whole network of stimuli deriving from several cellular types present in the microenvironment. They regulate stem cells fate or their maintenance, having a pivotal role in GBM progression and resistance to therapy. Only considering GBM cells together with stem cells in strict contact to the microenvironment will lead to optimize winning therapeutic strategies for GBM.

5 Therapeutic Targeting of Glioma Stem Cells

Neuro-oncology has experienced an explosion in the molecular modeling of GBM through tumor genetics and mouse modeling. The Cancer Genome Atlas (TCGA) confirmed the frequent mutational involvement of the p53, RB, and receptor tyrosine kinase (RTK) pathways in GBM (Cancer-Genome-Atlas-Research-Network 2008). Moreover, gene-expression studies divided GBM patients into distinct tumor subtypes – classical, mesenchymal, neural and proneural, each characterized by a peculiar mutational load in epidermal growth factor receptor (EGFR), neurofibromin 1 (NF1), platelet-derived growth factor receptor A (PDGFRA) and Isocitrate Dehydrogenase 1 (IDH1) (Verhaak et al. 2010). Although this large scale effort suggested a number of possible GBM targets, only few of these genetic findings have entered into clinical practice to date (Yan et al. 2013).

As previously outlined, GBM display high resistance to conventional radiotherapy and chemotherapy (Sanai and Berger 2008). Indeed, soon after their initial description, GSC resistance to treatments have been described (Bao et al. 2006; Liu et al. 2006), thus suggesting them as one the principal contributors to GBM tumor recurrence. GSCs have been demonstrated to be more resistant to radiation than the non-stem glioma cells (Bao et al. 2006). Indeed, chemotherapy with TMZ delays GBM tumor growth, but long term survivors are extremely rare and recurrence after TMZ therapy strongly indicates the presence of TMZ-resistant GSCs (Stupp et al. 2005). In an in vivo mouse model of GBM, TMZ treatment increased tumor side population (SP), a cell population that have been described to be enriched in CSCs, suggesting that TMZ treatment could even favor tumor recurrence (Chua et al.

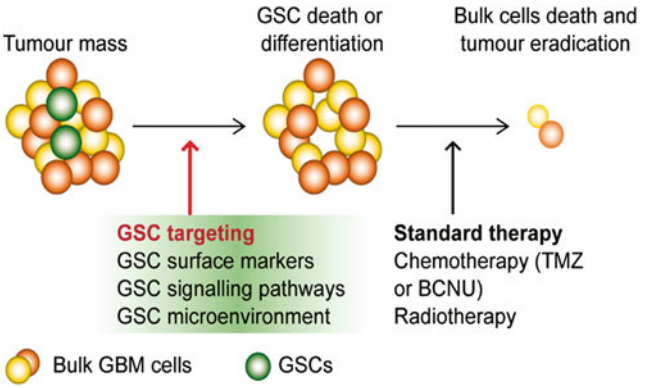

Fig. 11.2 *Targeted therapy in Glioblastoma.* Conventional therapies (surgery, radiotherapy, chemotherapy) target the tumor bulk, but display no efficacy toward the GSC compartment. Microenvironmental factors and activation of specific signaling pathways are able to sustain the little population of remaining GSC, allowing for GBM relapse. Latest studies have been trying to generate new targeted therapies (*green box*) able to differentiate or eliminate the GSCs or signals from the microenvironment able to maintain this cell pool

2008). For these reasons, it is now widely accepted that GSCs contribute to GBM recurrence after conventional therapies. Thus, there is a urgent need to develop more effective therapies based on the specific targeting of signaling pathways involved in the maintenance of GSCs functions (Fig. 11.2).

Initial models of GSC regulation have been based on neural stem cell (NSC) biology, the probable normal cellular correlate. Despite, GSCs seem to be governed by pathways active in brain development, including Notch, Wnt, bone morphogenetic protein (BMP), transforming growth factor-β (TGF-β), and RTK pathways (Binda et al. 2014), our knowledge of the mechanisms underlying GSC maintenance and resistance to therapy are still in early development, thus preventing their complete understanding. Moreover, recent evidence support the idea that using GSC enriched cell cultures derived from human GBM biopsies could be a better strategy to setup more appropriate drug discovery programs, although with some caveats in terms of inter – and intra-tumoral GSC heterogeneity, their isolation and proper long term expansion (Romaguera-Ros et al. 2012). Despite these limitations, potentially important therapeutic targets in GSCs have been published on a frequent basis. Here, some of previously identified GSC targets and possible novel therapeutic strategies against them are discussed (Fig. 11.2).

5.1 Targeting GSC Surface Molecules

Based on the suggestive but still debated hypothesis that a unique surface marker expression would be able to define the entire GSC population (Perez Castillo et al. 2008), one target of particular interest to the field is CD133. A functional role for

CD133 has been reported in GSCs and other tumors, as regulator of the PI3K–Akt pathway via its interactions with the p85 subunit of PI3K (Wei et al. 2013) and consequently involving Erk1/2 and MAPK signaling (Dong et al. 2010). Upstream of this cascade, RET has been identified as a crucial mediator of CD133 intracellular functions in neuroblastoma cells (Takenobu et al. 2011). As a cell surface protein, CD133 has been targeted with antibodies in preclinical studies and a vaccine against CD133 (ICT-121) is entering clinical trials (Yan et al. 2013). Moreover, direct targeting of GSCs cell surface molecules has been investigated by a lentiviral preparation expressing a shRNA for L1 cell adhesion molecule (L1CAM), a molecule preferentially expressed in $CD133^+$ GBM cells, which is able to suppress GBM cell growth in vitro and in vivo (Bao et al. 2008).

5.2 Overcoming Radiation and Drug Resistance

DNA repair mechanisms can restore the integrity of damaged DNA bases and thus contribute to drug and radiation resistance. In this context, cancer stem cells have been reported to possess enhanced DNA repair capacity (Johannessen et al. 2008). One of the first studies in this field was published by Rich's group, reporting that CD133 cells survived ionizing radiation in greater proportions compared to cells that lacked CD133 expression (Bao et al. 2006). This effect has been associated to the over-activation of Chk1 and Chk2 DNA damage checkpoint kinases in the $CD133^+$ GSC population. In fact, conventional radiation is able to exert phosphorylation of these cell cycle effectors in $CD133^+$ cells, but not in $CD133^-$, suggesting a constitutive activation of multiple cell cycle checkpoints in GSCs that may further up-regulate in response to DNA damage (Nakai et al. 2009). Chk1 and 2 activation can be inhibited by a specific inhibitor debromohymenialdisine (DBH) representing an intriguing target for GSC treatment (Bao et al. 2006).

Resistance of $CD133^+$ GSCs is also probably sustained by the combined higher expression of drug resistance, DNA repair enzymes and anti-apoptosis proteins such as breakpoint cluster region pseudogene 1 (BCRP1), *O*-6-methylguanine-DNA methyltransferase (MGMT) and FAS-associating death domain (FADD)-like antiapoptotic molecule (FLIP), respectively (Liu et al. 2006). In this context, our group previously reported that O(6)-benzylguanine (6-BG), a nontoxic pseudosubstrate inhibitor of MGMT, treatment is able to sensitize GSCs to chemotherapy with TMZ (Pistollato et al. 2010).

This high resistance of GSCs to radiation and anticancer drugs has been investigated by many authors and associated to both DNA repair and non-DNA-repair mechanisms including heat shock protein-90 (HSP-90) inhibition, synergizing with radiation and/or TMZ (Sauvageot et al. 2009), treatment with anti epidermal growth factor receptor (EGFR) antibodies (cetuximab and nimotuzumab), able to increase radiosensitivity (Michelakis et al. 2010) or blockade of chloride transport, enhancing chemotherapy-mediated cell death (Kang and Kang 2008).

5.3 Targeting GSC Signaling Pathways

Self-renewal and survival of Neural Stem Cells (NSCs) are mainly regulated starting from embrional development by both the Notch family proteins and by epidermal growth factor (EGF)-activated signaling pathways (Aguirre et al. 2010). In particular Notch pathway activation is the primary responsible for NSC maintenance and differentiation inhibition, whereas EGFR sustains proliferation and migration of newly derived precursors from NSCs. Thus, maintenance of the balance between stemness and differentiation can result from the dynamic interplay between Notch and EGFR pathways.

5.4 Notch

Similar to what happens during normal neural development, it has been documented that Notch is a critical regulator of CSC maintenance in several types of tumors, including GBM. Fan et al. showed that Notch blockade by γ-secretase inhibitors reduced neurosphere growth and clonogenicity of GSCs in vitro (Chen et al. 2010; Fan et al. 2010; Ulasov et al. 2013). Moreover, Notch blockade has been correlated to GSC chemotherapy sensitization and to inhibition of xenograft recurrence (Gilbert et al. 2010). Hovinga et al. also emphasized that the Notch pathway plays a critical role in linking angiogenesis and CSC self-renewal and thus is a potential therapeutic target (Hovinga et al. 2010). Also Notch ligands such as Delta-like Ligand 4 (DLL4) have been associated with tumorigenesis and GSC maintenance (Li et al. 2011). Overall, the inhibition of Notch signaling should be considered as a promising therapeutic target for GSCs.

5.5 EGFR and PI3K/AKT

EGFR is overexpressed and/or mutated in many carcinomas, including lung, breast, colon, head and neck, prostate, ovarian, but displays some specific mutations also in GBM (Inda et al. 2010). PI3K/Akt/mTOR pathway, being aberrantly activated by EGFR amplification or the presence of the EGFRvIII ligand-independent variant, is thus often up-regulated in GSCs (Bleau et al. 2009), conferring them survival and/or proliferative advantages. The targeting of this important signaling cascade at different levels (by blocking EGFR, PI3K or directly AKT) might overcome the unsatisfactory results observed in clinical studies when RTK inhibitors have been used alone (Florio and Barbieri 2012). Particularly interesting are results obtained with A-443654, able to inhibit GSC proliferation in vitro and in vivo (Gallia et al. 2009) and the combination between the mTOR inhibitor temsirolimus and perifosine (Pitter et al. 2011). Recent findings from Kitanaka's group suggest that PI3K/Akt/

mTOR and MEK/ERK pathways coordinately regulate the differentiation and tumorigenicity of GCSs. Also in this case, concomitant inhibition of both pathways more potently suppress their survival signals rather single inhibitions (Sunayama et al. 2010). In this study FoxO3a was reported as fundamental for the differentiation of GSCs induced by Akt and Erk inhibition and that its constitutive activation is sufficient to induce differentiation and to inhibit GSC self-renewal and tumorigenicity, suggesting that FoxO3a may be a potential therapeutic target (Persano et al. 2013; Sunayama et al. 2011). Finally, knockdown of CD133 in GSCs causes down-regulation of Akt phosphorylation, highlighting the strict link between stem cell surface markers and activation of intracellular signaling (Eyler et al. 2008; Gallia et al. 2009).

5.6 Shh

Sonic hedgehog (Shh)-Gli signaling is another of the key regulator pathway in the NSC niche during embryogenesis (Binda et al. 2014) and, being critical for NSC maintenance is often aberrantly activated in GBM thus supporting GSC growth and maintenance (Clement et al. 2007). Indeed the potent Shh antagonist cyclopamine depletes GSCs, reducing self-renewal and the tumorigenic potential of GBM stem cells, increasing also TMZ and radiation-mediated cell death (Bar et al. 2007; Merchant and Matsui 2012). Clinical trials with another Shh signaling antagonist, vismodegib, are ongoing in comparison with standard chemotherapy (Lorusso et al. 2011).

5.7 Ephrins

Of the numerous receptors that have been implicated in GSC biology, the Ephrin (Eph) RTKs have been investigated in cancer and stem cell biology. Indeed, they regulate a wide range of physiological processes in the CNS during development and, in adult neurogenesis, they affect NSCs survival and proliferation (Depaepe et al. 2005; Holmberg et al. 2005; Pasquale 2008). Recently, it has been shown that GSCs are a major site of EphA2 overexpression and that EphA2 expression correlates with both the size and tumorigenic potential of the GSC pool. Furthermore, forced down-regulation of EphA2 expression suppresses GSC self-renewal and intracranial tumor-initiating ability, showing that this receptor may represent a selective molecular target for potential therapeutic purposes (Binda et al. 2012, 2014). Moreover, EphA3 was found to be specifically expressed in mesenchymal GSCs and appeared to modulate downstream mitogen-activated protein kinase (MAPK) signaling, thus appearing as another possible Eph signaling target (Day et al. 2013).

5.8 Induction of Differentiation

Differentiation therapy forcing GSCs to differentiate might be a promising and notably non-cytotoxic strategy for GSC targeting. In this regard BMPs may be potential soluble factors in the treatment of gliomas (Persano et al. 2012). BMPs are members of the Transforming Growth Factor-β (TGF-β) family of ligands, but exerting opposite effects. In fact, TGF-β has been shown to have regulatory effects on GSC differentiation by inducing the Smad-2⁄3 transcriptional complex thus preventing GSC, but not normal neural stem⁄progenitor cells differentiation (Penuelas et al. 2009).

The prototypic receptors for BMP in mammals are the type II receptor, BMPR2, and type I receptors, BMPR1A and BMPR1B (Chen and Panchision 2007). It has been reported that BMP2 and 4 act as neuroepithelial proliferation/differentiation signals at different stages of embryonic central nervous system development, an effect mainly mediated by BMPR1A and BMPR1B respectively (Chen and Panchision 2007). For this reason, BMPs have been used as pro-differentiating factors for GBM treatment. Despite, we and others recently reported on the role of BMPs, in particular BMP2 and BMP4, in promoting astroglial differentiation and in reducing cell growth of GBM-derived cells (Persano et al. 2012; Piccirillo et al. 2006), considering BMPs treatment a promising therapeutic approach for brain cancer, enthusiasm has been weaken by a study showing that GSC may epigenetically reduce BMPR1B expression thus evading BMP-induced differentiation (Binello and Germano 2011; Lee et al. 2008).

Recently, Chirasani et al. clearly demonstrated in vivo and in vitro that BMP7, another member of the bone morphogenetic protein family, released by neural precursor cells induces differentiation and represses proliferation, self-renewal and tumor initiation of GSCs (Chirasani et al. 2010). Moreover, a BMP7 variant have been shown to inhibit GBM growth in vitro and in vivo (Tate et al. 2012).

These results suggest to explore further if the inhibitory effects mediated by BMPs on cell growth are targeted specifically on the CSC population, and whether other soluble factors are useful to selectively inhibit cancer stem cells growth. Overall, mimicking events induced by BMP2,4,7 and their effectors remains a potential important therapeutic tool and clinical trials using BMPs are being designed.

For further information, we report also treatment with all-trans retinoic acid (ATRA), Interferon-β (IFN-β) and agonists of peroxisome proliferator-activated receptor (PPAR) c as all able to induce GSC differentiation with different mechanisms involving activation of nuclear retinoic acid receptor (RAR) and STAT-3 signaling pathway respectively (Campos et al. 2010; Chearwae and Bright 2008; Yuki et al. 2009).

6 Conclusion and Future Perspectives

A great number of advances have been made in trying to setup better therapeutic strategies for GBM patients care. The rise of models explaining GBM origin and progression by the involvement of GSCs, and their sharing by the scientific community has led, in the recent years, to a downright explosion of interest in this field, also rising some concerns about the real efficacy of standard treatments applied for GBM. TMZ chemotherapy, despite introducing a real increase of patients' survival, is nevertheless based on an old concept of anti-cancer drugs targeting highly proliferating cells. Indeed, TMZ is a DNA alkylating agent able to effectively get through the blood–brain barrier, that, since it is orally administered, highly meets with patients compliance. The high rate of relapse after surgery, radiation and chemotherapy raises the consciousness that these standard treatments are still not sufficient. Thus a novel class of drugs is urgently needed to overcome GBM intrinsic resistance to therapy. Although many compounds demonstrated strong efficacy in preclinical studies, none or only few of them showed similar effects during clinical trials, due to negligible anti-tumoral activity or severe side effects. This could be due to the GBM tumor intrinsic heterogeneity and for this reason a better understanding of GSCs behavior, phenotype and signaling activation status must be improved. Thus, future therapies should be validated on GSCs rather than cell lines. Next years will be fundamental to validate recent developed agents or novel delivery strategies for future patients care, trying to counteract this almost incurable disease.

References

Aguirre A, Rubio ME, Gallo V (2010) Notch and EGFR pathway interaction regulates neural stem cell number and self-renewal. Nature 467(7313):323–327

Altman FA (1965) The origin of the first cancer cell. Arch Geschwulstforsch 25(1):21–31

Altman J, Das GD (1965) Post-natal origin of microneurones in the rat brain. Nature 207(5000):953–956

Artavanis-Tsakonas S, Simpson P (1991) Choosing a cell fate: a view from the Notch locus. Trends Genet 7(11–12):403–408

Bao S, Wu Q, McLendon RE, Hao Y, Shi Q, Hjelmeland AB, Dewhirst MW, Bigner DD, Rich JN (2006) Glioma stem cells promote radioresistance by preferential activation of the DNA damage response. Nature 444(7120):756–760

Bao S, Wu Q, Li Z, Sathornsumetee S, Wang H, McLendon RE, Hjelmeland AB, Rich JN (2008) Targeting cancer stem cells through L1CAM suppresses glioma growth. Cancer Res 68(15):6043–6048

Bar EE, Chaudhry A, Lin A, Fan X, Schreck K, Matsui W, Piccirillo S, Vescovi AL, DiMeco F, Olivi A, Eberhart CG (2007) Cyclopamine-mediated hedgehog pathway inhibition depletes stem-like cancer cells in glioblastoma. Stem Cells 25(10):2524–2533

Bar EE, Lin A, Mahairaki V, Matsui W, Eberhart CG (2010) Hypoxia increases the expression of stem-cell markers and promotes clonogenicity in glioblastoma neurospheres. Am J Pathol 177(3):1491–1502

Binda E, Visioli A, Giani F, Lamorte G, Copetti M, Pitter KL, Huse JT, Cajola L, Zanetti N, DiMeco F, De Filippis L, Mangiola A, Maira G, Anile C, De Bonis P, Reynolds BA, Pasquale EB, Vescovi AL (2012) The EphA2 receptor drives self-renewal and tumorigenicity in stem-like tumor-propagating cells from human glioblastomas. Cancer Cell 22(6):765–780

Binda E, Reynolds BA, Vescovi AL (2014) Glioma stem cells: turpis omen in nomen? (The evil in the name?). J Intern Med 276(1):25–40

Binello E, Germano IM (2011) Targeting glioma stem cells: a novel framework for brain tumors. Cancer Sci 102(11):1958–1966

Bleau AM, Hambardzumyan D, Ozawa T, Fomchenko EI, Huse JT, Brennan CW, Holland EC (2009) PTEN/PI3K/Akt pathway regulates the side population phenotype and ABCG2 activity in glioma tumor stem-like cells. Cell Stem Cell 4(3):226–235

Calabrese C, Poppleton H, Kocak M, Hogg TL, Fuller C, Hamner B, Oh EY, Gaber MW, Finklestein D, Allen M, Frank A, Bayazitov IT, Zakharenko SS, Gajjar A, Davidoff A, Gilbertson RJ (2007) A perivascular niche for brain tumor stem cells. Cancer Cell 11(1):69–82

Campos B, Wan F, Farhadi M, Ernst A, Zeppernick F, Tagscherer KE, Ahmadi R, Lohr J, Dictus C, Gdynia G, Combs SE, Goidts V, Helmke BM, Eckstein V, Roth W, Beckhove P, Lichter P, Unterberg A, Radlwimmer B, Herold-Mende C (2010) Differentiation therapy exerts antitumor effects on stem-like glioma cells. Clin Cancer Res 16(10):2715–2728

Cancer-Genome-Atlas-Research-Network (2008) Comprehensive genomic characterization defines human glioblastoma genes and core pathways. Nature 455(7216):1061–1068

Cayuso J, Ulloa F, Cox B, Briscoe J, Marti E (2006) The Sonic hedgehog pathway independently controls the patterning, proliferation and survival of neuroepithelial cells by regulating Gli activity. Development 133(3):517–528

Charles N, Holland EC (2010) The perivascular niche microenvironment in brain tumor progression. Cell Cycle 9(15):3012–3021

Chearwae W, Bright JJ (2008) PPARgamma agonists inhibit growth and expansion of CD133+ brain tumour stem cells. Br J Cancer 99(12):2044–2053

Chen HL, Panchision DM (2007) Concise review: bone morphogenetic protein pleiotropism in neural stem cells and their derivatives–alternative pathways, convergent signals. Stem Cells 25(1):63–68

Chen R, Nishimura MC, Bumbaca SM, Kharbanda S, Forrest WF, Kasman IM, Greve JM, Soriano RH, Gilmour LL, Rivers CS, Modrusan Z, Nacu S, Guerrero S, Edgar KA, Wallin JJ, Lamszus K, Westphal M, Heim S, James CD, VandenBerg SR, Costello JF, Moorefield S, Cowdrey CJ, Prados M, Phillips HS (2010) A hierarchy of self-renewing tumor-initiating cell types in glioblastoma. Cancer Cell 17(4):362–375

Cheng L, Huang Z, Zhou W, Wu Q, Donnola S, Liu JK, Fang X, Sloan AE, Mao Y, Lathia JD, Min W, McLendon RE, Rich JN, Bao S (2013) Glioblastoma stem cells generate vascular pericytes to support vessel function and tumor growth. Cell 153(1):139–152

Chirasani SR, Sternjak A, Wend P, Momma S, Campos B, Herrmann IM, Graf D, Mitsiadis T, Herold-Mende C, Besser D, Synowitz M, Kettenmann H, Glass R (2010) Bone morphogenetic protein-7 release from endogenous neural precursor cells suppresses the tumourigenicity of stem-like glioblastoma cells. Brain 133(Pt 7):1961–1972

Chua C, Zaiden N, Chong KH, See SJ, Wong MC, Ang BT, Tang C (2008) Characterization of a side population of astrocytoma cells in response to temozolomide. J Neurosurg 109(5):856–866

Clement V, Sanchez P, de Tribolet N, Radovanovic I, Ruiz i Altaba A (2007) HEDGEHOG-GLI1 signaling regulates human glioma growth, cancer stem cell self-renewal, and tumorigenicity. Curr Biol 17(2):165–172

Coskun V, Wu H, Blanchi B, Tsao S, Kim K, Zhao J, Biancotti JC, Hutnick L, Krueger RC Jr, Fan G, de Vellis J, Sun YE (2008) CD133+ neural stem cells in the ependyma of mammalian postnatal forebrain. Proc Natl Acad Sci USA 105(3):1026–1031

Day BW, Stringer BW, Al-Ejeh F, Ting MJ, Wilson J, Ensbey KS, Jamieson PR, Bruce ZC, Lim YC, Offenhauser C, Charmsaz S, Cooper LT, Ellacott JK, Harding A, Leveque L, Inglis P, Allan S, Walker DG, Lackmann M, Osborne G, Khanna KK, Reynolds BA, Lickliter JD, Boyd AW (2013) EphA3 maintains tumorigenicity and is a therapeutic target in glioblastoma multiforme. Cancer Cell 23(2):238–248

Della Puppa A, Persano L, Masi G, Rampazzo E, Sinigaglia A, Pistollato F, Denaro L, Barzon L, Palu G, Basso G, Scienza R, d'Avella D (2012) MGMT expression and promoter methylation status may depend on the site of surgical sample collection within glioblastoma: a possible pitfall in stratification of patients? J Neurooncol 106(1):33–41

Depaepe V, Suarez-Gonzalez N, Dufour A, Passante L, Gorski JA, Jones KR, Ledent C, Vanderhaeghen P (2005) Ephrin signalling controls brain size by regulating apoptosis of neural progenitors. Nature 435(7046):1244–1250

Dirks PB (2008) Brain tumor stem cells: bringing order to the chaos of brain cancer. J Clin Oncol 26(17):2916–2924

Dong L, Qi N, Ge RM, Cao CL, Lan F, Shen L (2010) Overexpression of CD133 promotes the phosphorylation of Erk in U87MG human glioblastoma cells. Neurosci Lett 484(3):210–214

Ernst A, Hofmann S, Ahmadi R, Becker N, Korshunov A, Engel F, Hartmann C, Felsberg J, Sabel M, Peterziel H, Durchdewald M, Hess J, Barbus S, Campos B, Starzinski-Powitz A, Unterberg A, Reifenberger G, Lichter P, Herold-Mende C, Radlwimmer B (2009) Genomic and expression profiling of glioblastoma stem cell-like spheroid cultures identifies novel tumor-relevant genes associated with survival. Clin Cancer Res 15(21):6541–6550

Eyler CE, Foo WC, LaFiura KM, McLendon RE, Hjelmeland AB, Rich JN (2008) Brain cancer stem cells display preferential sensitivity to Akt inhibition. Stem Cells 26(12):3027–3036

Facchino S, Abdouh M, Bernier G (2011) Brain cancer stem cells: current status on glioblastoma multiforme. Cancers (Basel) 3(2):1777–1797

Fan X, Khaki L, Zhu TS, Soules ME, Talsma CE, Gul N, Koh C, Zhang J, Li YM, Maciaczyk J, Nikkhah G, Dimeco F, Piccirillo S, Vescovi AL, Eberhart CG (2010) NOTCH pathway blockade depletes CD133-positive glioblastoma cells and inhibits growth of tumor neurospheres and xenografts. Stem Cells 28(1):5–16

Florio T, Barbieri F (2012) The status of the art of human malignant glioma management: the promising role of targeting tumor-initiating cells. Drug Discov Today 17(19–20):1103–1110

Gallia GL, Tyler BM, Hann CL, Siu IM, Giranda VL, Vescovi AL, Brem H, Riggins GJ (2009) Inhibition of Akt inhibits growth of glioblastoma and glioblastoma stem-like cells. Mol Cancer Ther 8(2):386–393

Gilbert CA, Daou MC, Moser RP, Ross AH (2010) Gamma-secretase inhibitors enhance temozolomide treatment of human gliomas by inhibiting neurosphere repopulation and xenograft recurrence. Cancer Res 70(17):6870–6879

Gonzalez-Gomez P, Sanchez P, Mira H (2011) MicroRNAs as regulators of neural stem cell-related pathways in glioblastoma multiforme. Mol Neurobiol 44(3):235–249

Hambardzumyan D, Becher OJ, Holland EC (2008a) Cancer stem cells and survival pathways. Cell Cycle 7(10):1371–1378

Hambardzumyan D, Squatrito M, Carbajal E, Holland EC (2008b) Glioma formation, cancer stem cells, and akt signaling. Stem Cell Rev 4(3):203–210

Harris AL (2002) Hypoxia–a key regulatory factor in tumour growth. Nat Rev Cancer 2(1):38–47

Hoelzinger DB, Demuth T, Berens ME (2007) Autocrine factors that sustain glioma invasion and paracrine biology in the brain microenvironment. J Natl Cancer Inst 99(21):1583–1593

Holmberg J, Armulik A, Senti KA, Edoff K, Spalding K, Momma S, Cassidy R, Flanagan JG, Frisen J (2005) Ephrin-A2 reverse signaling negatively regulates neural progenitor proliferation and neurogenesis. Genes Dev 19(4):462–471

Hovinga KE, Shimizu F, Wang R, Panagiotakos G, Van Der Heijden M, Moayedpardazi H, Correia AS, Soulet D, Major T, Menon J, Tabar V (2010) Inhibition of notch signaling in glioblastoma targets cancer stem cells via an endothelial cell intermediate. Stem Cells 28(6):1019–1029

Ignatova TN, Kukekov VG, Laywell ED, Suslov ON, Vrionis FD, Steindler DA (2002) Human cortical glial tumors contain neural stem-like cells expressing astroglial and neuronal markers in vitro. Glia 39(3):193–206

Ikushima H, Todo T, Ino Y, Takahashi M, Miyazawa K, Miyazono K (2009) Autocrine TGF-beta signaling maintains tumorigenicity of glioma-initiating cells through Sry-related HMG-box factors. Cell Stem Cell 5(5):504–514

Ikushima H, Todo T, Ino Y, Takahashi M, Saito N, Miyazawa K, Miyazono K (2011) Glioma-initiating cells retain their tumorigenicity through integration of the Sox axis and Oct4 protein. J Biol Chem 286(48):41434–41441

Inda MM, Bonavia R, Mukasa A, Narita Y, Sah DW, Vandenberg S, Brennan C, Johns TG, Bachoo R, Hadwiger P, Tan P, Depinho RA, Cavenee W, Furnari F (2010) Tumor heterogeneity is an active process maintained by a mutant EGFR-induced cytokine circuit in glioblastoma. Genes Dev 24(16):1731–1745

Jackson EL, Alvarez-Buylla A (2008) Characterization of adult neural stem cells and their relation to brain tumors. Cells Tissues Organs 188(1–2):212–224

Jiang X, Xing H, Kim TM, Jung Y, Huang W, Yang HW, Song S, Park PJ, Carroll RS, Johnson MD (2012) Numb regulates glioma stem cell fate and growth by altering epidermal growth factor receptor and Skp1-Cullin-F-box ubiquitin ligase activity. Stem Cells 30(7):1313–1326

Johannessen TC, Bjerkvig R, Tysnes BB (2008) DNA repair and cancer stem-like cells–potential partners in glioma drug resistance? Cancer Treat Rev 34(6):558–567

Kang MK, Kang SK (2008) Pharmacologic blockade of chloride channel synergistically enhances apoptosis of chemotherapeutic drug-resistant cancer stem cells. Biochem Biophys Res Commun 373(4):539–544

Kaur B, Khwaja FW, Severson EA, Matheny SL, Brat DJ, Van Meir EG (2005) Hypoxia and the hypoxia-inducible-factor pathway in glioma growth and angiogenesis. Neuro Oncol 7(2):134–153

Kim Y, Kim KH, Lee J, Lee YA, Kim M, Lee SJ, Park K, Yang H, Jin J, Joo KM, Lee J, Nam DH (2012) Wnt activation is implicated in glioblastoma radioresistance. Lab Invest 92(3):466–473

Koh MY, Powis G (2012) Passing the baton: the HIF switch. Trends Biochem Sci 37(9):364–372

Lee J, Kotliarova S, Kotliarov Y, Li A, Su Q, Donin NM, Pastorino S, Purow BW, Christopher N, Zhang W, Park JK, Fine HA (2006) Tumor stem cells derived from glioblastomas cultured in bFGF and EGF more closely mirror the phenotype and genotype of primary tumors than do serum-cultured cell lines. Cancer Cell 9(5):391–403

Lee J, Son MJ, Woolard K, Donin NM, Li A, Cheng CH, Kotliarova S, Kotliarov Y, Walling J, Ahn S, Kim M, Totonchy M, Cusack T, Ene C, Ma H, Su Q, Zenklusen JC, Zhang W, Maric D, Fine HA (2008) Epigenetic-mediated dysfunction of the bone morphogenetic protein pathway inhibits differentiation of glioblastoma-initiating cells. Cancer Cell 13(1):69–80

Li Z, Bao S, Wu Q, Wang H, Eyler C, Sathornsumetee S, Shi Q, Cao Y, Lathia J, McLendon RE, Hjelmeland AB, Rich JN (2009) Hypoxia-inducible factors regulate tumorigenic capacity of glioma stem cells. Cancer Cell 15(6):501–513

Li Z, Wang J, Gong L, Wen Z, Xu C, Huang X (2011) Correlation of delta-like ligand 4 (DLL4) with VEGF and HIF-1alpha expression in human glioma. Asian Pac J Cancer Prev 12(1):215–218

Lino MM, Merlo A, Boulay JL (2010) Notch signaling in glioblastoma: a developmental drug target? BMC Med 8:72

Liu G, Yuan X, Zeng Z, Tunici P, Ng H, Abdulkadir IR, Lu L, Irvin D, Black KL, Yu JS (2006) Analysis of gene expression and chemoresistance of CD133+ cancer stem cells in glioblastoma. Mol Cancer 5:67

Lorusso PM, Jimeno A, Dy G, Adjei A, Berlin J, Leichman L, Low JA, Colburn D, Chang I, Cheeti S, Jin JY, Graham RA (2011) Pharmacokinetic dose-scheduling study of hedgehog pathway inhibitor vismodegib (GDC-0449) in patients with locally advanced or metastatic solid tumors. Clin Cancer Res 17(17):5774–5782

Louis DN, Ohgaki H, Wiestler OD, Cavenee WK, Burger PC, Jouvet A, Scheithauer BW, Kleihues P (2007) The 2007 WHO classification of tumours of the central nervous system. Acta Neuropathol 114(2):97–109

Majmundar AJ, Wong WJ, Simon MC (2010) Hypoxia-inducible factors and the response to hypoxic stress. Mol Cell 40(2):294–309

McCord AM, Jamal M, Shankavaram UT, Lang FF, Camphausen K, Tofilon PJ (2009) Physiologic oxygen concentration enhances the stem-like properties of CD133+ human glioblastoma cells in vitro. Mol Cancer Res 7(4):489–497

McLendon RE, Rich JN (2011) Glioblastoma stem cells: a neuropathologist's view. J Oncol 2011:397195

Merchant AA, Matsui W (2012) Targeting hedgehog–a cancer stem cell pathway. Clin Cancer Res 16(12):3130–3140

Michelakis ED, Sutendra G, Dromparis P, Webster L, Haromy A, Niven E, Maguire C, Gammer TL, Mackey JR, Fulton D, Abdulkarim B, McMurtry MS, Petruk KC (2010) Metabolic modulation of glioblastoma with dichloroacetate. Sci Transl Med 2(31):31–34

Mizutani K, Yoon K, Dang L, Tokunaga A, Gaiano N (2007) Differential Notch signalling distinguishes neural stem cells from intermediate progenitors. Nature 449(7160):351–355

Nakai E, Park K, Yawata T, Chihara T, Kumazawa A, Nakabayashi H, Shimizu K (2009) Enhanced MDR1 expression and chemoresistance of cancer stem cells derived from glioblastoma. Cancer Invest 27(9):901–908

Nishie A, Ono M, Shono T, Fukushi J, Otsubo M, Onoue H, Ito Y, Inamura T, Ikezaki K, Fukui M, Iwaki T, Kuwano M (1999) Macrophage infiltration and heme oxygenase-1 expression correlate with angiogenesis in human gliomas. Clin Cancer Res 5(5):1107–1113

Ohgaki H, Kleihues P (2005) Epidemiology and etiology of gliomas. Acta Neuropathol 109(1):93–108

Pasquale EB (2008) Eph-ephrin bidirectional signaling in physiology and disease. Cell 133(1):38–52

Penuelas S, Anido J, Prieto-Sanchez RM, Folch G, Barba I, Cuartas I, Garcia-Dorado D, Poca MA, Sahuquillo J, Baselga J, Seoane J (2009) TGF-beta increases glioma-initiating cell self-renewal through the induction of LIF in human glioblastoma. Cancer Cell 15(4):315–327

Perez Castillo A, Aguilar-Morante D, Morales-Garcia JA, Dorado J (2008) Cancer stem cells and brain tumors. Clin Transl Oncol 10(5):262–267

Persano L, Rampazzo E, Della Puppa A, Pistollato F, Basso G (2011) The three-layer concentric model of glioblastoma: cancer stem cells, microenvironmental regulation, and therapeutic implications. Sci World J 11:1829–1841

Persano L, Pistollato F, Rampazzo E, Della Puppa A, Abbadi S, Frasson C, Volpin F, Indraccolo S, Scienza R, Basso G (2012) BMP2 sensitizes glioblastoma stem-like cells to Temozolomide by affecting HIF-1alpha stability and MGMT expression. Cell Death Dis 3:e412

Persano L, Rampazzo E, Basso G, Viola G (2013) Glioblastoma cancer stem cells: role of the microenvironment and therapeutic targeting. Biochem Pharmacol 85(5):612–622

Pfenninger CV, Roschupkina T, Hertwig F, Kottwitz D, Englund E, Bengzon J, Jacobsen SE, Nuber UA (2007) CD133 is not present on neurogenic astrocytes in the adult subventricular zone, but on embryonic neural stem cells, ependymal cells, and glioblastoma cells. Cancer Res 67(12):5727–5736

Piccirillo SG, Reynolds BA, Zanetti N, Lamorte G, Binda E, Broggi G, Brem H, Olivi A, Dimeco F, Vescovi AL (2006) Bone morphogenetic proteins inhibit the tumorigenic potential of human brain tumour-initiating cells. Nature 444(7120):761–765

Pistollato F, Rampazzo E, Abbadi S, Della Puppa A, Scienza R, D'Avella D, Denaro L, Te Kronnie G, Panchision DM, Basso G (2009) Molecular mechanisms of HIF-1alpha modulation induced by oxygen tension and BMP2 in glioblastoma derived cells. PLoS One 4(7):e6206

Pistollato F, Abbadi S, Rampazzo E, Persano L, Della Puppa A, Frasson C, Sarto E, Scienza R, D'Avella D, Basso G (2010) Intratumoral hypoxic gradient drives stem cells distribution and MGMT expression in glioblastoma. Stem Cells 28(5):851–862

Pitter KL, Galban CJ, Galban S, Tehrani OS, Li F, Charles N, Bradbury MS, Becher OJ, Chenevert TL, Rehemtulla A, Ross BD, Holland EC, Hambardzumyan D (2011) Perifosine and CCI 779 co-operate to induce cell death and decrease proliferation in PTEN-intact and PTEN-deficient PDGF-driven murine glioblastoma. PLoS One 6(1):e14545

Qiang L, Yang Y, Ma YJ, Chen FH, Zhang LB, Liu W, Qi Q, Lu N, Tao L, Wang XT, You QD, Guo QL (2009) Isolation and characterization of cancer stem like cells in human glioblastoma cell lines. Cancer Lett 279(1):13–21

Rampazzo E, Persano L, Pistollato F, Moro E, Frasson C, Porazzi P, Della Puppa A, Bresolin S, Battilana G, Indraccolo S, Te Kronnie G, Argenton F, Tiso N, Basso G (2013) Wnt activation promotes neuronal differentiation of glioblastoma. Cell Death Dis 4:e500

Rampazzo E, Della Puppa A, Frasson C, Battilana G, Bianco S, Scienza R, Basso G, Persano L (2014) Phenotypic and functional characterization of glioblastoma cancer stem cells identified trough 5-aminolevulinic acid-assisted surgery. J Neurooncol 116(3):505–513

Reynolds BA, Weiss S (1992) Generation of neurons and astrocytes from isolated cells of the adult mammalian central nervous system. Science 255(5052):1707–1710

Reynolds BA, Weiss S (1996) Clonal and population analyses demonstrate that an EGF-responsive mammalian embryonic CNS precursor is a stem cell. Dev Biol 175(1):1–13

Roggendorf W, Strupp S, Paulus W (1996) Distribution and characterization of microglia/macrophages in human brain tumors. Acta Neuropathol 92(3):288–293

Romaguera-Ros M, Peris-Celda M, Oliver-De La Cruz J, Carrion-Navarro J, Perez-Garcia A, Garcia-Verdugo JM, Ayuso-Sacido A (2012) Cancer-initiating enriched cell lines from human glioblastoma: preparing for drug discovery assays. Stem Cell Rev 8(1):288–298

Rossi M, Magnoni L, Miracco C, Mori E, Tosi P, Pirtoli L, Tini P, Oliveri G, Cosci E, Bakker A (2011) Beta-catenin and Gli1 are prognostic markers in glioblastoma. Cancer Biol Ther 11(8):753–761

Sanai N, Berger MS (2008) Glioma extent of resection and its impact on patient outcome. Neurosurgery 62(4):753–764, discussion 264–756

Sauvageot CM, Weatherbee JL, Kesari S, Winters SE, Barnes J, Dellagatta J, Ramakrishna NR, Stiles CD, Kung AL, Kieran MW, Wen PY (2009) Efficacy of the HSP90 inhibitor 17-AAG in human glioma cell lines and tumorigenic glioma stem cells. Neuro Oncol 11(2):109–121

Schiffer D, Annovazzi L, Caldera V, Mellai M (2010) On the origin and growth of gliomas. Anticancer Res 30(6):1977–1998

Semenza GL (2002) HIF-1 and tumor progression: pathophysiology and therapeutics. Trends Mol Med 8(4 Suppl):S62–S67

Sequerra EB, Costa MR, Menezes JR, Hedin-Pereira C (2013) Adult neural stem cells: plastic or restricted neuronal fates? Development 140(16):3303–3309

Shahi MH, Lorente A, Castresana JS (2008) Hedgehog signalling in medulloblastoma, glioblastoma and neuroblastoma. Oncol Rep 19(3):681–688

Singh SK, Clarke ID, Terasaki M, Bonn VE, Hawkins C, Squire J, Dirks PB (2003) Identification of a cancer stem cell in human brain tumors. Cancer Res 63(18):5821–5828

Singh SK, Hawkins C, Clarke ID, Squire JA, Bayani J, Hide T, Henkelman RM, Cusimano MD, Dirks PB (2004) Identification of human brain tumour initiating cells. Nature 432(7015):396–401

Soda Y, Marumoto T, Friedmann-Morvinski D, Soda M, Liu F, Michiue H, Pastorino S, Yang M, Hoffman RM, Kesari S, Verma IM (2011) Transdifferentiation of glioblastoma cells into vascular endothelial cells. Proc Natl Acad Sci USA 108(11):4274–4280

Son MJ, Woolard K, Nam DH, Lee J, Fine HA (2009) SSEA-1 is an enrichment marker for tumor-initiating cells in human glioblastoma. Cell Stem Cell 4(5):440–452

Spence AM, Muzi M, Swanson KR, O'Sullivan F, Rockhill JK, Rajendran JG, Adamsen TC, Link JM, Swanson PE, Yagle KJ, Rostomily RC, Silbergeld DL, Krohn KA (2008) Regional hypoxia in glioblastoma multiforme quantified with [18F]fluoromisonidazole positron emission tomography before radiotherapy: correlation with time to progression and survival. Clin Cancer Res 14(9):2623–2630

Stupp R, Mason WP, van den Bent MJ, Weller M, Fisher B, Taphoorn MJ, Belanger K, Brandes AA, Marosi C, Bogdahn U, Curschmann J, Janzer RC, Ludwin SK, Gorlia T, Allgeier A, Lacombe D, Cairncross JG, Eisenhauer E, Mirimanoff RO (2005) Radiotherapy plus concomitant and adjuvant temozolomide for glioblastoma. N Engl J Med 352(10):987–996

Sunayama J, Matsuda K, Sato A, Tachibana K, Suzuki K, Narita Y, Shibui S, Sakurada K, Kayama T, Tomiyama A, Kitanaka C (2010) Crosstalk between the PI3K/mTOR and MEK/ERK pathways involved in the maintenance of self-renewal and tumorigenicity of glioblastoma stem-like cells. Stem Cells 28(11):1930–1939

Sunayama J, Sato A, Matsuda K, Tachibana K, Watanabe E, Seino S, Suzuki K, Narita Y, Shibui S, Sakurada K, Kayama T, Tomiyama A, Kitanaka C (2011) FoxO3a functions as a key integrator of cellular signals that control glioblastoma stem-like cell differentiation and tumorigenicity. Stem Cells 29(9):1327–1337

Takenobu H, Shimozato O, Nakamura T, Ochiai H, Yamaguchi Y, Ohira M, Nakagawara A, Kamijo T (2011) CD133 suppresses neuroblastoma cell differentiation via signal pathway modification. Oncogene 30(1):97–105

Tate CM, Pallini R, Ricci-Vitiani L, Dowless M, Shiyanova T, D'Alessandris GQ, Morgante L, Giannetti S, Larocca LM, di Martino S, Rowlinson SW, De Maria R, Stancato L (2012) A BMP7 variant inhibits the tumorigenic potential of glioblastoma stem-like cells. Cell Death Differ 19:1644–1654

Uchida N, Buck DW, He D, Reitsma MJ, Masek M, Phan TV, Tsukamoto AS, Gage FH, Weissman IL (2000) Direct isolation of human central nervous system stem cells. Proc Natl Acad Sci USA 97(26):14720–14725

Uhrbom L, Dai C, Celestino JC, Rosenblum MK, Fuller GN, Holland EC (2002) Ink4a-Arf loss cooperates with KRas activation in astrocytes and neural progenitors to generate glioblastomas of various morphologies depending on activated Akt. Cancer Res 62(19):5551–5558

Ulasov I, Thaci B, Sarvaiya P, Yi R, Guo D, Auffinger B, Pytel P, Zhang L, Kim CK, Borovjagin A, Dey M, Han Y, Baryshnikov AY, Lesniak MS (2013) Inhibition of MMP14 potentiates the therapeutic effect of temozolomide and radiation in gliomas. Cancer Med 2(4):457–467

Verhaak RG, Hoadley KA, Purdom E, Wang V, Qi Y, Wilkerson MD, Miller CR, Ding L, Golub T, Mesirov JP, Alexe G, Lawrence M, O'Kelly M, Tamayo P, Weir BA, Gabriel S, Winckler W, Gupta S, Jakkula L, Feiler HS, Hodgson JG, James CD, Sarkaria JN, Brennan C, Kahn A, Spellman PT, Wilson RK, Speed TP, Gray JW, Meyerson M, Getz G, Perou CM, Hayes DN (2010) Integrated genomic analysis identifies clinically relevant subtypes of glioblastoma characterized by abnormalities in PDGFRA, IDH1, EGFR, and NF1. Cancer Cell 17(1):98–110

Vescovi AL, Galli R, Reynolds BA (2006) Brain tumour stem cells. Nat Rev Cancer 6(6):425–436

Wang J, Sakariassen PO, Tsinkalovsky O, Immervoll H, Boe SO, Svendsen A, Prestegarden L, Rosland G, Thorsen F, Stuhr L, Molven A, Bjerkvig R, Enger PO (2008) CD133 negative glioma cells form tumors in nude rats and give rise to CD133 positive cells. Int J Cancer 122(4):761–768

Wang R, Chadalavada K, Wilshire J, Kowalik U, Hovinga KE, Geber A, Fligelman B, Leversha M, Brennan C, Tabar V (2010) Glioblastoma stem-like cells give rise to tumour endothelium. Nature 468(7325):829–833

Wei Y, Jiang Y, Zou F, Liu Y, Wang S, Xu N, Xu W, Cui C, Xing Y, Liu Y, Cao B, Liu C, Wu G, Ao H, Zhang X, Jiang J (2013) Activation of PI3K/Akt pathway by CD133-p85 interaction promotes tumorigenic capacity of glioma stem cells. Proc Natl Acad Sci USA 110(17):6829–6834

Westphal M, Lamszus K (2011) The neurobiology of gliomas: from cell biology to the development of therapeutic approaches. Nat Rev Neurosci 12(9):495–508

Yan K, Yang K, Rich JN (2013) The evolving landscape of glioblastoma stem cells. Curr Opin Neurol 26(6):701–707

Yang L, Lin C, Wang L, Guo H, Wang X (2012) Hypoxia and hypoxia-inducible factors in glioblastoma multiforme progression and therapeutic implications. Exp Cell Res 318(19):2417–2426

Yoon K, Gaiano N (2005) Notch signaling in the mammalian central nervous system: insights from mouse mutants. Nat Neurosci 8(6):709–715

Yuki K, Natsume A, Yokoyama H, Kondo Y, Ohno M, Kato T, Chansakul P, Ito M, Kim SU, Wakabayashi T (2009) Induction of oligodendrogenesis in glioblastoma-initiating cells by IFN-mediated activation of STAT3 signaling. Cancer Lett 284(1):71–79