

REVIEW

Cancer stem cells in glioblastoma — molecular signaling and therapeutic targeting

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Received May 6, 2010 Accepted June 8, 2010

ABSTRACT

Glioblastomas (GBMs) are highly lethal primary brain tumors. Despite current therapeutic advances in other solid cancers, the treatment of these malignant gliomas remains essentially palliative. GBMs are extremely resistant to conventional radiation and chemotherapies. We and others have demonstrated that a highly tumorigenic subpopulation of cancer cells called GBM stem cells (GSCs) promotes therapeutic resistance. We also found that GSCs stimulate tumor angiogenesis by expressing elevated levels of VEGF and contribute to tumor growth, which has been translated into a useful therapeutic strategy in the treatment of recurrent or progressive GBMs. Furthermore, stem cell-like cancer cells (cancer stem cells) have been shown to promote metastasis. Although GBMs rarely metastasize beyond the central nervous system, these highly infiltrative cancers often invade into normal brain tissues preventing surgical resection, and GSCs display an aggressive invasive phenotype. These studies suggest that targeting GSCs may effectively reduce tumor recurrence and significantly improve GBM treatment. Recent studies indicate that cancer stem cells share core signaling pathways with normal somatic or embryonic stem cells, but also display critical distinctions that provide important clues into useful therapeutic targets. In this review, we summarize the current understanding and advances in glioma stem cell research, and discuss potential targeting strategies for future development of anti-GSC therapies.

KEYWORDS cancer stem cell, glioblastoma, therapeutic resistance, molecular targeting, tumor angiogenesis, hypoxia response, stem cell niche

INTRODUCTION

Glioblastomas (WHO grade IV gliomas) are the most common type of malignant tumors in central nervous system (CNS) in adults. Glioblastoma (GBMs) remains one of the most fatal and least successfully treated solid tumors (Furnari et al., 2007; Wen and Kesari, 2008). The median survival of GBM patients treated with multimodal therapies including surgical resection, radiation and chemotherapy is less than 16 months (Stupp et al., 2005; Furnari et al., 2007). This poor prognosis for GBM patients has not improved significantly over decades, underscoring the difficulties and challenges in effectively detecting and treating these lethal cancers. The fundamental problem of these malignancies is their highly infiltrative nature and extreme resistance to conventional treatments. Aggressive invasion of GBM cancer cells into the normal brain tissue and spinal cord often prevents complete removal of tumor cells through surgical resections. Invading cancer cells appear to be particularly resistant to current therapies and are often protected by the neurovascular niche (Furnari et al., 2007). In addition, the majority of patients suffer treatment failure within 2–3 cm of the original resection cavity, indicating that therapeutic resistance is a common feature of GBM tumors. Collectively, these difficulties have propelled the reevaluation of current treatments in order to achieve maximal efficacy with minimized toxicities or side-effects. While chemotherapy has been used for several decades in neuro-oncology, the oral DNA methylating agent temozolomide (TMZ) has shown effective when used concurrently with radiation and then as adjuvant chemotherapy such that it is now a standard practice (Stupp et al., 2005; Wen and Kesari, 2008). Several targeted therapies have been tested in trials of malignant gliomas, but to date only bevacizumab (Avastin) has been approved by the FDA to treat GBMs (Vredenburgh et al., 2007a, b; Friedman et al., 2009). Immunotherapies and

toxin-ligand conjugates have shown promise in early clinical trials but so far lack definitive efficacy in phase III trials. Thus, development of more effective treatments is urgently needed, which will require new paradigms in cancer biology and more insight into the mechanisms underlying the cancer invasion, therapeutic resistance and tumor recurrence in GBMs.

It has been well recognized that most solid tumors consist of heterogeneous cancer cells, as well as vasculatures, stromal elements and inflammatory cells (Hanahan and Weinberg, 2000). GBM displays remarkable intratumoral heterogeneity and cellular hierarchy not only morphologically but also in differentiation status. Cancer cell heterogeneity and hierarchy may be resulted from both genetic and/or epigenetic causes. Increasing evidence from hematopoietic malignancies and solid tumors (including brain, breast, colorectal, head and neck cancers) has strongly supported the concept that a subpopulation of cancer cells in each tumor has greater potential of cancer initiation and repopulation (Lapidot et al., 1994; Bonnet and Dick, 1997; Al-Hajj et al., 2003; Hemmati et al., 2003; Singh et al., 2003; Galli et al., 2004; Singh et al., 2004; Ricci-Vitiani et al., 2007; O'Brien et al., 2007; Dalerba et al., 2007; Prince et al., 2007; Schatton et al., 2008). These cells were called cancer stem cells (CSCs) or tumor-initiating or propagating cells because they share some critical characteristics with normal stem cells, including the capacities for self-renewal, multi-lineage differentiation, and maintained proliferation (Reya et al., 2001; Vescovi et al., 2006; Bao et al., 2006a; Rosen and Jordan, 2009; Park and rich, 2009; Heddleston et al., 2010; Frank et al., 2010). Although cancer stem cell or stem cell-like cancer cell terminology is imperfect due to its distinct differences from normal stem cell, but it does capture the shared characteristics with normal stem cells especially somatic stem cells. In this review, we used "cancer stem

cell" (CSC) terminology, which does not implicate that the cell-of-origin of cancer stem cells has to be normal stem cell or progenitor, although several recent studies have demonstrated that normal tissue stem cells can be transformed to be cancer stem cells (Barker et al., 2009; Zhu et al., 2009). Cancer stem cells in malignant gliomas that were called glioma stem cells or GBM stem cells (GSCs) have been identified and characterized by several groups including our group (Hemmati et al., 2003; Galli et al., 2004; Singh et al., 2004; Bao et al., 2006a, b, 2008; Lee et al., 2006; Li et al., 2009). These cells are functionally defined with self-renewal (Fig. 1), cell differentiation *in vitro* (Fig. 2), and tumor propagation *in vivo* (Fig. 3B). Studies from a number of groups including ours have demonstrated that GSCs display much greater tumorigenic potential than matched non-stem tumor cells when xenotransplanted into the brains of immunocompromised rodents (Fig. 3B) (Singh et al., 2004; Bao et al., 2006a; Lee et al., 2006; Li et al., 2009). We and others have shown that GSCs have the potential to differentiate into astrocytes, oligodendrocytes and neurons (Fig. 2) (Hemmati et al., 2003; Galli et al., 2004; Singh et al., 2004; Bao et al., 2006a; Calabrese et al., 2007), though GSCs commonly display aberrant differentiation signatures with multiple lineage markers expressed in one differentiated cell. GSCs may be resulted from genetic and epigenetic changes in neural stem/progenitor cells or the differentiated cells such as astrocytes after a series of mutations or epigenetic reprogram. Although the origin of GSCs is not clearly defined, GSCs share similar properties with normal neural stem cells (NSCs) that endow these cells with key traits in carcinogenesis. These properties include enhanced potentials for proliferation, angiogenesis, invasion and modulating immune responses.

GSCs have been implicated in several malignant behaviors

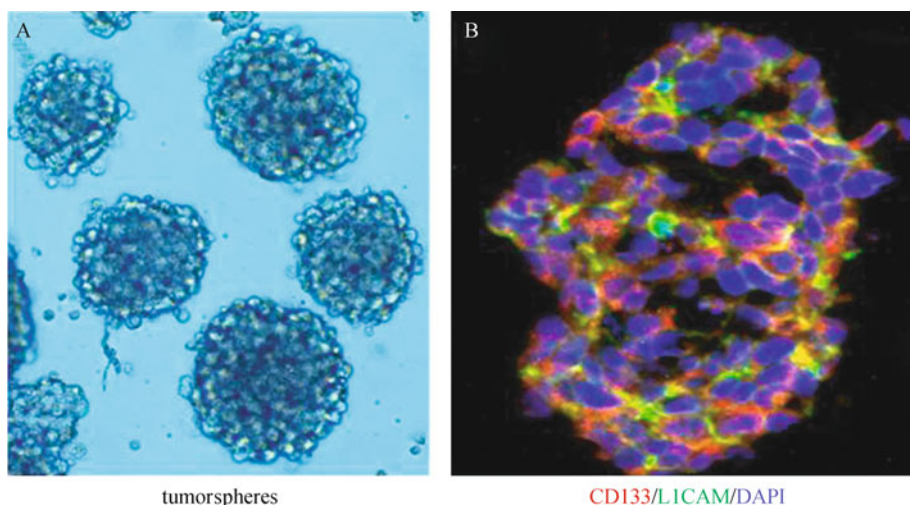


Figure 1. GBM stem cells (GSCs) formed tumorspheres and expressed L1CAM and CD133 markers. (A) GSCs isolated from a glioblastoma surgical specimen formed tumorspheres (neurospheres). (B) The section of GSC tumorsphere was immuno-stained with anti-L1CAM (green) and anti-CD133 (red) antibodies, and counterstained with DAPI for nuclear DNA (blue).

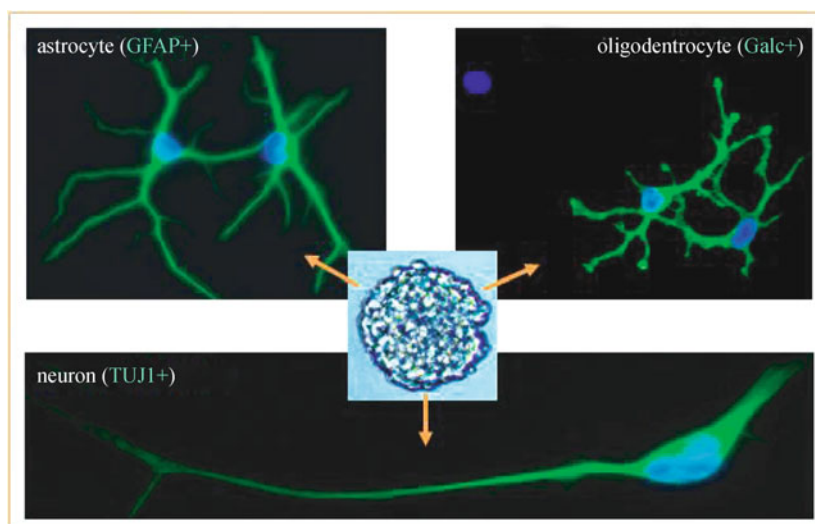


Figure 2. GBM stem cells (GSCs) displayed capacity of multiple-lineage differentiation. GSCs derived from a GBM tumor specimen formed tumorspheres and differentiated into cells expressing markers for astrocyte (GFAP+), oligodentrocyte (Galc+) and neuron (TUJ1+) upon induction of differentiation.

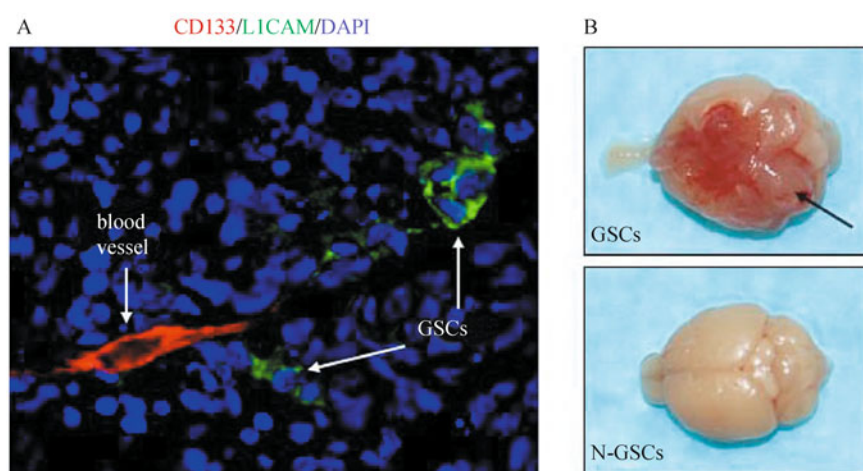


Figure 3. GBM stem cells (GSCs) are localized in vascular niches in the tumor tissue and are highly tumorigenic *in vivo*. (A) The frozen section of a GBM surgical specimen was immuno-stained with anti-L1CAM antibody for GSCs (in green) and anti-CD31 antibody for endothelial cells of blood vessel (in red), and counterstained with DAPI for DNA (blue). GSCs (L1CAM+, in green) are located near the blood vessel (CD31+, in red). (B) GSCs display greater potential of tumor formation than the matched non-stem cancer cells (N-GSCs) in xenograft models. GSCs isolated from a primary GBM specimen formed a large and highly vascular tumor (indicated by arrow) in mouse brain.

associated with GBM tumor progression. We demonstrated that GSCs express elevated levels of VEGF (vascular endothelial growth factor) to enhance tumor angiogenesis (Bao et al., 2006b). This finding has been confirmed by other groups determining that SDF-1 (stromal-derived factor-1, also known as CXCL12) is an additional pro-angiogenic ligand expressed by GSCs (Folkins et al., 2009). This is particularly significant as a humanized anti-VEGF antibody called bevacizumab (Avastin) has demonstrated a promising efficacy against glioblastomas, and it was recently approved by the United States FDA for the treatment of recurrent or

progressive GBMs (Vredenburgh et al., 2007a, b; Friedman et al., 2009). Moreover, we also found that GSCs are relatively resistant to radiation due to preferential response of the DNA damage checkpoint and the enhanced DNA repair capacity (Bao et al., 2006a), while other groups have shown relative resistance of GSCs to chemotherapies such as temozolomide (Liu et al., 2006; Bleau et al., 2009). These studies may help understand in part why those GBM patients with a promising radiographic response universally suffer recurrence and/or progression of their cancers. Thus, molecular targeting of GSCs may directly improve the efficacy of current

cytotoxic therapies and anti-angiogenic therapies. In this review, we summarize the roles of GSCs in tumor progression and malignant behaviors of GBMs with attention to signaling pathways and molecular regulators involved in maintaining the GSC phenotype, and discuss molecular strategies targeting GSCs for the development of novel therapeutics to improve the survival of GBM patients in the future.

CANCER STEM CELLS AND THERAPEUTIC RESISTANCE OF GLIOBLASTOMA

GBMs are highly infiltrative tumors that display extreme resistance to conventional radiotherapy and chemotherapy, and often recur rapidly in a local fashion despite maximal surgical resection (Furnari et al., 2007; Wen and Kesari, 2008). We and others have shown that GSCs contribute to therapeutic resistance and likely are responsible for GBM tumor recurrence (Bao et al., 2006a; Liu et al., 2006; Rich and Bao, 2007; Bleau et al., 2009). The presence of GSCs within GBM tumor responsible for generating the entire mass of cancer cells has important implications for understanding the efficacy of current therapies and the resistance issue. Although the hierarchical relationship between GSC population and bulk tumor remains controversial (Park et al., 2010), it is possible that GSCs contribute to tumor repopulation after conventional therapies. Radiation is the most effective non-surgical therapy for GBMs, but it is palliative indicating that GBMs contain resistant cancer cell populations. Indeed, we demonstrated that GSCs are more resistant to radiation than the matched non-stem glioma cells (Bao et al., 2006a). In response to DNA damage induced by radiation or the radio-mimic drug neocarzinostatin (NCS), GSCs preferentially activate DNA damage checkpoint response as measured by the activating phosphorylation of several critical checkpoint proteins (ATM, Rad17, Chk2 and Chk1) (Bao et al., 2006a). As a consequence of the preferential checkpoint activation, GSCs are more efficient in repairing the damaged DNA and more rapidly recover from the genotoxic stress than the matched non-stem tumor cells. Thus, GSCs display more resistance to radiation-induced apoptosis than the non-stem cancer cells *in vitro* and *in vivo* (Bao et al., 2006a). Moreover, a low molecular weight inhibitor (Debromohymenialdisine, DBH) of Chk2 and Chk1 kinases abolishes the radio-resistance of GSCs, suggesting that targeting the DNA damage checkpoint activation may sensitize GSCs to radiotherapy and thus overcome the radioresistance of GBMs. Although checkpoint inhibitors may be used as radiosensitizers of GSC population, the effects of the checkpoint inhibitors on normal stem cells and other normal cells must also be considered, because long-term inhibition of checkpoint activation may lead to new oncogenesis. Recently, it has also been shown that inhibition of the Notch signaling pathway by the γ -secretase inhibitor or Notch shRNA renders GSCs more sensitive to radiation (Wang

et al., 2010), suggesting that Notch pathway may serve as another potential target for reducing GBM radioresistance. In addition, recent studies suggested that targeting SirT1 expression or HSP90 activity can also attenuate radioresistance of GSC population (Chang et al., 2009; Sauvageot et al., 2009). It seems that multiple molecular mechanisms regulate GSC radioresistance, perhaps with intertumoral variation. Interestingly, decreased radiosensitivity in breast cancer stem cells has been linked to lower levels reactive oxygen species (ROS) (Diehn et al., 2009) or enhanced activity of Wnt/ β -catenin signaling (Woodward et al., 2007). Although these mechanisms have not been reported in GSCs, they give hope that several molecular regulators may be targeted synergistically in GSCs in order to effectively overcome radioresistance.

The current standard of treatment for GBMs also includes adjuvant chemotherapy with temozolomide (TMZ) that is a commonly used oral methylating chemotherapeutic agent (Stupp et al., 2005; Wen and Kesari, 2008). TMZ achieves significant cytotoxic effect on cancer cells mainly by methylating the O^6 position of guanine in DNA. This DNA adduct can be removed by the repair enzyme O^6 -methylguanine-DNA-methyltransferase (MGMT) that is expressed in varied levels in GBMs. MGMT expression is likely regulated at several levels but recent attention has focused on promoter methylation that has been linked to greater sensitivity to TMZ treatment due to reduced MGMT levels (Hegi, et al., 2005). The addition of TMZ to GBM therapy has been potentially most effective by a radiosensitization effect (Stupp et al., 2005), but TMZ is commonly used as adjuvant therapy as well. Although therapy with TMZ may slow GBM tumor growth and increases the proportion of patients surviving for two years, long-term survivors are still rare due to drug resistance and tumor recurrence. Invariable recurrence after TMZ therapy indicates the presence of TMZ-resistant cancer cells in GBM. It has been shown that GSCs also contribute to the chemoresistance to TMZ (Liu et al., 2006). In a genetically engineered glioma mouse model, TMZ treatment increased the side population (SP), a potential measure of cancer stem cells (Bleau et al., 2009). Although TMZ can eliminate MGMT-negative GSCs (Beier et al., 2008), TMZ does not inhibit self-renewal of GSCs with normal levels of MGMT (Clement et al., 2007). Several other potential mechanisms underlying the drug resistance of GSCs have also been reported. Increased expression of drug transporters including the ABC (ATP binding cassette) transporters that pump out chemotherapeutic agents may be one of critical mechanisms. Some studies suggested that the expression of ABC transporters increase in cancer stem cells (Schatten et al., 2008). A side population (SP) of cancer cells isolated from tumors may represent a class of cancer stem cells with high drug efflux capacity and thus show inherently high resistance to chemotherapeutic agents (Hirschmann-Jax et al., 2004). SP cells express elevated levels of the ABC drug

transporters such as ABCG2 and ABCA3 in GBM cell lines (Hirschmann-Jax et al., 2004), suggesting that targeting these drug transporters may reduce chemoresistance of GSC populations in GBM.

GLIOBLASTOMA STEM CELLS AND TUMOR ANGIOGENESIS

Active tumor angiogenesis is one of hallmarks of glioblastomas (Plate and Risau, 1995; Furnari et al., 2007). Florid neovascularization plays crucial roles in providing nutrition and oxygen and removing waste to facilitate the rapid growth and progression of malignant gliomas. The degree of vascularization is significantly correlated with the glioma malignancy, tumor aggressiveness, and clinical prognosis (Plate and Risau, 1995). Based on this background, a number of laboratories have investigated the relationship between the tumor vasculature and GSC population. We have determined that GSCs formed tumors with greater vascularity than the non-stem tumor cells (Bao et al., 2006b). We demonstrated that GSCs promote tumor angiogenesis partially through elevated expression of the most important pro-angiogenic factor, vascular endothelial growth factor (VEGF) (Bao et al., 2006b; Li et al., 2009). GSCs express much higher levels of VEGF than the matched non-stem tumor cells and display greater angiogenic potential *in vitro* and *in vivo*. Targeting VEGF through bevacizumab specifically blocked GSC pro-angiogenic effects both *in vitro* and *in vivo*. Other groups showed that GSCs promote both tumor angiogenesis and vasculogenesis via VEGF and SDF-1 (Folkins et al., 2009). To understand the molecular mechanisms that drive VEGF expression in GSCs, we interrogated the role of hypoxia and the hypoxia inducible factors (HIFs) in the GSC-mediated angiogenesis. As expected, hypoxic condition induced VEGF expression in both GSCs and non-stem glioma cells but the levels were consistently higher in GSCs (Li et al., 2009; Heddleston et al., 2010). Interestingly, HIF-1 α and HIF-2 α specifically controlled VEGF expression in GSCs in a non-redundant manner (Li et al., 2009). In addition, hypoxia can promote the expansion of GSC fraction and regulate expression of stem cell markers (Heddleston et al., 2009; McCord et al., 2009; Soeda et al., 2009). Thus, hypoxia may enhance tumor progression and therapeutic resistance through its promotion of a cancer stem cell phenotype and induction of VEGF and other pro-angiogenic factors.

The powerful pro-angiogenic effect of GSCs on tumor vascularization suggests that targeting GSCs should disrupt tumor angiogenesis. Furthermore, recent studies demonstrated that the relationship between GSCs and the vasculature is complex and bi-directional. Normal neural stem cells (NSCs) reside in perivascular locations called vascular niches that provide essential pro-survival and maintenance cues (Shen et al., 2008; Tavazoie et al., 2008; Mirzadeh et al., 2008). In a seminal study, Gilbertson group demonstrated that

cancer stem cells in brain tumors also reside in perivascular niches (Calabrese et al., 2007). They further showed that endothelial cells increase survival of brain tumor stem cells and targeting the tumor vasculature with bevacizumab reduces the number of cancer stem cells in treated tumors. In addition, they found that co-transplantation of endothelial cells with GSCs accelerates tumor initiation and progression (Calabrese et al., 2007). We have observed that glioma cells expressing GSC markers, CD133, HIF2 α or L1CAM (CD171), are localized near blood vessels (Bao et al., 2006a, 2008; Li et al., 2009) (Fig. 3A). Collectively, these studies suggest that the prevascular niche may provide a specific microenvironment for the maintenance of GSCs. The symbiotic relationship between GSCs and vasculatures may explain the promising efficacy of anti-angiogenesis therapy with cediranib (AZD2171, a VEGFR inhibitor) or bevacizumab for GBM patients in the clinical trials (Vredenburgh et al., 2007a, b; Batchelor et al., 2007; Friedman et al., 2009). Thus, anti-angiogenic treatment may actually function as an anti-GSC therapy (Folkins et al., 2007). It is likely that anti-angiogenic drugs might not only inhibit tumor vascularization to suppress GBM growth, but also directly disrupt the niches for the maintenance of GSCs, therefore weakening the "tumor roots" or eliminating the "tumor seeds" to block further tumor progression.

HYPOXIC RESPONSES IN GLIOBLASTOMA STEM CELLS

Hypoxia is commonly present in many types of solid tumors including GBMs. Hypoxic condition was thought to have a negative impact on tumor growth. However, now it has been well recognized that hypoxia actually promotes tumor angiogenesis, cancer invasion and therapeutic resistance such as radioresistance in GBMs (Jensen, 2009; Heddleston et al., 2009). Moreover, recent works from our group and others have demonstrated that hypoxic niches play critical roles in the maintenance of cancer stem cells in tumor tissue (Li et al., 2009; Heddleston et al., 2009; Pietras et al., 2008, 2009). Similarly, hypoxic niches are also involved in the maintenance of normal stem cells. For example, hematopoietic stem cells are maintained in hypoxic niches in bone marrow (Parmar et al., 2007). Interestingly, hypoxia also prevents the differentiation of neural stem cells and promotes the maintenance of self-renewal potential of embryonic stem (ES) cells (Studer et al., 2000; Morrison et al., 2000; Ezashi et al., 2005; Santilli et al., 2010). In addition, restricted oxygen concentrations have been shown to enhance the production of induced pluripotent stem cells (iPSC) (Yoshida et al., 2009). GBMs frequently display areas of necrosis that occurs in avascular and low oxygen regions. Surprisingly, necrosis serves as a grading criterion for GBMs in clinical diagnosis. While brain tumor stem cells have been linked to a perivascular niche, we have found an additional enrichment

of GSCs around necrotic regions that often display hypoxic condition (Li et al., 2009). Several groups have found that restricted oxygen promotes a GSC phenotype (McCord et al., 2009; Soeda et al., 2009). Our group also found that restricted oxygen conditions increase expression of GSC markers and the indicators of self-renewal, suggesting that the state of cancer stem cell may be plastic and that microenvironmental conditions can promote the acquisition of a stem cell-like phenotype (Heddleston et al., 2009, 2010). These studies suggest that disrupting the microenvironment of GSCs, like the hypoxic niches, may offer a new approach targeting GSCs in glioblastomas.

The cellular responses to hypoxia are mainly mediated through the hypoxia inducible factors (HIFs). In response to hypoxia, both cancer cells and normal cells undergo significant transcription modification that leads to alterations of cellular activities, but different cells may display varied hypoxic responses. Recently, we demonstrated that hypoxia responses in GSCs differ from that in non-stem cancer cells. Hypoxia differentially induces HIF2 α in GSCs but not in non-stem GBM cancer cells, while HIF1 α is induced in both GSCs and the non-stem GBM tumor cells (Li et al., 2009). Furthermore, HIF2 α was essential only in GSCs and was not expressed by normal neural stem or progenitor cells, suggesting that HIF2 α may represent a specific target for GSCs or other cancer stem cells. Under hypoxic conditions, GSCs display a specific gene expression profile different from that induced in the non-stem cancer cells. In addition to the increased VEGF expression, HIF2 α and several HIF2 α transcriptional targets such as Oct4, Glut1 and Serpin B9 are specifically upregulated in GSCs under hypoxia (Li et al., 2009). GSCs display high levels of HIF2 α under oxygen concentration as high as 5% that is within the physiologic range of oxygen in the brain and most tumor tissues, whereas HIF1 α is induced only at severely hypoxic conditions (< 1% oxygen) in both GSCs and the non-stem tumor cells (Li et al., 2009). Functional studies through RNA interference demonstrated that both HIF2 α and HIF1 α are required for GSC proliferation *in vitro* and GSC tumor formation *in vivo*, but only HIF1 α is required for the growth of non-stem GBM cancer cells, suggesting that GSCs use both HIF1 α and HIF2 α for the hypoxia response and may be able to survive better under stress conditions. Other studies have shown that HIF1 α functions in the hypoxia-driven expansion of GSCs (Soeda et al., 2009). Moreover, a statistical analysis of HIFs expression in the REMBRANDT National Cancer Institute patient database indicated that HIF2 α but not HIF1 α expression levels informed negative survival of patients. In addition, overexpression of HIF2 α actually promotes cancer stem state in GBM (Heddleston et al., 2009). These data suggest that HIF2 α represents a potential target specific for GSCs, since HIF2 α is not expressed in normal neural progenitors. However, the role HIF2 α in other normal stem cells needs to be elucidated, in order to understand whether targeting

HIF2 α have any negative impact on other normal adult stem cells. Differential hypoxic response in GSCs may provide a new strategy to target cancer stem cells in malignant gliomas.

SIGNALING PATHWAYS IN GLIOBLASTOMA STEM CELLS

The identification of cancer stem cells and the therapeutic targeting of these cells to improve cancer treatment have generated excitement (Zhou et al., 2009), but our understanding on the molecular signaling of cancer stem cells is still in early development. Although cancer stem cells share some critical characteristics with normal somatic stem or progenitor cells, cancer stem cells are clearly distinct from the normal stem cells at genetic/epigenic and molecular signaling levels. It is obvious that elucidation of specific signaling pathways involved in the maintenance and functions of GSCs will be useful to develop novel strategies to improve GBM treatment. A number of signaling pathways associated with the maintenance of GSC phenotypes have been reported (Vescovi et al., 2006; Park and Rich, 2009; Zhou et al., 2009). Here we discuss a few of critical signaling transduction pathways mediated from external signals to nucleus in GSCs.

Notch signaling

Notch proteins include four members (Notch 1–4) of transmembrane receptors. They mediate short-range cellular communication through interaction with ligands (Jagged-1, -2, and Delta-like-1, -3, and -4). The Notch-mediated signaling pathway is essential for the maintenance of somatic stem and progenitor cells by promoting self-renewal and repressing differentiation (Lathia et al., 2008). It is well known that the activation of Notch requires sequential proteolytic cleavages by the γ -secretase complex to release Notch intracellular domain from membrane to nucleus (Mizutani et al., 2007; Lathia et al., 2008). The nuclear translocation of the cleaved Notch further leads to Notch-dependent transcription. The important roles of Notch signaling in regulating self-renewal and determining cell fate have been well established in neural stem or progenitor cells (Lathia et al., 2008). Notch signaling potently promotes the proliferation of normal neural stem cell (NSC) and is required for the maintenance of neural progenitors both *in vitro* and *in vivo* (Mizutani et al., 2007). Aberrant Notch signaling has been found in a number types of tumors including gliomas (Purow et al., 2005; Kanamori et al., 2007). The role of Notch signaling in brain tumor stem cells was initially identified in medulloblastomas. Blockade of Notch signaling by a γ -secretase inhibitor (GSI-18) induces differentiation and apoptosis of stem-like cells derived from medulloblastomas and impairs the tumorigenic potential of these cells (Fan et al., 2006). Recently, the function of Notch signaling has been linked to cancer stem cells in malignant gliomas, as inhibition of Notch signaling in GSCs attenuates

the formation of neurosphere-like colonies (Fan et al., 2010). Furthermore, Notch overexpression in a K-ras-induced glioblastoma mouse model increased expression of NSC marker Nestin and induced glioma formation in the NSC-rich subependymal zone in brain (Shih et al., 2006). As mentioned above, Notch signaling has been linked to radioresistance of GSCs (Wang et al., 2010), suggesting that inhibition of Notch signaling may not only disrupt the maintenance of GSCs but also reduce the radioresistance of GSCs. Now γ -secretase inhibitors are in early clinical development for brain cancers. In addition, other regulators of Notch signaling, including Delta/Notch-like epidermal growth factor-related receptor (DNER) and the Notch ligand Delta-like 4 (DLL4), can also regulate GBM tumor growth (Li et al., 2007; Sun et al., 2009; Jeon et al., 2008). Anti-DLL4 therapies have demonstrated anti-cancer stem cell activity (Hoey et al., 2009) but concern has been raised as chronic DLL4 targeting can induce neoplasia as well (Yan et al., 2010). Further, other signaling regulators such as ID4 (inhibitor of differentiation 4) and CXCR4 also functionally interact with Notch signaling in brain tumors (Jeon et al., 2008; Williams et al., 2008). It is important to understand the role of these interactions in maintaining the stem-like phenotype of GSCs.

Hedgehog-Gli signaling

The Sonic Hedgehog signaling is one of key regulatory pathways critical for the maintenance of several types of adult stem cells, including neural stem cells (Ruiz i Altaba et al., 2007). The binding of Hedgehog ligands to the PTCH receptor activates Gli signal transducers that then translocate into the nucleus to activate or repress transcription of its downstream genes. Aberrant Hedgehog signaling has been linked to the development of medulloblastomas, the common childhood tumors (Goodrich et al., 1997; Vorechovský et al., 1997; Shahi et al., 2008). Active Hedgehog-Gli signaling is also associated with gliomas (Shahi et al., 2008). In fact, the key intracellular mediator Gli was originally discovered in a glioma (Kinzler et al., 1987). Moreover, Gli activity correlates with tumor grade in a genetically engineered mouse model (Becher et al., 2008). Several groups have investigated the role of Hedgehog-Gli signaling in GSCs and found that this signaling pathway regulates self-renewal and tumorigenic potential of GSCs (Clement et al., 2007; Ehtesham et al., 2007; Bar et al., 2007; Xu et al., 2008). Treatment of GSCs with the Hedgehog inhibitor cyclopamine or Gli RNA interference suppresses self-renewal and proliferation while increases apoptotic cell death. Importantly, inhibition of Hedgehog-Gli signaling enhances the efficacy of TMZ to inhibit GSC proliferation and induce cell death. Several studies demonstrated that the inhibition of Hedgehog signaling pathway blocks GSC tumor growth, and the viable neoplastic cells after the cyclopamine treatment failed to propagate tumors *in vivo*. Furthermore, cyclopamine

treatment has been shown to improve the effect of radiation on GSCs. Taken together, these studies indicated that Hedgehog-Gli signaling pathway is critical for the GSC maintenance and targeting this pathway with pharmacologic inhibitors may inhibit GSC growth and improve the efficacy of conventional therapies against GBMs. Although the toxicity of these inhibitors on normal stem cells needs to be carefully evaluated, recent clinical studies with the Hedgehog inhibitor GDC-0449 have shown promising responses with acceptable toxicity in brain tumor patients (Rudin et al., 2009; Yauch et al., 2009).

RTK-Akt signaling

Receptor Tyrosine Kinases (RTKs) mediate signal transduction of multiple oncogenic cytokines or growth factors, including the epidermal growth factor (EGF) and basic fibroblast growth factor (bFGF) that are used in culturing GSCs *in vitro* (Lee et al., 2006). Among these RTK pathways, the EGFR-mediated growth signaling through phosphoinositide 3-kinase (PI3K)/Akt is one of the most critical and best characterized pathways in malignant gliomas. GBMs frequently display EGFR amplification and/or expression of the constitutively active variant EGFRvIII that leads to increased EGFR-Akt signaling in GBM cancer cells (Moscatello et al., 1998; Choe et al., 2003). Overexpression of EGFRvIII in genetically engineered models induces glioma-like tumors (Holland et al., 1998; Ding et al., 2003). It is not then surprising that EGFR activity is required for the maintenance of GSCs as EGFR kinase inhibitors attenuates GSC proliferation and neurosphere formation *in vitro* (Soeda et al., 2008; Griffero et al., 2009). A number of intracellular pathways are activated upon EGFR activation, but prominently the PI3K-Akt axis has been strongly linked to GSC biology (Dreesen et al., 2007; Eyler et al., 2008). It has been recently demonstrated that GSCs are more dependent on Akt signaling than the non-stem GBM tumor cells (Eyler et al., 2008). Functional inhibition of Akt with the pharmacologic inhibitors disrupts GSC tumorsphere formation, reduces migration and invasion, induces apoptosis *in vitro*, and significantly delays intracranial tumor formation of GSCs (Eyler et al., 2008; Bleau et al., 2009; Gallia et al., 2009). Although targeting EGFR-PI3K-Akt signaling pathway may have specific effects on GSCs to reduce their tumorigenic potential, the results to date in clinical trials of EGFR inhibitors have been disappointing, suggesting that EGFR inhibition alone is an insufficient therapeutic paradigm and prompting greater focus on PI3K inhibitors.

Bone morphogenetic proteins (BMPs)/transforming growth factor- β (TGF- β)

The BMPs and TGF- β superfamily includes a large number of proteins that regulate a wide range of cellular activities during

development and injury responses. The BMPs play crucial roles to instruct cell fate during neural development. Based on this background, Vescovi group performed a seminal study and demonstrated that BMPs can activate their canonical receptors on GSCs to induce differentiation and inhibit GBM tumor growth in xenograft models (Piccirillo et al., 2006). This study showed that direct implantation of BMP-bearing beads into glioblastomas slowed tumor growth laying the foundation for a potential therapeutic strategy. The role of BMPs in GSCs became more nuanced after the Fine group showed that GSCs derived from some cases of GBMs epigenetically regulate BMP receptors to shift toward a fetal phenotype to escape the pro-differentiation effects of BMPs (Lee et al., 2008). In contradistinction, most members of TGF- β serve as oncogenic stimuli in GBM growth through induction of angiogenesis, immune evasion, and invasion (Wick et al., 2006). Recent studies have added a new dimension in TGF- β oncogenesis as autocrine and paracrine loops that function to maintain GSCs through induction of leukemia inhibitory factor (LIF) and the SOX family members (Peñuelas et al., 2009; Ikushima et al., 2009). TGF- β inhibitors have already entered into clinical trial and BMPs are being considered.

Wnt- β -Catenin signaling

The Wnt- β -catenin signaling has been well studied in several types of cancers such as colon cancer. The canonical Wnt cascade is one of critical regulators in embryonic stem cells and adult stem cells. Wnt- β -catenin signaling has clearly defined roles in both normal stem cells and cancer stem cells (Grigoryan et al., 2008). In brain, the Wnt signaling pathway regulates brain development as well as proliferation and self-renewal of neural stem or progenitor cells in the fetal ventricular zone, the postnatal subventricular zone and hippocampus (McMahon et al., 1990; Thomas et al., 1990; Lie et al., 2005; Adachi et al., 2007; Kalani et al., 2008). The alterations of Wnt signaling pathway have been linked to medulloblastoma (Koch et al., 2001; Yokota et al., 2002). Wnt signaling is activated predominantly in medulloblastoma of the classic subtype (Thompson et al., 2006). Recent studies indicated that Wnt- β -catenin signaling may contribute to radioresistance in cancer stem cells (Woodward et al., 2007). Whether Wnt- β -catenin signaling is associated with GSC maintenance and radioresistance requires further investigation, but it is possible that Wnt blockade can effectively and specifically target cancer stem cells in glioblastomas.

STAT3 signaling

The signal transducer and activator of transcription 3 (STAT3) is a crucial transcriptional regulator involved in a wide range of cellular activities in the central nervous system development, immune response, stem cell maintenance and tumorigenesis.

The link between STAT3 activation and glioma biology has become increasingly evident in recent years (de la Iglesia et al., 2009). Hyper-activation of STAT3 has been detected in many types of cancers including solid tumors and hematopoietic malignancies. The oncogenic function of STAT3 depends on its specific phosphorylation on Tyr-705 that can be attributed to aberrant activity of various upstream kinases. STAT3 in conjunction with C/EBP β correlates with mesenchymal transformation of GBMs and inversely related to patient outcome (Carro et al., 2010). Based on this background, several groups have investigated roles of STAT3 in GSCs. Inhibition of STAT3 with specific inhibitors or targeting STAT3 with specific shRNAs disrupts proliferation and maintenance of GSCs (Sherry et al., 2009; Cao et al., 2010). Moreover, the phosphorylated active form of STAT3 on Tyr-705 and Ser-727 is present in the GSC population, and this active form of STAT3 decreases to undetectable level after differentiation induction of GSCs (Sherry et al., 2009). Several pathways upstream of STAT3 are active in GSCs. It has been shown that interleukin-6 (IL6), erythropoietin and Notch signaling can regulate STAT3 activation. Targeting these pathways inhibits STAT3 activation, and cell growth and self-renewal in GSCs (Cao et al., 2010; Wang et al., 2009). Interestingly, STAT3 also contributes to the immune regulation by GSCs (Wei et al., 2010). Because STAT3 is involved in many cellular activities in a wide range of cell types including normal stem cells, STAT3 may not be a specific target for GSCs, although STAT3 inhibitors are undergoing clinical development.

SPECIFIC CELL SURFACE MOLECULES IN GLIOBLASTOMA STEM CELLS

Cell surface molecules differentially expressed in GSCs and functionally associated with the maintenance of GSCs may be ideal markers for sorting or targeting GSC population. Several molecules, including CD133 (Singh et al., 2003, 2004), CD15 (Read et al., 2009; Son et al., 2009; Ward et al., 2009), A2B5 (Ogden et al., 2008; Tchoghandjian et al., 2010) and L1CAM (Bao et al., 2008), have been identified on cell surface of GSCs (Fig. 1B, 3A). Although CD133 (prominin-1) has been widely used as a marker for enriching GSC subpopulations from GBM primary tumors or xenografts, many normal cells such as neural stem cells or progenitors express CD133 potentially limiting its utility as a target, and the reliability of CD133 to discriminate GSCs is not absolute (Beier et al., 2007). CD15 (stage-specific embryonic antigen-1, SSEA-1; also called Lewis X antigen) originally identified as a surface marker of mouse embryonic stem cells (Solter and Knowles, 1978; Damjanov et al., 1982) has recently been used as an alternative marker to enrich GSCs from some GBM tumors in which CD133 is not an informative marker for GSC population (Read et al., 2009; Son et al., 2009; Ward et al., 2009). But whether CD15 can be used as a target for GSCs is not clear because the function of CD15 in normal

stem cells and cancer stem cells remains poorly understood, and CD15 is a carbohydrate antigen expressed by normal neural and progenitor cells (Capela and Temple, 2002) rather than a distinct protein target. Other surface markers such as A2B5 have been used for the enrichment of GSC population (Ogden et al., 2008; Tchoghandjian et al., 2009), but further investigations are needed to determine whether this surface marker can be used for targeting GSCs in GBMs.

In the search for specific functional targets that are uniquely expressed on cell surface of GSCs, we have identified L1CAM as a differentially expressed surface glycoprotein that plays critical roles in the maintenance, survival and cellular functions of GSCs (Bao et al., 2008). L1CAM was originally identified as a cell adhesion molecule in the nervous system and plays critical roles during nervous system development (Maness and Schachner, 2007; Schmid and Maness, 2008). This protein contains a cytoplasmic tail, a transmembrane domain and an extracellular domain that can interact with another L1CAM molecule through homophilic binding, or EGFR, FGFR, neuropilin-1, $\alpha 5\beta 1$ and $\alpha v\beta 3$ integrins, and a number of extracellular matrix proteins through heterophilic interaction (Maness and Schachner, 2007; Schmid and Maness, 2008; Raveh et al., 2009). L1CAM mediated intra- and inter-cellular signaling plays crucial roles in regulating cell adhesion, migration, growth, survival, and cancer cell invasion. We found that L1CAM is preferentially expressed in GSCs relative to the non-stem tumor cells and neural progenitor cells (Bao et al., 2008). Targeting L1CAM with shRNAs specifically disrupts tumor-sphere formation and growth of GSCs *in vitro*. Furthermore, L1CAM knockdown in GSCs remarkably suppressed the tumor growth and increased the survival of mice bearing intracranial GBM xenografts (Bao et al., 2008). We have determined the molecular mechanism by which L1CAM promotes GSC maintenance and tumor growth. L1CAM upregulates Olig2 to suppress expression of p21^{WAF1/CIP1}. In addition, a number of studies showed that L1CAM is associated with chemoresistance of ovarian and pancreatic cancers (Stoeck et al., 2007; Sebens Mürköster et al., 2007; Gavert et al., 2008; Raveh et al., 2009). We have recently found that differential expression of L1CAM in GSCs contributes to therapeutic resistance. Our data indicate that L1CAM may represent a novel target for the development of effective anti-GSC specific therapeutics.

SPECIFIC TRANSCRIPTION FACTORS AND THE MAINTENANCE OF GLIOBLASTOMA STEM CELLS

Since cancer stem cells in GBMs share some critical characteristics with normal neural stem cells and embryonic stem cells, some important stem cell transcription factors (SCTFs) involved in regulating normal stem cells are also required for the maintenance of a GSC phenotype. These stem cell transcription factors such as Sox2, Oct4, Nanog,

c-Myc, Olig2 and Bmi1 are critical for maintaining the self-renewal, proliferation, survival, and multi-lineage differentiation potential of GSCs. Here we discuss some of them that have been relatively well studied in GSCs.

Sox2, Oct4 and Nanog are core components of stem cell transcription factor network and play crucial roles in maintaining embryonic stem cells and somatic stem cells (Ellis et al., 2004; Loh et al., 2006; Tay et al., 2008; Fong et al., 2008). They are also critical factors for cell reprogram and the generation of inducible pluripotent stem cells (iPS) (Takahashi and Yamanaka, 2006; Park et al., 2008). These SCTFs are differentially expressed in GSC subpopulation and are important for GSC maintenance. Knockdown of Sox2, Oct4 or Nanog with specific shRNAs attenuated the GSC phenotype and induced GSC differentiation. Although targeting these transcription factors leads to differentiation of GSCs, it is a challenge to apply these factors as molecular targets for eliminating GSC population, because these common stem cell transcription factors are also crucial for the maintenance of normal stem cells, such as hematopoietic stem cells and neural stem cells.

Olig2 is a basic helix-loop-helix (bHLH) transcription factor that is uniquely expressed in neural stem cells or progenitors in the central nervous system (CNS). Olig2 is highly expressed in neural progenitors that give rise to oligodendrocytes and several subtypes of neurons (Lu et al., 2002). A number of studies have shown that Olig2 is widely expressed in astrocytomas and is required for tumor initiation and growth *in vivo* (Ligon et al., 2004; 2007), suggesting a functional link between Olig2 expression and cancer stem cells in gliomas. Indeed, we found that Olig2 is differentially expressed in GSCs relative to the non-stem tumor cells isolated from almost all cases of GBM tumor specimens obtained in our group, which indicates that Olig2 is a common marker for GSC subpopulation (Bao et al., 2008; Li et al., 2009). This bHLH transcription factor is required for the maintenance of multi-lineage differentiation potential of neural progenitors and GSCs. It has been shown that Olig2 mediates GSC proliferation and maintenance in part through suppression of p21^{WAF1/CIP1}, a key regulator in cell cycle control (Ligon et al., 2007; Bao et al., 2008).

c-Myc, one of most well known oncoproteins, has been extensively studied for its critical role in the proliferation of both normal stem cells and cancer cells. c-Myc may provide a functional link to study the relationship between "stemness" and tumorigenicity of cancer stem cells. Recently, several studies demonstrated that c-Myc expression is elevated in GSCs and it is required for maintaining GSCs *in vitro* and their tumorigenic potential *in vivo* (Wang et al., 2008). Early study showed that c-Myc expression levels correlate with tumor grade in gliomas (Herms et al., 1999). Conditional over-expression of c-Myc in mouse astroglia leads to brain tumors that resemble human malignant gliomas (Jensen et al., 2003). In addition, c-Myc prevents cell differentiation and promotes

self-renewal of tumor cells derived from the pten/p53 double null mouse model (Zheng et al., 2008a). Taken together, these studies support an important role of c-Myc in GSC maintenance. However, the widespread effects of c-Myc in normal physiology must be considered.

Bmi1 is one of polycomb group genes that normally function as epigenetic silencers. Bmi1 has been implicated in stem cell fate determination in several tissues (Molofsky et al., 2003). This protein functions as a positive regulator of neural stem or progenitor cells. It has been demonstrated that Bmi1 is required for the malignant transformation of both neural stem cells and differentiated astrocytes (Bruggeman et al., 2007). Transformation of Bmi1 wild-type neural stem cells lead to high grade gliomas *in vivo*, but transformed Bmi1-deficient neural stem cells only give rise to less malignant type of gliomas with fewer cells expressing stem cell markers (Bruggeman et al., 2007). Furthermore, Bmi1 is frequently overexpressed in several types of human cancers including malignant gliomas. Recently, Bmi1 has been shown to be highly expressed in GSCs and required for GSC self-renewal (Abdouh et al., 2009). In addition, similar finding for an essential role of Bmi1 in maintaining cancer stem cell in hepatocellular carcinomas has been reported (Chiba et al., 2008). These studies suggest that Bmi1 may be a common maker for cancer stem cells in several types of human cancers.

REST, the repressor element 1-silencing transcription factor also called NRSF (neuron-restricted silencing factor), is a master neuronal repressor that plays crucial roles in maintaining neural stem cells by inhibiting neuronal differentiation (Ballas et al., 2005). REST contains a zinc-finger domain that recognizes a conserved RE-1 element (21–23 base pairs) within regulatory regions (promoters) of target genes to suppress the transcription of critical genes associated with neuronal differentiation. Interestingly, REST can be targeted for proteasomal degradation by the ubiquitin E3 ligase SCF β -TRCP, which promotes neural differentiation (Westbrook et al., 2008; Zhang et al., 2009). It has been shown that REST promotes oncogenesis in medulloblastomas and neuroblastomas that usually arise from neural progenitors (Lietz et al., 1998; Lawinger et al., 2000). REST is also highly expressed in glioblastomas and neuroblastomas (Blom et al., 2006). We have observed that REST is differentially expressed in GSCs isolated from some cases of GBM tumor specimens, suggesting that targeting REST may induce GSC differentiation. Thus, REST is a potential target for GSCs.

REGULATION OF GLIOBLASTOMA STEM CELLS BY MICRO RNAS

miRNAs are a group of small non-coding RNAs that potently silence gene expression through post-transcriptional modification on target mRNAs. Since a single miRNA may regulate several or many distinct mRNAs, miRNAs are

powerful regulators of gene expression. The roles of miRNAs in regulating embryonic stem cells, somatic stem cells or cancer stem cells have received much attention in recent years. miRNAs are emerging as crucial regulators of cellular proliferation and differentiation. They can function as either tumor suppressors or oncogenes in various tissues or tumors. miRNA has been shown to be critical in the regulation of cancer cell functions in malignant gliomas. For example, miRNA-21 is overexpressed in GBM tumors and functional blockade of this miRNA induces apoptotic cell death (Conti et al., 2009). However, levels of miR-124, miR-137 and miR-451 are significantly reduced in malignant gliomas (both grade III and grade IV) relative to normal brain (Silber et al., 2008; Gal et al., 2008). The roles of miRNAs in GSCs have been demonstrated in two recent studies showing that miR-124, miR-137 and miR-451 levels are significantly reduced in GSCs relative to non-stem tumor cells (Silber et al., 2008; Gal et al., 2008). Moreover, overexpression of these miRNAs in GSCs suppresses proliferation and induces differentiation of GSCs, indicating that these miRNAs have important roles in maintaining a GSC phenotype. Further, external expression of miR-451 disrupts tumorsphere formation and suppresses tumor growth of GSCs *in vivo*, suggesting a tumor suppressor role of miR-451 in GBMs (Gal et al., 2008). These studies indicate that some critical miRNAs can be potentially used as molecular targets or therapeutic agents for targeting GSCs. However, we may face a great challenge to deliver these miRNAs into cancer cells in tumor tissue and to make these miRNAs as stable targeting agents for GSCs.

DIFFERENTIATION OF GLIOBLASTOMA STEM CELLS

One of important characteristics that GSCs share with normal neural stem cells is their multi-lineage differentiation potential, although differentiation capacity is not considered to be one of essential property for cancer stem cells in other tumors. We and others have demonstrated that GSCs isolated from GBM primary tumors or xenografts have the potential to differentiate into cells with the marker profiles and morphologies of astrocytes, oligodendrocytes and neurons (Fig. 2) (Bao et al., 2006a). These differentiated cells usually lose long-term repopulation potential *in vitro* and fail to propagate tumors *in vivo*, suggesting that inducing GSC differentiation may be a practical strategy to eliminate GSC population in GBMs. Thus, understanding signal transduction pathways controlling stem cell and cancer stem cell differentiation is of importance. A number of signaling regulators involved in differentiation induction of cancer stem cells have been identified. As mentioned above, the members of BMP family induce GSC differentiation into astroglial and neuron-like cells and thus inhibit GSC proliferation and deplete GSC population (Piccirillo et al., 2006). Study from the Viscovi group has shown that targeting GSCs with BMP4 *in vivo* significantly

suppressed GBM tumor growth and reduced tumor invasion (Piccirillo et al., 2006). Recent study by the Fine group has confirmed that BMPs promote glial differentiation of GSCs (Lee et al., 2008), but they also observed that GSCs derived from some GBM tumor samples displayed enhanced cell proliferation rather than differentiation in response to BMP treatment. This is because GSCs from these tumor samples lost BMPR1B expression due to epigenetic silencing by an EZH2-dependent mechanism (Lee et al., 2008). Ectopic expression of BMPR1B restored the BMP4-induced differentiation in these GSCs. These studies suggested that epigenetic characteristics of individual tumor may determine GSC response to the differentiation-inducing agents, and BMPs in combination with epigenetic modulators may be able to enhance differentiation of GSCs.

In addition, there are other signaling pathways or regulators that have been implicated in controlling GSC differentiation. Recent studies demonstrated that inactivation of PTEN (a well-known tumor suppressor) promotes undifferentiated state of GSCs (Zheng et al., 2008a, b), suggesting PTEN may promote GSC differentiation. PTEN is a phosphatase with dual-specificity for both protein and lipid and is often mutated in GBMs (Fan et al., 2002; Zheng et al., 2008a). It has been well known that PTEN deletion or functional loss is linked to progression and/or immunoresistance of malignant gliomas (Parsa et al., 2007). Inactivation of PTEN leads to increased expression of c-Myc that is critical for maintaining GSC proliferation and self-renewal, suggesting promoting PTEN function may suppress the "stemness" of GSCs and induce GSC differentiation. In another study, Sox11 has been shown to induce GSC differentiation (Hide et al., 2009). Over-expression of Sox11 inhibits tumorigenic potential of GSCs by promoting neuronal differentiation. Moreover, epigenetic silence of Sox11 expression was detected in GSCs derived from some GBM tumors when a gene expression profile was analyzed between tumorigenic and non-tumorigenic clones of glioma cancer cells. These studies suggest that inducing differentiation is an attractive strategy to target GSCs, although the molecular mechanisms underlying the control of GSC differentiation is not fully understood.

CONCLUSIONS

Functional characterization of cancer stem cells in tumor progression and therapeutic resistance has altered our understanding of tumor biology, which led to a reevaluation of conventional therapies for malignant gliomas and other cancers. Although controversy still exists as to the methods for isolating and characterizing GSCs and defining their exact roles in malignant behaviors *in vivo*, GSCs represent a subpopulation of cancer cells with extraordinary capacities to promote tumor angiogenesis, invasion, therapeutic resistance and repopulation after treatment, making them a crucial cell population that should be targeted for anti-GBM

therapies. Novel therapies directed against GSCs may significantly improve the currently poor record of clinical activity with conventional treatments. Cancer cure requires elimination of both GSC and non-stem tumor cell populations. As non-stem tumor cells may be able to reprogram into stem cell-like cancer cells under certain conditions, we believe that eradicating both GSC and non-GSC cancer cell populations in GBM is essential to achieve therapeutic success (Fig. 4). Recent advances in this exciting research area have allowed us to gain remarkable insights into the molecular mechanisms or signaling pathways that are differentially present or regulated in GSCs or non-stem tumor cells. We have discussed several key signaling pathways or molecular targets that are potentially useful for the future development of anti-GSC therapeutics. Most of them are still far away from the clinical application at this point, although the anti-vascular niche treatment has shown promising results in clinical trials leading to FDA approval for bevacizumab for the treatment of recurrent or progressive GBMs. Additional translational research is needed to validate the clinical relevance of these laboratory findings and better apply these new concepts to clinical practice. For example, current radiographic endpoints examine total tumor volume rather than specific cancer cell subpopulations. But the properties of tumor heterogeneity and cellular hierarchy within a solid tumor indicate that the nature of the surviving cancer cells after treatment may determine the scope of tumor recurrence and its lethality. Cellular and molecular analysis of tumor heterogeneity may accelerate biomarker development and the application of personalized medical therapy. However, great challenges lay ahead as GSC populations themselves are also heterogeneous (Piccirillo et al., 2009) and the GSCs may evolve over time within a GBM patient. As the genetics of gliomas are becoming increasingly defined with clear subgroups of tumors evolving from low grade to high grade with greater malignancy, our understanding of GSC diversity and GBM heterogeneity will certainly become more nuanced. It is very clear that micro-environment is crucial to maintain GSC population. GSCs interact with not only vascular niche but also non-stem tumor cells, stromal elements and immune cells. The emerging concepts and roles of cancer stem cells are still rapidly evolving. Recent studies demonstrated that the epithelial-mesenchymal transition (EMT) plays an important role in the acquisition of malignant and stem cell traits of cancer cells (Mani et al., 2008; Radisky and LaBarge, 2008; Gupta et al., 2009). It has been well known that EMT promotes tumor invasion and metastasis (Kalluri and Weinberg, 2009; Thiery et al., 2009). Thus, the stem cell-like phenotype may contribute to tumor invasion and metastasis. These paradigms are exciting as they may provide new avenues for developing novel therapeutics to improve tumor treatment and reduce tumor metastasis and recurrence that cause most cancer deaths. Since the origin of GSCs in GBM from different patient may vary and they may also display

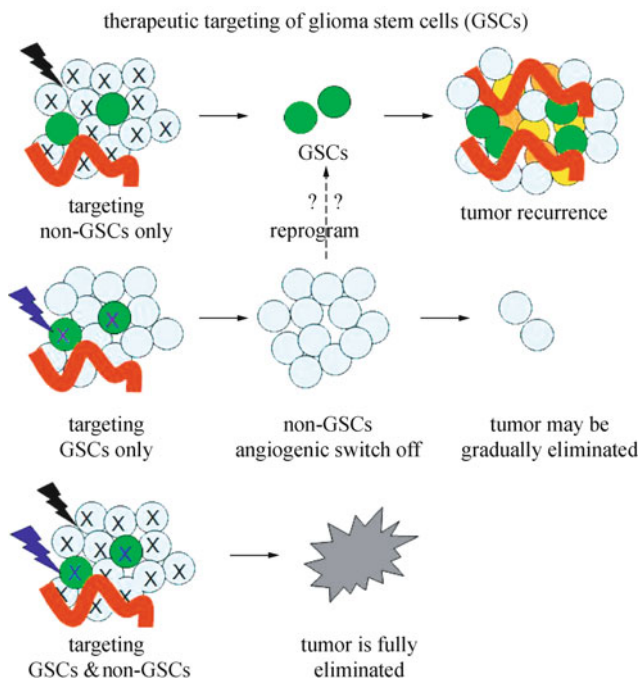


Figure 4. Therapeutic targeting of both GBM stem cells (GSCs) and non-stem cancer cells (non-GSCs) is critical in GBM treatment. Molecular targeting of both GSC and non-GSC populations in malignant gliomas will be important to eliminate the entire tumor, as GSCs are resistant to current therapies causing tumor recurrence, and non-stem tumor cells (non-GSCs) may be able to reprogram into GSCs under certain micro-environment or specific conditions.

different genetic and epigenetic changes in complex tumor tissues, future treatment for GBM and other tumors may rely on a unique combination of several targeted therapies based on the cellular, molecular, genetic and epigenetic information of the tumor in the individual patient. It is likely that the exciting advances in these emerging areas of cancer research may bring new opportunities for a group of cancer patients who lack effective treatment options.

ABBREVIATIONS

BMPs, bone morphogenetic proteins; CSC, cancer stem cell; CNS, central nervous system; EGF, epidermal growth factor; GBMs, glioblastomas; GSCs, GBM stem cells; TGF- β , transforming growth factor- β

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