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Glioma Stem Cells

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1. Introduction

Malignant gliomas are the most common primary brain tumor, with median survival of less than 15 months from the time of diagnosis. (Stupp R, et al., 2005) Most malignant tumors contain a small subpopulation of cells that are highly tumorigenic and share features with normal stem cells, including the ability for extensive self-renewal and to differentiate into multiple lineages. Many tumors, including glioblastomas, are activated by these cancer stem cells, making them resistant to conventional therapies such as radiation and chemotherapies. In addition, these cancer stem cells adopt the signaling pathways of normal neural stem and progenitor cells, thereby playing a critical and complex role in tumorigenesis, allowing the tumor to rapidly progress, proliferate, and metastasize. Thus, the pathologic pathways directed by these cancer stem cells make gliomas hard to treat and regulate. Therefore, in order to understand gliomas as well as the cancer treatment-related neurotoxicity on a cellular level, it is crucial to be familiar with the concept of glioma stem cells and their lineage relationships with the central nervous system. Furthermore, to effectively target these cancer stem cells, an understanding of the molecular profiling of well-characterized cancer cell populations is necessary to identify novel biomarkers that will provide the foundation to track their targeted pathways. This will help evaluate and personalize treatment options to help advance our knowledge in the biology of glioma and translate these concepts into the clinical arena. Thus, this chapter will focus on the current understanding on progenitor cells and neural stem cells and highlight important findings regarding the identification and characterization of glioma stem cells, and the development of novel-stem-cell-based treatment strategies for brain tumors.

2. Glioma and stem cells

Malignant gliomas, aside from being the most common brain tumor, are very challenging to treat with median survival times of less than 15 months from time of diagnosis (Stupp R, et al., 2005). Many of the malignant gliomas, especially glioblastomas, display aberrant genetic abnormalities that contribute to their pathologic cellular and morphological heterogeneity (Tabatabai & Weller, 2011). This tumor heterogeneity thus poses a critical obstacle in treating malignant gliomas because different cell populations within the tumor tissues respond differently to treatments such as conventional chemotherapy and radiation (Tabatabai & Weller, 2011; Bao, et al., 2006; Dietrich et al., 2008). In addition, the tumor's tendency to aggressively infiltrate into the surrounding brain parenchyma, therefore

preventing a complete surgical tumor resection, hinders the treatment of gliomas and results in fatal tumor recurrences. (Bao, et al., 2006; Cheng L, et al., 2011; Dietrich et al., 2008).
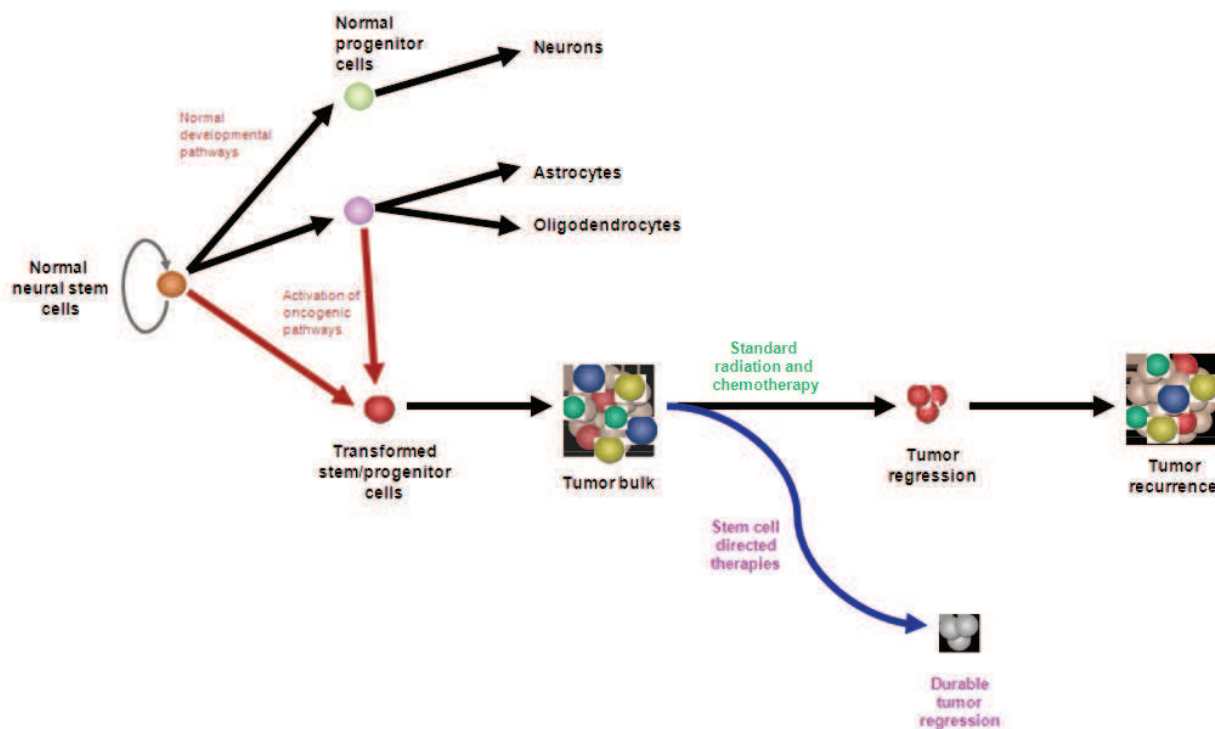
Fig. 1. Resistance mechanisms in glioma cells

Normal neural stem cells self-renew and give rise to multipotential progenitor cells that form neurons, oligodendroglia, and astrocytes. Glioma stem cells arise from the transformation of either neural stem cells or progenitor cells (red) or, less likely, from differentiation of a oligodendrocytes or astrocytes (red arrows) and lead to malignant gliomas. Glioma stem cells are relatively resistant to standard treatments such as radiation and chemotherapy and lead to regrowth of the tumor after treatment. Therapies directed at stem cells can deplete these cells and potentially lead to more durable tumor regression (blue).

To complicate the matters further, early and effective detection of these cancers are extremely difficult, resulting in a poor prognosis for the patients diagnosed with malignant gliomas (Huang Z et al., 2010). Then, these difficulties collectively warrant for reevaluation of current treatments in order to achieve optimal efficacy, prolong the survival, and improve and maximize the quality of life for the patients.

Recent studies suggest that in order to understand the pathologic nature of malignant gliomas, it is crucial to acknowledge the role of cancer stem cells that are involved in the processes of tumor initiation, tumor progression, angiogenesis and resistance to therapy (Huang Z et al., 2010). In a study done by Park et al., even though the correlational relationship between gliomas stem cells and massive tumor is not clear, it is possible that the stem cells contribute to the tumor recurrence after the initial, conventional therapies (Park & Rich, 2009). These cancer stem cells are also known as the tumor-initiating or propagating cells because they display and share some important characteristics with normal stem cells, including self-renewal, multi-lineage differentiation, and maintained proliferation (Huang Z

et al., 2010; Rosen and Jordan 2009; Park & Rich, 2009; Heddleston et al., 2010). Also similar to neural stem cells, cancer stem cells appear to be organized and depend on vascular and nonvascular elements (Dietrich, et al., 2008, Calabrese,et al.,2007). Although the exact origin of the gliomas stem cells is still controversial, there seems to be a consensus that these cancer stem cells arise from genetic and epigenetic changes in neural stem and progenitor cells after many mutations or epigenetic transcription (Huang Z et al., 2010).

Therefore, exploring the signaling pathways and molecular mechanisms that drive these tumor-initiating cells in malignant gliomas is necessary to identify and provide promising novel treatment strategies. In order to understand the exact mechanisms that govern cancer stem cells' role in tumor angiogenesis and extensive proliferation, a careful insight into the cancer stem cell signaling mechanisms warrants further analysis and investigation to optimize brain tumor treatment.

3. Glioma stem cell signaling

The main factor that contributes to the cancer cells' extensive proliferation within their perivascular niche is the cell-extrinsic and cell-intrinsic signals. Therapeutic targeting of these signals within their niche, along with the tumor-associated vasculature may significantly interfere with glioma cancer stem cell growth (Park & Rich, 2009; Dietrich et al., 2010). In clinical studies done by Kreisl et al., and Vredenburgh et al., the administration of bevacizumab, a monoclonal antibody targeting vascular endothelial growth factor (VEGF) demonstrated a clinical effectiveness in achieving therapeutic response (Kreisl et al., 2009; Vredenburgh et al., 2007). As evident by this and other ongoing studies, providing therapies that target these glioma stem cell signaling pathways not only provide effective treatment by directly targeting these stem cells but also gives us valuable insights into the pharmacodynamics of cancer stem cells' dependence on the perivascular niche for survival, growth, and proliferation (Park & Rich, 2009). Therefore, the following major signal transduction pathway cascades will be extensively reviewed to provide highly discriminating targets and tumor biomarkers of therapeutic response: epidermal growth factor (EGF), platelet-derived growth factor (PDGF), vascular endothelial growth factor (VEGF), RAS/Raf/MAPK & AKT/PI3K pathways, Sonic hedgehog (Shh), and PTEN.

3.1 Epidermal growth factor signaling

Epidermal growth factor related signaling has probably been the most extensively studied pathway in malignant gliomas. Epidermal growth factor receptor (EGFR) is a tyrosine kinase that is activated upon ligand binding which induces receptor dimerization. EGFR signaling is involved in many aspects of cellular events including corticogenesis, neural cell survival, proliferation, differentiation, and migration (Ayuso-Sacido et al., 2009; Zhu G et al., 2000). The overexpression of EGFR plays a significant role in GBM and other malignant gliomas' pathologic progression (Ayuso-Sacido et al., 2009). In addition, EGFR is important in neural cell fate and astrocyte differentiation. EGFR expression is often mutated in more than 50% of glioblastomas, with EGFRvIII, a truncated extracellular domain within EGFR that transform into an active ligand-independent kinase, being the most common mutant encountered in gliomas and is responsible for enhanced tumorigenic behavior and genetic instability (Ayuso-Sacido et al., 2009; Frederick et al., 2000, Liu L et al., 2005; Rasheed BK et al., 1999; Ekstrand, 1991; Wong, 1992; Collins, 1993; Mellinghoff, 2005; Aldape, 2004; Pelloski, 2007; Huang, 2007; Li, 2009).

In experimental studies looking at the role of EGFR and the expression of EGFR vIII, the expression of EGFRvIII in the glial lineage in mouse models were significantly similar to human gliomas and in addition, these lesions were mainly occurring within the progenitor cells (Ayuso-Sacido et al., 2009; Dai C et al., 2001; Halatsch ME et al., 2000; Holland EC et al., 2004; Laywell ED et al., 2000). The critical importance of EGF-mediated signaling in gliomas is that these signaling have been shown to contribute to both normal stem cell behavior and to pathologic gliomagenesis. Activated EGFR signaling pathway induce normal stem cells to display characteristics that are seen in glioma stem cells including increased proliferation, migration and survival (Ayuso-Sacido et al., 2000). Consequently, it has been suggested that interference with EGFR and EGFRvIII signaling may offer an attractive strategy for selective glioma therapy (Huang, 2007; Shir, 2006; Zhu, 2009).

Selective small-molecules designed to inhibit EGF/EGFR signaling were among the first targeted molecular therapies used in glioblastoma patients. One of the drugs that is being investigated to treat gliomas is gefitinib (Iressa®), a selective EGFR inhibitor. However, in preclinical studies using gefitinib to observe its activity revealed that the drug showed selective efficacy in tumors that have mutations in exons 19 and 21 of the amplified HER1/EGFR-TK domain (Halatsch ME et al., 2006; Lynch T et al., 2004; Paez J et al., 2004). This result might be the reason why gefitinib is ineffective in treating malignant gliomas, including GBM, because these mutations have not been found in gliomas (Marie Y et al., 2005; Halatsch ME et al., 2006). In addition, preclinical data showed that gefitinib has very low or no activity against tumors that express EGFRvIII (Heimberger AB et al., 2002). Clinical data shows that gefitinib for glioma patients are generally well tolerated with few, minor side effects. However, when compared with historical data, event-free/progression-free survival and median overall survival were same and gefitinib failed to produce effective objective response (Wong et al., 1999; Rich,2004).

Erlotinib (Tarceva®, OSI-774) is another inhibitor of EGFR and the constitutively active mutant EGFRvIII. In pre-clinical studies, erlotinib suppressed GBM cell lines' anchorage-independent growth, suggesting that erlotinib may prevent or even delay GBM recurrence following surgical resection (Halatsch ME et al., 2006). For erlotinib, phase I/II clinical study data indicate that it was well tolerated and findings from phase I trials supported the data from phase II trials in terms of response rates and its antitumor effects (Brewer et al., 2005). The results of a large multicenter Phase II trial, in which patients with progressive glioblastoma were randomly assigned to erlotinib, or conventional chemotherapy with either temozolomide or carmustine (BCNU), revealed that erlotinib had insufficient activity when used as monotherapy (van den Bent, 2009).

Taken together, monotherapy with anti-EGFR agents such as gefitinib and erlotinib, thus far, have not demonstrated significant activity in patients with malignant glioma. However, recent studies suggest that combining the anti-EGFR agents with inhibitors of other molecular target may provide more effective approach in treating malignant gliomas. For example, dual-kinase inhibition of EGFR and ErbB-2 (e.g., lapatinib) (Spector, 2005; Giannopoulou, 2009), or EGFR and VEGF recepetor (VEGFR; e.g., AEE788; Zactima®/vandetanib/ZD6474) (Traxler, 2004; Goudar, 2005; Sandstrom, 2008). Since the stem cell biology suggests that glioma stem cells display a close resemblance to the normal neural cells, our current knowledge of normal stem cell can help analyze and deregulate the pathway of the cancer stem cells to provide promising, therapeutic results.

3.2 Platelet-derived growth factor signaling

Platelet-derived growth factor (PDGF) and its associated receptors play fundamental roles in the developing and adult brain. (Yeh,1993) Studies from *in vivo* animal models show that there is a correlational relationship between the abnormal PDGF signaling and glioma formation (Calzolari & Malatesta, 2009). Often times in gliomas, PDGF ligands are overexpressed along with their PDGFRα receptors. Hence these amplification-dependent receptors or the ligands give rise to aberrant, overactive PDGF signaling pathway (Calzolari & Malatesta, 2009). In the adult brain, PDGFR-a expression is found in the lateral subventricular zone, whereas PDGF is abundantly expressed by neurons and astrocytes (Oumesmar, 1997).

Also in animal model studies increased PDGF signaling blocked neuroblast generation and enhanced neural stem cell proliferation in the subventricular zone with formation of glioma-like hyperplasias (Jackson, 2006). Moreover, the inhibition of PDGF-mediated signaling decreases glioma cell proliferation both in vitro and in vivo (Lokker, 2002), supporting the role of both autocrine and paracrine mechanisms in glioma biology. The fact that many of the malignant gliomas display altered and overactive PDGF pathway suggest that this signaling axis plays an important role in gliomagenesis. Also it seems likely that PDGF signaling is involved and is crucial in tumor proliferation and survival by stimulating growth and supplying nutrients to underlying tumor cells (di Tomaso E et al., 2009).

Imatinibmesylate (Gleevec®) is a small-molecule, oral inhibitor of multiple tyrosine kinases including PDGFR α and β, c-KIT and the BCR-ABL onco-protein (Morris & Abrey 2010). In a phase I/II study done by Wen et al., Imatinib in a monotherapy setting showed disappointing results. Of the 105 patients who were enrolled, 68% of the patients had very low imatinib in their plasma when taking the drug with enzyme inducing anti-epileptic drugs (EIAEDs). This is a serious problem because most glioma patients are on anti-seizure medications, regardless of the treatments that they are receiving. In addition, to the low pharmacokinetic data, imatinib also induced intratumoral hemorrhages and showed no therapeutic responses to patients with anaplastic gliomas (Morris & Abrey 2010; Wen PY et al., 2006). Another phase II showed disappointing results with having 6-moth PFS rate of 16% in GBM, 9% in astrocytomas, and 4.0% in oligodendrogliomas (Raymond et al., 2008). In a combination therapy regimen, administering imatinib with temozolomide found to be tolerable in many patients. However in several clinical studies observing a combined treatment of imatinib with hydroxyurea, a ribonucleoside diphosphate reductase inhibitor, showed disappointing results (Reardon DA et al., 2005 & 2008).While other PDGFR pathway inhibitors are currently under clinical investigation (Roberts, 2005), dual kinase inhibitors, or combinational therapies with conventional cytotoxic agents are also developing including PDGFR and VEGFR dual inhibitors such as PTK787 (vatalanib), sorafenib (Nexavar), sunitinib, AEE788, AZD2171 (cediranib), TKI258, OSI-930 and pazopanib. Also, there are studies observing the efficacy of nilotinib (Tasigna®), which is an oral drug that has greater potency and selectivity for BCR-ABL than imatinib (Saglio G et al., 2010).

3.3 Vascular endothelial growth factor signaling

Antiangiogenic treatments examining VEGF and its associated signaling cascade have been an integral part in modern cancer therapy by targeting extensive tumor vasculatures of the malignant gliomas (Wick et al., 2011). Both endothelial cells and glioma cells may express

and upregulate VEGF and its receptors, resulting in both paracrine and autocrine loops that drive endothelial cell proliferation, tumor invasion, migration and permeability. (Ferrara, 2003; Millauer, 1994) Also, there are circumstantial evidence that elevated VEGF expression in gliomas is associated with the degree of malignancy of the tumor and overall tumor prognosis (Schmidt, 1999; Leon, 1996).

Glioma stem cells appear to be directly involved in this process by stimulating tumor angiogenesis through production of pro- angiogenic factors, such as VEGF (Bao, 2006). In many gliomas, especially in glioblastomas, the neural stem cells and their endothelial compartment closely interact with the vascular niche and by releasing VEGF and promoting angiogenesis (Ricci-Vitiani L et al., 2011). Thus, inhibition of tumor angiogenesis may especially target the cancer stem cell population with the hope of achieving more durable clinical responses. (Folkins, 2007) Antiangiogenic therapy that targets VEGF signaling has evolved into an important therapeutic treatment strategy. Bevacizumab (Avastin®) is a humanized anti-VEGF antibody that demonstrates promising results in treating patients with glioblastomas and have been approved by the United States FDA for the treatment of recurrent of progressive GBMs (Vredenburgh et al., 2007; Huang Z et al., 2010). In a retrospective study of 55 patients with GBM done by Norden et al., the result showed a promising data where the median 6-month progression survival rates were 42% (Brastianos & Batchelor, 2010; Norden AD et al., 2008). Similar promising results have also been reported in other subsequent studies in randomized phase II trials where the patients in the bevacizumab arm showed 42% 6-month progression survival (Therasse P et al., 2000).

Aflibercept (Regeneron®) is a potent, VEGF-trap that is fused to an immunoglobulin constant region. In a study done by De Groot et al., aflibercept brought therapeutic response to 30% of GBM patients. Future trials examining aflibercept in treating malignant gliomas is still underway (De Groot JF et al., 2008).

There are range of novel RTK inhibitors, such as cediranib, vandetanib, vatalanib, sorafenib, sunitinib, pazopanib, AE-788, and CT-322 that have been shown to influence angiogenesis and tumor growth through multiple targets and are currently in various stages of preclinical and clinical investigation. (Brastianos & Batchelor, 2010). Cediranib (Recentin®) is a potent pan-VEGFR, PDGFR and c-KIT inhibitor that showed objective response in 57% of the patients in a phase II trial (Batchelor TT et al., 2007). Vandetanib (Zactima®) is a selective inhibitor of VEGFR2 and EGFR and showed promising antiglioma effects in preclinical studies (Rich JN et al., 2005). Vatalanib is a an oral pan-VEGFR, PDGFR, c-Kit inhibitor that reduced the activity of VEGF-mediated glioma growth (Goldbrunner et al., 2004). In a phase I/II trial, vatalanib produced radiographic responses in 4% of the patients and resulted in stable disease response rate of 56% (Conrad C et al., 2004). Sorafenib (Nexavar®) is a multi-target inhibitor that targets VEGFR, PDGFR, c-KIT, and Raf. It is approved to be used in a single-agent, monotherapy setting and data from phase I trial indicates that patients well tolerated the drug (Jane EP et al., 2006; Nabors L et al., 2007). Sunitinib (Sutent®) is also a multi-target kinase inhibitor that displayed promising and efficacious antiangiogenic activity and antitumor activity in GBM models *in vitro* and *in vivo*. Pazopanib (Voltrient®) is another multi-target kinase inhibitor of VEGFR-1, -2, and -3, PDGFR and c-KIT and in a recent phase II study, pazopanib was well tolerated with median progression-free survival being 12 weeks (Iwamoto FM et al., 2010). Studies exploring the therapeutic efficaciousness of AE-788 and CT-322 are underway, which are oral inhibitor of EGFR, HER2 and VEGFR2 and pegylated protein inhibiting VEGFR-2 signaling pathway cascade, respectively (Brastianos & Batchelor, 2010).

Exploring VEGF signaling pathway is crucial in treating malignant glioma because these anti-VEGF agents can normalize the blood vessels, which will allow to improve the delivery of chemotherapy agents that will produce favorable radiation response. Even though there are many promising data published regarding the therapeutic efficacy of VEGF inhibitors in pre-clinical and clinical settings, more studies are in development to confirm these results and to understand the complexity of molecular signaling pathways.
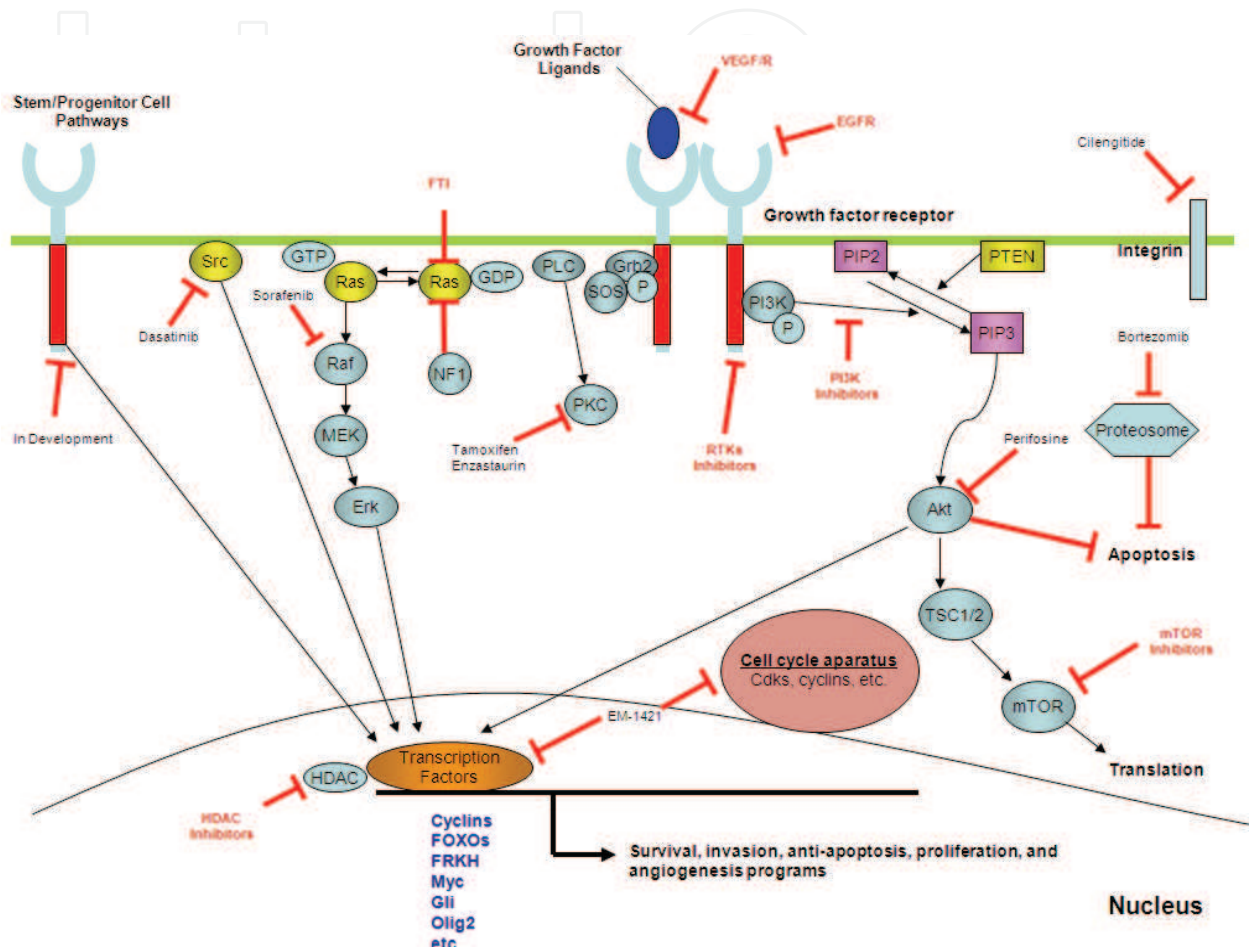

Fig. 2. Signaling pathways important for stem cell signaling

Pharmacological interference with these pathways promises to have therapeutic potential in targeting cancer stem cells. Receptor tyrosine kinase-mediated signaling (FGF, PDGF, EGF, VEGF) promotes progenitor cell proliferation and angiogenesis. PTEN acts as a tumor-suppressor gene and is frequently inactivated in gliomas. The
Wnt/b-catenin pathway leads to accumulation of intranuclear b-catenin and transcription of target genes critical for stem cell and progenitor cell function. Shh binds to its associated transmembrane receptor PTC1, releasing the membrane protein Smo, which results in downstream activation of the Gli proteins and transcription factors. Bmi1 controls maintenance of stem cells by repressing genes that promote differentiation or cell death, such as the tumor suppressors p16Ink4a and p19Arf. Binding of the cell membrane-associated Notch receptor proteins and their associated ligands (not shown) leads to cleaving of the intracellular domain of Notch (NICD) and transcription of target genes essential for maintenance and self-renewal of stem cells. PTC1: Patched 1; Shh: Sonic hedgehog; Smo: Smoothened.
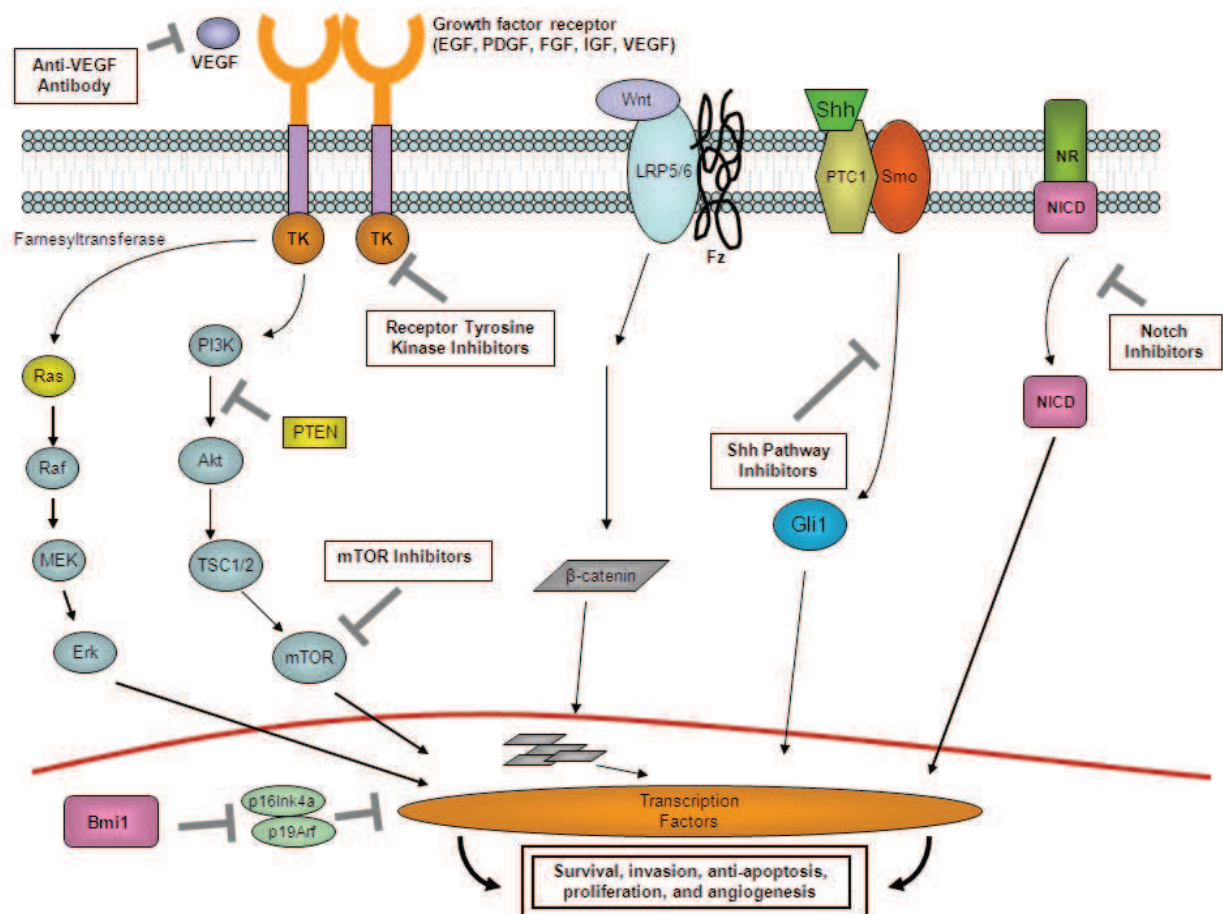
Fig. 3. Major signaling pathways in malignant gliomas and the corresponding targeted agents in development for glioblastoma

RTK inhibitors that target epidermal growth factor (EGF) receptor include gefitinib, erlotinib, lapatinib, BIBW2992, and vandetanib; those that target plateletderived growth factor (PDGF) receptor include imatinib, dasatinib, and tandutinib; those that target vascular endothelial growth factor (VEGF) receptor include cediranib, pazopanib, sorafenib, unitinib, vatalanib, vandetanib, and XL184. EGF receptor antibodies include cetuximab and anitumumab. Farnesyl transferase inhibitors include lonafarnib and tipifarnib; HDAC inhibitors include depsipeptide, vorinostat, and LBH589; PI3K inhibitors include BEZ235 and XL765; mTOR inhibitors include sirolimus, temsirolimus, everolimus, and deforolimus; and VEGF receptor inhibitors include bevacizumab, aflibercept (VEGF-trap), and CT-322. Growth factor ligands include EGF, PDGF, IGF, TGF, HGF/SF, VEGF, and FGF. Stem-cell pathways include SHH, wingless family, and Notch. Akt denotes murine thymoma viral oncogene homologue (also known as protein kinase B), CDK cyclin-dependent kinase, ERK extracellular signal-regulated kinase, FGF fibroblast growth factor, FTI farnesyl transferase inhibitors, GDP guanine diphosphate, Grb 2 growth factor receptor-bound protein 2, GTP guanine triphosphate, HDAC histone deacetylase, HGF/SF hepatocyte growth factor/scatter factor, IGF insulin-like growth factor, MEK mitogen-activated protein kinase kinase, mTOR mammalian target of rapamycin, NF1 neurofibromin 1, PIP2 phosphatidylinositol (4,5) biphosphate, PIP3 phosphatidylinositol 3,4,5-triphosphate, PI3K phosphatidylinositol 3-kinase, PKC protein kinase C, PLC phospholipase C, PTEN phosphatase and tensin homologue, RAF v-raf 1 murine leukemia viral oncogene

homologue 1, RAS rat sarcoma viral oncogene homologue, RTK receptor tyrosine kinase inhibitor, SHH sonic hedgehog, SOS son of sevenless, Src sarcoma (Schmidt-Ruppin A-2) viral oncogene homologue, TGF transforming growth factor family, and TSC1 and 2 tuberous sclerosis gene 1 and 2. Red text denotes inhibitors.

3.4 RAS/Raf/MAPK & AKT/PI3K pathways

Downstream effects of RTK signaling include activation of Ras/ Raf/MAPK and AKT/PI3K, promoting cell survival, cell proliferation, cell migration and angiogenesis. Malignant gliomas and glioblastomas have hyperactivated phosphatidylinositol-3-kinase (PI3K)-Akt , also known as protein kinase B) pathway that serve as the genesis of human cancers (Eyler C et al., 2008; Castellino RC et al., 2007; Wlodarski P et al., 2006; Knobbe CB et al., 2005). Once Akt signaling pathway is hyperactivated it results in the proliferation, invasion, and angiogenesis of tumor cells. AKT/PI3K activation through loss of PTEN in combination with constitutively active EGFR signaling has been shown to induce glial tumor formation along with genetic instability in experimental models (Eyler C et al., 2008). Interestingly, glioma stem cells appear to be more dependent on AKT signaling than non-stem glioma cells, suggesting that AKT inhibition is crucial in managing the tumor stem cells from expanding and treating brain tumors more effectively (Eyler C et al., 2008). Currently, there are various clinical trials observing tem- sirolimus (CCI-779), its active metabolite sirolimus (rapamycin), everolimus (certican), and AP23573, which are mTOR inhibitors that target PI3K signaling pathway (Galanis,2005; Chang,2005; Kuhn,2007).

3.5 Sonic hedgehog signaling

Sonic hedgehog (Shh) is a key regulator and a determinant that is crucial for the generation and maintenance of adult stem cells (Dave R et al., 2011). Hedgehog (Hh) signaling is also involved in patterning, growth, and cell fate determination in many developing organ systems. Upon secretion, the hedgehog molecules bind to Patched 1 protein (PTCH1) and inhibit the receptor. Once inactivated, PTCH1 accumulates Smoothened (SMOH) in its cytoplasm instead of releasing them (Dave R et al., 2011). These results in downstream activation of the Gli proteins – zinc-finger transcription factors that translocate to the nucleus and may either activate or repress downstream targets, such as Wnt, IGF and PDGFR-a, myc and cyclin D1. Shh is important in regulating neural stem cells, neural tube patterning, and neurogenesis (Machold, 2003; Park, 2003; Lai, 2003; Palma, 2005; Cai, 2008; Han, 2008; Komada, 2008). Recent studies show that SHH-neutralizing antibodies help in inhibiting tumor cell growth and reducing its proliferation rates (Chen YJ et al., 2007; Thayer SP et al., 2003; Berman DM et al., 2003; Karhadkar SS et al., 2004). Early clinical trials (e.g., GDC-0449) are ongoing to evaluate inhibitors of Shh signaling (Rudin,2009). Collectively, Shh-mediated signaling is critically important in stem cell and tumor biology and may constitute another attractive target for therapy of malignant brain tumors.

3.6 PTEN

Phosphatase and tensin (PTEN) homologue gene has important functions in both normal neural stem cell physiology and oncogenic processes. It acts mainly as a tumor suppressor gene and is involved in many cellular functions including cell cyle progression, angiogenesis, migration, invasions, and stem cell regulation (Cheng RB et al., 2010; Alexiou GA et al., 2010). However, in many malignant gliomas, PTEN genes are often deleted or

mutated, which contributes to the pathologic progression of the tumor cells. This loss of PTEN leads to constitutive activation of AKT and resistance to apoptosis (Maehama T et al., 1998; Radu A et al., 2003; Stiles B et al., 2002). In turn, this down-regulation of PTEN results in aggressive tumor expansion and poor prognosis. PTEN inactivation in combination with EGFR amplification is sufficient to cause invasive gliomas in experimental mouse models, supporting the critical role of PTEN inactivation in gliomagenesis. Furthermore, it has been suggested that loss of PTEN enhances resistance to EGF RTK inhibitors in glioblastoma patients (Mellinghoff, 2007).

4. Targeted molecular therapy

In recent years, there has been tremendously increased understanding of the molecular abnormalities occurring in malignant gliomas (Maher,et al.,2001; Kitange,et al.,2003; Konopka&Bonni,2003). Molecular analysis of gliomas shows a step-wise progression of genetic changes involving overexpression of proto-oncogenes and loss of tumor suppressor genes. Low-grade astrocytomas (World Health Organization [WHO] grade II) tend to have inactivating mutations of TP53 and overexpression of platelet-derived growth factor (PDGF) and their receptors (PDGFR). Progression to anaplastic astrocytomas (WHO grade III) is associated with inactivation of the p16-cdk4-Rb pathway and allelic loss of 19q, whereas progression further to a secondary glioblastoma (WHO grade IV) is associated with loss of chromosome 10 and other changes. Primary glioblastomas, which originate de novo, often have loss of phosphatase and tensin homologue deleted on chromosome 10 (PTEN), together with amplification, mutation, and overexpression of the epidermal growth factor receptors (EGFR). There is increasing work on molecular profiling of malignant gliomas using a variety of different techniques.

These approaches are beginning to enable genes that are important in tumor progression to be identified (van den Boom et al., 2003). In addition, morphologically indistinguishable malignant gliomas can be differentiated into molecular subtypes that may eventually be used for identifying potential targets for treatment (Mischel et al., 2003; Rao et al., 2003), for patient stratification in clinical studies (Shai et al., 2003), and for determining prognosis (Nutt et al., 2003).

Recently, inhibitors of oncogenes and signaling pathways have shown promising therapeutic potential in the treatment of several systemic cancers (Drucker, 2002). The prototypic targeted molecular agent is imatinib mesylate (Gleevec; Novartis, Basel, Switzerland), a small molecule inhibitor of the abl, c-kit, and PDFGR tyrosine kinases. It has demonstrated significant antitumor activity in chronic myelogenous leukemia (CML) by inhibiting the abl tyrosine kinase and in gastrointestinal stromal tumors (GIST) by inhibiting c-kit (Drucker, 2002). The success of imatinib in these tumors demonstrates the potential of these agents in tumors with well defined molecular targets. Although the complexity of the molecular abnormalities in malignant gliomas and the redundancy of the signaling pathways make it unlikely that single agents will achieve the same success as imatinib in CML, there has been significant interest in this approach (Karpati et al., 2003; Newton, 2004; Mischel, 2003). Over the past years, several of the first generation trials evaluating targeted molecular agents in malignant gliomas have reached maturity, and it is possible to draw some preliminary conclusions. In general, these agents have been well tolerated, but unfortunately only small subsets of patients have benefited.

Molecular therapeutic target	Name of agent
EGFR	Gefitinib (ZD1839; Iressa®)
EGFR	Erlotinib (OSI-774; Tarceva®)
EGFR+Erb-B2	Lapatinib (GW-572016)
EGFR+VEGFR	AEE788
EGFR + VEGFR + RET	Vandetanib (ZD6474; Zactima®)
EGFR	Nimotuzumab
EGFR	Cetuximab (C225; Erbitux®)
PDGFR	Nilotinib (Tasigna)
PDGFR	Imatinib mesylate (ST1571; Gleevec®)
PDGFR + VEGFR	Vatalanib (PTK787; ZK222584)
PDGFR + Src + c-kit + Bcr-Abl	Dasatinib (Sprycel®)
PDGFR + c-Kit + FLT-3	Tandutinib (MLN-518
PDGFR + VEGFR + c-Kit + FLT-3	Sunitinib (Sutent®)
PDGFR	CP-673, 451
PDGFR + VEGFR + c-kit + Raf	AMG706
PDGFR + VEGFR + c-kit	Pazopanib (GW-786034)
PDGFR + VEGFR + c-kit	SUO11248
PDGFR + VEGFR + c-Kit + Raf	Sorafenib (Bay43-9006; Nexavar®)
PDGFR + VEGFR	OSI-930
PDGFR + VEGFR	TKI258
VEGF-A/B Ab	Aflibercept (VEGF-Trap)
VEGFR + c-Met	XL184
VEGFR, PDGFR, c-Kit	Pazopanib (GW786034)
VEGFR + PDGFR + c-Kit	Cediranib (AZD2171; Recentin®)
VEGFR + PDGFR + c-Kit + Raf	Sorafenib (Bay43-9006; Nexavar®)
VEGFR + EGFR + Ret	Vandetanib (ZD6474; Zactima®)
VEGFR + PDGFR + c-kit	Vatalanib (PTK787; ZK222584)
VEGFR + PDGFR + c-Kit	Sunitinib (Sutent®)
VEGFR + EGFR	AEE788
VEGFR + PDGFR + c-kit	SUO11248
VEGFR + FGFR + PDGFR + c-kit	SU6668
VEGFR + PDGFR	OSI-930
VEGFR + PDGFR	TKI258
Raf + VEGFR + PDGFR	Sorafenib (Bay43-9006; Nexavar®)
Raf	Bay549805
Raf + PDGFR + VEGFR + c-kit	AMG706
Raf, VEGFR	AAL881
PKC-b2 + Akt	Enzastaurin
Akt	Perifosine
mTOR	Temsirolimus (CCI-779)
mTOR	Everolimus (RAD001; Certican®)
mTOR	Sirolimus (rapamycin; Rapimmune®)
mTOR	AP23573

Table 1. Selected small-molecule inhibitors with activity against signaling pathways relevant to cancer stem cells in malignant gliomas

5. Conclusion

The discovery that malignant tumors contain small subpopulations of cells that are highly tumorigenic and share features with normal stem cells has stimulated the field of cancer

research and established a novel concept in tumor biology – that most cancers, including glioblastomas, are driven by 'cancer stem cells' responsible for tumorigenesis and resistance to conventional therapies. Understanding the mechanisms and signaling pathways that govern cancer stem cells will be a key to identifying effective therapies to eventually improve tumor control and clinical outcome. A number of problems will have to be overcome in the development of effective therapies targeting cancer stem cells. Cancer stem cells are typically slowly cycling cells, express high-levels of drug-resistance genes and may not necessarily depend on oncogenes and their gene products targeted by small-molecule inhibitors. Further progress in glioma research will come from the molecular profiling of well-characterized cancer cell populations (e.g., after FACS analysis) and the identification of novel cellular markers that will provide the foundation to track cancer stem cells *in vitro* and *in vivo.* There is significant need to improve our ability to monitor treatment response with novel biomarkers so that patients who are resistant to therapy may be identified early in the treatment course. Moreover, novel biomarkers or surrogate markers of activity, and advances in molecular imaging in combination with tumor tissue analysis from patients enrolled into clinical trials will be important to evaluate treatment response and to understand treatment failure.

Several signaling pathways that orchestrate normal neural stem and progenitor cells are adopted by cancer stem cells and drive tumor cell proliferation, migration and treatment resistance.

Further elucidation of the molecular circuitry driving tumorigenesis and treatment resistance will be essential to advance our knowledge in glioma biology and to translate these concepts into the clinical arena. Both targeting the cancer stem cell compartment and individualizing patient treatment based on the unique signaling features in a given tumor have the greatest potential to translate into a successful treatment strategy.

While the first generation of molecular targeted therapies have shown promising results in preclinical studies, most agents have failed to translate into significant clinical benefit in early clinical trials. Preliminary clinical studies suggest that inhibition of a single pathway may not be sufficient to inhibit glioma growth in order to prolong patient survival (Pillay, et al., 2009). Therefore, targeting multiple pathways and signaling components in combined treatment approaches promises to be more successful.

However, given the increasing number of putative targets and agents, and the exponentially increasing number of potential combinations used in patients, it will be important to identify the most promising combinations and to carefully design and plan clinical trials. Recent genomic studies highlight the fact that gliomas are heterogeneous tumors. (Parsons,et al.,2008) Consequently, it will be important to integrate information derived from large genomic studies and combine it with our increasing understanding of mechanisms relevant to cancer stem cells in order to effectively treat brain tumor patients.

With recent efforts to individualize cancer treatment in patients, molecular targeted therapies directed to cancer stem cells and their signaling pathways will be increasingly used in the near future by clinicians and oncologists. An important issue of concern has come from recent studies on the cell-biological analysis of cancer therapy-associated neurotoxicity. Both radiation and cytotoxic therapies have been shown to be highly toxic to neural progenitor cell populations important for the maintenance of normal brain function, and may disrupt neurogenesis and white matter integrity (Dietrich, et al., 2006; Han, et al., 2008; Monje, 2002; Dietrich, et al., 2008). As multiple new molecular agents have been developed to specifically target signaling pathways employed by normal neural stem and

progenitor cells, serious neurotoxic adverse effects may be encountered in long-term survivors. Another concern has come from recent experimental studies demonstrating that molecular targeted therapies with antiangiogenic compounds may promote tumor cell migration and metastasis (Holash et al., 1999; Rubenstein et al., 2000; Loges et al., 2009; Ebos et al., 2009; Paez-Ribes et al., 2009).

Regardless, targeted molecular therapies hold great promise and will become an important component of combined treatment approaches in our effort to fight cancer.

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Cancer Stem Cells - The Cutting Edge

Edited by Prof. Stanley Shostak

Over the last thirty years, the foremost inspiration for research on metastasis, cancer recurrence, and increased resistance to chemo- and radiotherapy has been the notion of cancer stem cells.The twenty-eight chapters assembled in Cancer Stem Cells - The Cutting Edge summarize the work of cancer researchers and oncologists at leading universities and hospitals around the world on every aspect of cancer stem cells, from theory and models to specific applications (glioma), from laboratory research on signal pathways to clinical trials of bio-therapies using a host of devices, from solutions to laboratory problems to speculation on cancersâ€™ stem cellsâ€™ evolution. Cancer stem cells may or may not be a subset of slowly dividing cancer cells that both disseminate cancers and defy oncotoxic drugs and radiation directed at rapidly dividing bulk cancer cells, but research on cancer stem cells has paid dividends for cancer prevention, detection, targeted treatment, and improved prognosis.

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