Targeting glioblastoma cancer stem cells: the next great hope?

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Glioblastoma multiforme (GBM) is the most common primary brain tumor and is notorious for its poor prognosis. The highly invasive nature of GBM and its inherent resistance to therapy lead to very high rates of recurrence. Recently, a small cohort of tumor cells, called cancer stem cells (CSCs), has been recognized as a subset of tumor cells with self-renewal ability and multilineage capacity. These properties, along with the remarkable tumorigenicity of CSCs, are thought to account for the high rates of tumor recurrence after treatment. Recent research has been geared toward understanding the unique biological characteristics of CSCs to enable development of targeted therapy. Strategies include inhibition of CSC-specific pathways and receptors; agents that increase sensitivity of CSCs to chemotherapy and radiotherapy; CSC differentiation agents; and CSC-specific immunotherapy, virotherapy, and gene therapy. These approaches could inform the development of newer therapeutics for GBM.
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GLIOMASTOMA multiforme (GBM) is the most common primary brain tumor in adults and is associated with poor outcomes. Even with optimized excision, chemotherapy, and radiotherapy, the median survival time is approximately 14 months.¹³¹ Because of their inherently resistant nature, along with their remarkably invasive behavior, most of these tumors recur after treatment. Despite being the focus of intense study, therapeutic advances in the treatment of this disease are almost absent, which has led investigators to double their focus on research into the basic biology of GBM to unearth new and more effective targets. Brain tumors have been described as complex organ systems comprising a neoplastic component with associated vasculature, inflammatory cells, and reactive cellular and extracellular components. Genetic analyses of GBMs have also demonstrated regional variation in gene expression and chromosomal aberrations. Use of differentiation markers has shown that GBMs display multiple states and variations.¹¹⁵ The discovery and characterization of a cohort of tumor cells, called cancer stem cells (CSCs), has garnered increasing interest, and these cells are thought to hold the key to an understanding of the resilient nature of GBMs.^{40,123,124}

Abbreviations used in this paper: Akt = protein kinase B; BCNU = 1,3-bis(2-chloroethyl)-1-nitrosourea; CSC = cancer stem cell; EGFR = epidermal growth factor receptor; GBM = glioblastoma multiforme; MGMT = methylguanine-DNA methyltransferase; STAT3 = signal transducer and activator of transcription 3; TGFβ = transforming growth factor-β.

The CSC hypothesis is predicated on the presence of a small cohort of cancer cells that have properties of neural stem cells. Accordingly, these cells demonstrate self-renewal ability and multilineage capacity.²² Self-renewal of CSCs maintains the small cohort of these cells, and the ability to differentiate into downstream progenitor cells gives rise to the diverse progeny that constitutes the bulk of the tumor. CSCs are thus thought to be the source of all GBM cells. CSCs are also known as tumor-initiating cells because of their potent ability to generate tumors in xenograft models (approximately 1000-fold more efficient than traditional [non-CSC] GBM cell lines).^{70,140} Additionally, recent research has shown that CSCs are generally more resistant to conventional cytotoxic therapies and are invasive. Therefore, CSCs are thought to be the major driving force behind GBM resistance to therapy and high rates of GBM recurrence (Fig. 1).

In the arena of stem cell research, reliable recognition and characterization of putative CSCs is of paramount importance.³² Functional assays such as serial implantation in nude mice are considered the gold standard.²² Other techniques include Hoechst dye exclusion,^{38,59,67,122,127} aldehyde dehydrogenase 1 assay,¹¹⁴ spectroscopy,^{76,77,130} and neurosphere cultures.^{53,138} Cell sorting is another commonly used potent tool to enrich the population of CSCs (Table 1).

Among cell surface markers used to identify CSCs and neural stem cells, one of the most commonly used is CD133 (prominin-1).⁹³ CD133 was initially used to enrich CSCs in patients with leukemia^{15,60} and has been observed

FIG. 1. Increased frequency of CD133 in recurrent de novo glioblastomas after radiotherapy and chemotherapy. **A:** Immunohistochemistry for CD133 in sections from primary tumors after external-beam radiation therapy and Gamma Knife surgery in a patient. *Arrowhead* designates CD133-positive cells. **B:** Immunohistochemistry for CD133 in sections from recurrent tumors after external-beam radiation therapy and Gamma Knife surgery in the same patient. **C–H:** Contrast-enhanced axial MR images of another patient, at the initial diagnosis (**C**), at recurrence after resection and irradiation (**D**), and in the terminal stage (**E**), and photomicrographs indicating no CD133 immunopositivity in the primary surgery specimen (**F**) but extensive immunopositivity in pseudopalisade formations in the section obtained from the remote-site recurrence (*arrow* in **E**) at autopsy (**G** and **H**). Bar = 100 μ m. Reproduced from Tamura et al.: Expansion of CD133-positive glioma cells in recurrent de novo glioblastomas after radiotherapy and chemotherapy. *J Neurosurg* 119:1145–1155, 2013. Published with permission.

in various other CSCs from different cancers.⁹⁸ Because CD133 appears on neural stem cells, Singh et al. conducted in vitro and in vivo studies showing the presence of CSC on CD133-positive cells in gliomas and medulloblastomas.^{123,124} Nestin is another marker for CSCs in the CNS.²⁸ Initially described as an antigen of rat-401 against embryonic spinal cord, nestin was later identified as a class VI intermediate filament protein.^{58,78}

Treatment Considerations

The current paradigm for treating GBM consists of a generally uniform regimen. According to the Stupp protocol, maximal safe resection is followed by radiotherapy and chemotherapy containing temozolomide.¹³¹ Temozolomide exercises cytotoxicity against GBM cells by creating O6-methylguanine lesions, which lead to DNA fragmen-

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TABLE 1: Commonly used glioma CSC markers

Marker
cell surface
CD133
CD15
CD44
CXCR4
integrin alpha-6
protein (cytoplasmic & nuclear)
nestin
Musashi-1
Bmi-1
transcriptional factor
Sox2
enzyme
ALDH1

tation and disruption of DNA replication. The resulting effects include tumor suppression and apoptosis of tumor cells.¹⁵⁵ Response to temozolomide, however, is not consistent in all patients. Long-term GBM survivors have been shown to harbor tumors with reduced or absent expression of methylguanine-DNA methyltransferase (MGMT).⁵² MGMT removes the methyl groups added by temozolomide, thereby preventing GBM cell death. Hence, with decreased expression of MGMT, because of methylation of its promoter, the therapeutic efficacy of temozolomide is enhanced.⁸⁹

That patient survival times differ according to MGMT status underscores the significance of understanding the diversity of tumor biology. Such an understanding can be of immense importance with regard to CSCs because activity of MGMT has been shown to be 32- to 56-fold higher in CD133-positive than in CD133-negative cells, leading to increased resistance to temozolomide.^{21,84} Others have reported that resistance to conventional anticancer drugs, such as doxorubicin, etoposide, carboplatin, and 1,3-bis(2-chloroethyl)-1-nitrosourea (BCNU), is stronger in glioma CSCs than in non-CSCs because of enhanced expression of multidrug resistance protein 1.⁹⁶ CSCs also upregulate mRNA of Fas-associated death domain–like antiapoptotic molecules Bcl-2 and Bcl-X.^{102,108,118} Higher expression of *BCRP1* (a drug-resistant gene) and antiapoptosis proteins and inhibitors also confer a protective advantage to CSCs.²¹

In addition, the fact that a higher proportion of CD133-positive glioma cells survive, relative to most other tumor cells, points to their increased resistance to ionizing radiotherapy.¹²⁰ By preferentially activating the DNA damage checkpoint in response to radiation, CSCs are able to repair radiation-induced DNA damage more effectively than are CD133-negative tumor cells. With exposure to conventional radiation, CD133-positive cells also exhibit enhanced activation of 3 key mediators of cell cycle checkpoint proteins: rad17, chk1, and chk2.^{9,116} If exposed to specific inhibitors of the chk1 and chk2 check-

point proteins, CSCs become more radiosensitive, akin to CD133-negative tumor cells.⁹

Because of their inherent resistance to established therapeutic measures, CSCs are valuable targets for the development of specific treatment modalities for improving the overall response of GBMs.¹⁰⁰ Increasing work is now uncovering the unique biological processes of glioma CSCs. Supplementing CSC-specific treatments with existing approaches may increase the effectiveness of overall treatment. We will further review some of the strategies being used to target glioma CSCs.

CSC-Specific Pathways and Receptors

Therapeutic targeting of CSC pathways and receptors responsible for tumor proliferation, maintenance, and drug resistance is of considerable interest. The notch signaling pathway is one of the major targets for CSC-specific therapies. Notch ligands, receptors, and targets have been found in a wide variety of neoplasms, including but not limited to lung, breast, cervix, renal, pancreas, medulloblastoma, and GBM.^{4,27,61,62,92,99,105,107,112,157} In addition, in many of these tumors, increased notch activity has been shown to promote tumor growth, and blockade of the pathway inhibits proliferation. In the context of the CNS, the notch signaling pathway regulates neural stem cells in the brain, and high activity of the notch pathway has been demonstrated in glioma CSCs.⁴⁰ Targeting the notch pathway by gamma-secretase inhibitors depletes glioma CSCs through reduced proliferation and increased apoptosis associated with decreased protein kinase B (Akt) and signal transducer and activator of transcription 3 (STAT3) phosphorylation.³⁴

Notch signaling may also play a major role in linking CSC renewal and angiogenesis in GBMs. Using a 3D organotypic explant system of surgical GBM specimens, Hovinga et al. inhibited notch signaling and reported not only decreased proliferation and self-renewal of tumor cells but also decreased endothelial cells.⁶² A more recent study suggested that the brain microvascular endothelial cells are the source of notch ligands that lead to CSC sustenance and renewal.¹⁵⁷ Currently, notch signaling pathway inhibitor RO4929097 is being tested in clinical trials for recurrent and progressive GBMs (NCT01122901 at clinicaltrials.gov).

The hedgehog pathway plays an essential role in the development of the cerebellum.^{26,146} This pathway has also been identified as being important in the pathogenesis of gliomas. In addition, Gli is a component of the sonic hedgehog signaling pathway and is amplified in gliomas.⁷³ Cyclopamine, a well-known teratogen and inhibitor of hedgehog signaling, has been shown to deplete CSCs in GBM.¹¹ Similarly, Clement et al. reported that interference of hedgehog signaling through lentivirus-mediated silencing resulted in decreased self-renewal and tumorigenicity of CSCs.²³ SANT-1 inhibition of the hedgehog pathway has also been shown to reduce proliferation of CSCs.³¹ Furthermore, there is increasing evidence of an intimate relationship between hedgehog signaling and endothelial migration.¹⁵⁰ Vismodegib (GDC-0449) is a small molecule antagonist of the hedgehog pathway⁹⁰ and is cur-

rently being tested in a clinical trial for recurrent GBM (NCT00980343 at clinicaltrials.gov).

Because inflammation is thought to be an integral part of the pathophysiology of GBM, Sharma et al. investigated the role of cyclooxygenase-2 in glioma CSCs. They reported that administration of celecoxib significantly reduced self-renewal capacity and increased apoptosis of glioma CSCs.¹²¹ Celecoxib has also been shown to reduce radioresistance in glioma CSCs, leading to reduced tumor growth and increased mean rates of survival among mice.⁸⁶

Glioma CSCs also produce high levels of vascular endothelial growth factor, which induces endothelial cell migration.¹⁰ However, treatment of CD133-positive GBM cells with bevacizumab blocks the tumor cells' ability to stimulate endothelial cell migration and initiate tumors *in vivo*.¹⁰ Calabrese et al. demonstrated that treatment of GBMs with bevacizumab depleted tumor blood vessels, significantly reduced the number of CSCs, and decreased the growth rate of the tumors.¹⁶ Because of the efficacy of antiangiogenic agents as determined in preclinical studies, they have been tested in clinical trials. Unfortunately, bevacizumab has failed to show improvement in the overall survival times for patients with newly diagnosed GBM.⁴³ Similarly, cediranib, either as monotherapy or in combination with lomustine, did not prolong progression-free survival times for patients with recurrent GBM more than lomustine alone.¹²

Amplification and mutation of receptor tyrosine kinases, such as epidermal growth factor receptor (EGFR), are other common genetic alterations that occur in GBMs.^{17,106} Recent studies have determined the presence of a constitutively active EGFR mutant (EGFRvIII) in glioma CSCs. This genetic alteration pathway potentiates tumor growth and heterogeneity through interleukin-6-mediated notch signaling,^{63,64} and Src family kinase-dependent phosphorylation of DOCK180 (dedicator of cytokinesis).^{35,36} Clinical trials investigating the efficacy of EGFR inhibitors in GBM, however, have yielded disappointing results so far.^{50,51,113}

Aberrant Wnt signaling is molecularly linked to many human cancers, including colorectal, breast, ovarian, hepatocellular carcinoma, neuroectodermal, and glioma.^{6,83,85,111} Dysregulation of the Wnt pathway in glioma CSCs has also been reported.^{71,119} Other similar targets being investigated for glioma CSC treatment include the homeobox family,²¹ phosphatase and tensin,³⁰ telomerase,⁸⁸ efflux transporters,^{24,91} and micro-RNA.^{20,46,79}

Chemotherapy Sensitizers

Compared with other tumor cells, CSCs are more refractory to chemotherapy, and resistance to temozolomide remains a significant hurdle in the treatment of GBMs. To circumvent this issue, investigators have reported several ways to potentiate the cytotoxicity of chemotherapeutic agents. These include cell-cycle checkpoint abrogation^{54,55} and depletion in the expression of antiapoptosis proteins¹²⁵ and DNA repair enzymes.⁵⁶

A molecular chaperone, 90-kD heat shock protein, has recently been associated with increased resistance to

chemotherapy and is expressed at 2–10-fold higher levels in tumors than in normal tissues.³⁷ Consequently, drugs that inhibit this protein expression potentiate the cytotoxicity of chemotherapeutic agents in human glioma cell lines.¹⁰¹

GPI 15427, a novel poly(adenosine diphosphate–ribose) polymerase–1 inhibitor, has been tested to show that its systemic administration shortly before temozolomide administration significantly increases the life span of tumor-bearing mice.¹³² Oral administration of GPI 15427 also effectively increases sensitivity to temozolomide.^{133,134} More recently, the effect of secreted frizzled-related protein 4, a Wnt signaling antagonist, was shown to sensitize glioma CSCs to doxorubicin and cisplatin.¹⁴⁵ Similarly, another study used a proteasome inhibitor, bortezomib, and revealed that combination therapies based on bortezomib and bevacizumab offered an increased benefit when the 2 agents were used in combination.¹³

Xu et al. targeted CD44, which is upregulated in GBM and is a CSC marker, and reported that its depletion impeded the growth of GBMs and sensitized the tumor cells to cytotoxic drugs *in vivo*.¹⁴⁹ Tyrosine kinase inhibitors have also been examined for sensitization of the tumor. Wachsberger et al. investigated the chemotherapy sensitization efficacy of cediranib (a potent receptor tyrosine kinase inhibitor) and found that it enhanced the effectiveness of temozolomide.¹³⁹

One of the most commonly used chemotherapeutic agents in the treatment of GBM is BCNU, but it often fails to eradicate CSCs. Recent work has uncovered an overexpression of multiple ion channel genes that are related to drug efflux. When a chloride channel blocker, 4,4'-diisothiocyanostilbene-2,2'-disulfonic acid, is used in combination with BCNU, the effect of BCNU synergistically increases.⁶⁹

Radiation Sensitizers

For developing approaches to sensitize CSCs, a concentrated effort has been made to understand the biology of CSC radioresistance. Because transforming growth factor- β (TGF β) is a known modifier of radiation responses, a TGF- β receptor type I kinase inhibitor, LY2109761, has been used in combination with radiotherapy to increase the radiosensitivity in glioma cell lines, including CSCs.¹⁵⁶ Similarly, LY364947, another small-molecule TGF β receptor type I kinase inhibitor, improved response when administered before radiotherapy.⁴⁸ LY2157299, a TGF β inhibitor, is being evaluated in an ongoing clinical trial (NCT01220271 at clinicaltrials.gov).

EGFR activation has also been implicated in the radioresistance of many cancers, including gliomas. Georger et al. used gefitinib (a tyrosine kinase inhibitor) in EGFR-amplified gliomas and reported a positive trend toward superior antitumor activity when combined therapy (gefitinib plus radiation) was administered.⁴¹ On further investigation, Kang et al. found that gefitinib enhanced radiosensitivity by reducing activation of EGFR-Akt and expression of DNA-dependent protein kinase catalytic subunit.⁶⁸ ZD1839 (Iressa) is another selective EGFR tyrosine kinase inhibitor that demonstrates a significant ra-

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diosensitizing effect in GBMs.¹²⁸ In the clinical domain, patients who enrolled in a Phase I/II study of radiation therapy concurrent with gefitinib therapy for newly diagnosed GBMs showed good drug tolerance but no survival benefits.¹⁸ Other tyrosine kinase inhibitors investigated as radiosensitizers for GBM are erlotinib¹¹³ and vandetanib.³³

STAT3 is a member of a family of DNA-binding molecules, and the aberrant activity of the Janus kinase 2/STAT3 pathway is associated with glioma CSCs. Inhibition of this pathway leads to decreased proliferation of glioma CSCs.^{5,129} Yang et al. reported that resveratrol (inhibitor of the STAT3 axis) significantly improved the survival rate among patients in their xenotransplant model, in part by synergistically enhancing the radiosensitivity of CSCs.¹⁵²

Valproic acid is a commonly prescribed antiepileptic drug used for the management of seizures in brain tumor patients. Valproic acid is also an effective inhibitor of histone deacetylase and is involved in modulating chromatin structure and gene expression.^{44,75,109} Valproic acid increases chemosensitivity to temozolomide and enhances the effect of radiotherapy in glioma cell lines.¹³⁶ Pretreatment with N-acetylcysteine can partially recover the apoptotic effect of temozolomide/valproic acid combination treatment.¹⁹

Another approach to making glioma CSCs more radiosensitive is to inhibit the DNA damage responses that follow radiotherapy.⁹⁵ A dual phosphoinositide 3-kinase/mTOR inhibitor, NVP-BEZ235, inhibits two central DNA damage response kinases: DNA-dependent protein kinase catalytic subunit and ataxia telangiectasia mutated. By doing so, NVP-BEZ235 potentiates the damage caused by ionizing radiation in glioma cells.^{42,144} Some of the other targets that reportedly increase the radiosensitivity of CSCs include polo-like kinase 1,⁴⁹ cyclin-dependent kinase 6,¹⁴⁷ insulin-like growth factor-1 receptor,¹⁰³ DNA binding protein-1,⁴⁷ and miR-210.¹⁵¹

CSC Differentiation Agents

Differentiation-inducing agents can also be used to decrease the number of CSCs and make a tumor more susceptible to therapy. One of the first agents to be used as a differentiating agent for GBM CSCs was bone morphogenetic protein-4.¹¹⁰ Bone morphogenetic proteins play an instructive role in the adult brainstem cell niche and increase the acquisition of astroglial differentiation.^{82,104} Piccirillo et al. demonstrated that bone morphogenetic proteins trigger the Smad signaling cascade in GBM cells, leading to a decrease in the clonogenic ability and number of CD133-positive glioma cells.¹¹⁰

The oncogene *BMII*, which regulates gene expression by modifying chromatin organization, is highly expressed in CD133-positive cells. Recent research has demonstrated the role of *BMII* in the self-renewal of CD133-positive tumor cells.¹³⁷ Not surprisingly, knockdown of this gene by use of short-hairpin RNA-expressing lentiviruses has been shown to inhibit the clonogenic potential of CD133-positive cells in vitro and in vivo.¹

Metformin, a first-line drug for treatment of Type 2 diabetes, was recently reported to possess anticancer

properties affecting the survival of CSCs in breast cancer models.^{57,65,126} Würth et al. investigated the effect of metformin on glioma cells and reported CSC-specific inhibition of the Akt-dependent cell survival pathway affecting self-renewal mechanisms.¹⁴⁸ Metformin is being tested for the treatment of GBM (NCT02149459 and NCT01430351 at clinicaltrials.gov).

Induction of autophagy has also been shown to promote differentiation in glioma CSCs. Drugs such as rapamycin¹⁵⁸ and curcumin¹⁵⁹ trigger the differentiation cascade by activating autophagy. Other differentiating targets include girdin (an actin-binding protein)⁹⁷ and the vanilloid-2 cation channel.⁹⁴ Cannabinoids and sorafenib have also been documented to induce glioma CSC differentiation and deplete the CSC population.²

Immunotherapy

Another area of significant interest is the stimulation of host responses to selectively eradicate CSCs without affecting normal tissue. Systemic immunotherapy that uses dendritic cells has been shown to induce a potent antitumor response.⁸⁰ Dendritic cells are effective antigen-presenting cells that have the ability to prime naïve T cells. Although the number of dendritic cells in circulation is very low, various methods can be used to increase them in vitro with sources such as the bone marrow, cord blood, and peripheral blood.⁸⁰

Additionally, the ability of dendritic cells to cross the blood-brain barrier and enter into perivascular and parenchymal spaces makes them very suitable for glioma immunotherapy.¹¹⁷ Certainly, early clinical trials of dendritic cell vaccines demonstrated strong systemic and intracranial T-cell responses and robust infiltration of tumor with T cells.^{81,153,154} Glioma CSCs also demonstrate exquisite susceptibility to T cell-mediated immunity. By using tumor specimens, Avril et al. demonstrated that glioma CSCs were more sensitive to lysis mediated by natural killer and T cells than were corresponding serum-cultured GBM cells obtained from the same initial tumor specimen.⁷

T cells can also be modified to target CSC surface markers. Ahmed et al. generated T cells specific for EGFR2 by using gene transfer from GBM patients. They demonstrated potent antitumor activity against autologous EGFR2-positive tumors, including their putative stem cells.³ More recent evidence also suggests that dendritic cell-mediated immunotherapy can be enhanced via elimination of regulatory T cells.^{45,87}

Passive immunotherapy that uses monoclonal antibodies or Fc-fusion proteins has similarly shown great potential.¹⁴¹ One of the GBM receptors studied in this regard is interleukin-13 subunit alpha-2; several studies have revealed the ability of interleukin 13-zetakine-redirected T cells to cause regression of GBM and CSCs.^{14,66,74} Nanoparticles have also been used to deliver monoclonal antibodies to glioma CSCs. Wang et al. evaluated single-walled carbon nanotubes conjugated with CD133 monoclonal antibodies and found effective selective targeting of CD133-positive cells along with eradication with photothermolysis.¹⁴²

Virotherapy and Gene Therapy

Among the emerging therapeutic options targeting CSCs, virotherapy has shown noteworthy promise in terms of glioma CSCs.²⁹ Fueyo et al. constructed a tumor-selective adenovirus, Delta-24, that carried a 24-bp deletion in the early region 1A, responsible for binding retinoblastoma protein. In vivo and in vitro results from their study demonstrated a potent lytic effect on glioma cells.³⁹ A second-generation Delta-24 virus (Delta24-hyCD) also exhibited significant chemosensitization and glioma control when combined with 5-fluorocytosine.²⁵

In another study, a combination of adenoviral virotherapy and temozolomide chemotherapy demonstrated significant overexpression of autophagy markers, acidic vesicular organelles, and light-chain-3 proteins in vitro. In vivo studies showed that survival rates were significantly higher when combination therapy was used.¹³⁵

Gene-silencing techniques can also be used for a better understanding of the role of certain genes in the biology of CSCs and can be exploited for therapeutic purposes. Bao et al. investigated the role of a neuronal cell adhesion molecule, L1CAM, in glioma CSCs by using lentiviral-mediated short-hairpin RNA interference. They reported disrupted neurosphere formation, induced apoptosis, and inhibited growth of glioma CSCs.⁸ Similarly, Wang et al. investigated the significance of *c-Myc* expression in glioma CSCs by using short-hairpin RNA interference and demonstrated that decreased expression of the target decreased proliferation and survival of CSCs.¹⁴³

Another innovative approach that has shown considerable potential is the nanoparticle delivery platform for gene therapy. By using a systemically administered nanoparticle carrying the p53 gene, Kim et al. targeted GBM cells, including CSCs.⁷² They reported that the combination of the nanoparticle-carrying p53 and temozolomide increased the antitumor efficacy of temozolomide and enhanced survival benefits in a mouse model of high-ly temozolomide-resistant GBM.⁷²

Conclusions

CSCs play an integral role in the growth and development of GBMs. Although current treatment modalities might be able to eradicate most of the tumor mass, the CSCs are mostly left unperturbed, which accounts for the high rates of recurrence and resistance to therapy. Ongoing research to elucidate the unique properties of CSCs has led to the development of targeted therapeutics for elimination of the CSC population. Supplementation of traditional therapies with CSC-directed agents might improve the overall therapeutic response of GBMs.

Disclosure

The authors report no conflict of interest concerning the materials or methods used in this study or the findings specified in this paper.

Author contributions to the study and manuscript preparation include the following. Conception and design: both authors. Acquisition of data: both authors. Analysis and interpretation of data: both authors. Drafting the article: both authors. Critically revising

the article: both authors. Reviewed submitted version of manuscript: both authors. Approved the final version of the manuscript on behalf of both authors: Ehtesham. Study supervision: Ehtesham.

References

1. Abdouh M, Facchino S, Chatoo W, Balasingam V, Ferreira J, Bernier G: BMI1 sustains human glioblastoma multiforme stem cell renewal. *J Neurosci* **29**:8884–8896, 2009
2. Aguado T, Carracedo A, Julien B, Velasco G, Milman G, Mechoulam R, et al: Cannabinoids induce glioma stem-like cell differentiation and inhibit gliomagenesis. *J Biol Chem* **282**:6854–6862, 2007
3. Ahmed N, Salsman VS, Kew Y, Shaffer D, Powell S, Zhang YJ, et al: HER2-specific T cells target primary glioblastoma stem cells and induce regression of autologous experimental tumors. *Clin Cancer Res* **16**:474–485, 2010
4. Allenspach EJ, Maillard I, Aster JC, Pear WS: Notch signaling in cancer. *Cancer Biol Ther* **1**:466–476, 2002
5. Ashizawa T, Miyata H, Iizuka A, Komiya M, Oshita C, Kume A, et al: Effect of the STAT3 inhibitor STX-0119 on the proliferation of cancer stem-like cells derived from recurrent glioblastoma. *Int J Oncol* **43**:219–227, 2013
6. Augustin I, Goidts V, Bongers A, Kerr G, Vollert G, Radlwimmer B, et al: The Wnt secretion protein Evi/Gpr177 promotes glioma tumorigenesis. *EMBO Mol Med* **4**:38–51, 2012
7. Avril T, Vauleon E, Hamlat A, Saikali S, Etcheverry A, Delmas C, et al: Human glioblastoma stem-like cells are more sensitive to allogeneic NK and T cell-mediated killing compared with serum-cultured glioblastoma cells. *Brain Pathol* **22**:159–174, 2012
8. Bao S, Wu Q, Li Z, Sathornsumetee S, Wang H, McLendon RE, et al: Targeting cancer stem cells through L1CAM suppresses glioma growth. *Cancer Res* **68**:6043–6048, 2008
9. Bao S, Wu Q, McLendon RE, Hao Y, Shi Q, Hjelmeland AB, et al: Glioma stem cells promote radioresistance by preferential activation of the DNA damage response. *Nature* **444**:756–760, 2006
10. Bao S, Wu Q, Sathornsumetee S, Hao Y, Li Z, Hjelmeland AB, et al: Stem cell-like glioma cells promote tumor angiogenesis through vascular endothelial growth factor. *Cancer Res* **66**:7843–7848, 2006
11. Bar EE, Chaudhry A, Lin A, Fan X, Schreck K, Matsui W, et al: Cyclopamine-mediated hedgehog pathway inhibition depletes stem-like cancer cells in glioblastoma. *Stem Cells* **25**:2524–2533, 2007
12. Batchelor TT, Mulholland P, Neyns B, Nabors LB, Campone M, Wick A, et al: Phase III randomized trial comparing the efficacy of cediranib as monotherapy, and in combination with lomustine, versus lomustine alone in patients with recurrent glioblastoma. *J Clin Oncol* **31**:3212–3218, 2013
13. Bota DA, Alexandru D, Keir ST, Bigner D, Vredenburgh J, Friedman HS: Proteasome inhibition with bortezomib induces cell death in GBM stem-like cells and temozolomide-resistant glioma cell lines, but stimulates GBM stem-like cells' VEGF production and angiogenesis. Laboratory investigation. *J Neurosurg* **119**:1415–1423, 2013
14. Brown CE, Starr R, Aguilar B, Shami AF, Martinez C, D'Apuzzo M, et al: Stem-like tumor-initiating cells isolated from IL13Rα2 expressing gliomas are targeted and killed by IL13-zetakine-redredirected T Cells. *Clin Cancer Res* **18**:2199–2209, 2012
15. Bühring HJ, Seiffert M, Marxer A, Weiss B, Faul C, Kanz L, et al: AC133 antigen expression is not restricted to acute myeloid leukemia blasts but is also found on acute lymphoid leukemia blasts and on a subset of CD34+ B-cell precursors. *Blood* **94**:832–833, 1999
16. Calabrese C, Poppleton H, Kocak M, Hogg TL, Fuller C, Ham-

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- ner B, et al: A perivascular niche for brain tumor stem cells. **Cancer Cell** **11**:69–82, 2007
17. Cancer Genome Atlas Research Network: Comprehensive genomic characterization defines human glioblastoma genes and core pathways. **Nature** **455**:1061–1068, 2008
18. Chakravarti A, Wang M, Robins HI, Lautenschlaeger T, Curran WJ, Brachman DG, et al: RTOG 0211: a phase 1/2 study of radiation therapy with concurrent gefitinib for newly diagnosed glioblastoma patients. **Int J Radiat Oncol Biol Phys** **85**:1206–1211, 2013
19. Chen CH, Chang YJ, Ku MSB, Chung KT, Yang JT: Enhancement of temozolomide-induced apoptosis by valproic acid in human glioma cell lines through redox regulation. **J Mol Med (Berl)** **89**:303–315, 2011
20. Chen L, Zhang R, Li P, Liu Y, Qin K, Fa ZQ, et al: P53-induced microRNA-107 inhibits proliferation of glioma cells and down-regulates the expression of CDK6 and Notch-2. **Neurosci Lett** **534**:327–332, 2013
21. Cho DY, Lin SZ, Yang WK, Lee HC, Hsu DM, Lin HL, et al: Targeting cancer stem cells for treatment of glioblastoma multiforme. **Cell Transplant** **22**:731–739, 2013
22. Clarke MF, Dick JE, Dirks PB, Eaves CJ, Jamieson CHM, Jones DL, et al: Cancer stem cells—perspectives on current status and future directions: AACR Workshop on cancer stem cells. **Cancer Res** **66**:9339–9344, 2006
23. Clement V, Sanchez P, de Tribolet N, Radovanovic I, Ruiz i Altaba A: HEDGEHOG-GLI1 signaling regulates human glioma growth, cancer stem cell self-renewal, and tumorigenicity. **Curr Biol** **17**:165–172, 2007
24. Colen CB, Shen Y, Ghoddoussi F, Yu P, Francis TB, Koch BJ, et al: Metabolic targeting of lactate efflux by malignant glioma inhibits invasiveness and induces necrosis: an in vivo study. **Neoplasia** **13**:620–632, 2011
25. Conrad C, Miller CR, Ji Y, Gomez-Manzano C, Bharara S, McMurray JS, et al: Delta24-hyCD adenovirus suppresses glioma growth in vivo by combining oncolysis and chemosensitization. **Cancer Gene Ther** **12**:284–294, 2005
26. Dahmane N, Ruiz i Altaba A: Sonic hedgehog regulates the growth and patterning of the cerebellum. **Development** **126**:3089–3100, 1999
27. Dang L, Fan X, Chaudhry A, Wang M, Gaiano N, Eberhart CG: Notch3 signaling initiates choroid plexus tumor formation. **Oncogene** **25**:487–491, 2006
28. Dell'Albani P: Stem cell markers in gliomas. **Neurochem Res** **33**:2407–2415, 2008
29. Dey M, Ulasov IV, Tyler MA, Sonabend AM, Lesniak MS: Cancer stem cells: the final frontier for glioma virotherapy. **Stem Cell Rev** **7**:119–129, 2011
30. Di Cristofano A, Pandolfi PP: The multiple roles of PTEN in tumor suppression. **Cell** **100**:387–390, 2000
31. Dixit D, Ghildiyal R, Anto NP, Ghosh S, Sharma V, Sen E: Guggulsterone sensitizes glioblastoma cells to Sonic hedgehog inhibitor SANT-1 induced apoptosis in a Ras/NFκB dependent manner. **Cancer Lett** **336**:347–358, 2013
32. Dobbin ZC, Landen CN: Isolation and characterization of potential cancer stem cells from solid human tumors—potential applications. **Curr Protoc Pharmacol** **63**:14–28, 2013
33. Drappatz J, Norden AD, Wong ET, Doherty LM, Lafrankie DC, Ciampa A, et al: Phase I study of vandetanib with radiotherapy and temozolomide for newly diagnosed glioblastoma. **Int J Radiat Oncol Biol Phys** **78**:85–90, 2010
34. Fan X, Khaki L, Zhu TS, Soules ME, Talsma CE, Gul N, et al: NOTCH pathway blockade depletes CD133-positive glioblastoma cells and inhibits growth of tumor neurospheres and xenografts. **Stem Cells** **28**:5–16, 2010
35. Feng H, Hu B, Jarzynka MJ, Li Y, Keezer S, Johns TG, et al: Phosphorylation of dedicator of cytokinesis 1 (Dock180) at tyrosine residue Y722 by Src family kinases mediates EGFRvIII-driven glioblastoma tumorigenesis. **Proc Natl Acad Sci U S A** **109**:3018–3023, 2012
36. Feng H, Hu B, Vuori K, Sarkaria JN, Furnari FB, Cavenee WK, et al: EGFRvIII stimulates glioma growth and invasion through PKA-dependent serine phosphorylation of Dock180. **Oncogene** **33**:2504–2512, 2014
37. Ferrarini M, Heltai S, Zocchi MR, Rugarli C: Unusual expression and localization of heat-shock proteins in human tumor cells. **Int J Cancer** **51**:613–619, 1992
38. Feuring-Buske M, Hogge DE: Hoechst 33342 efflux identifies a subpopulation of cytogenetically normal CD34(+)CD38(−) progenitor cells from patients with acute myeloid leukemia. **Blood** **97**:3882–3889, 2001
39. Fueyo J, Gomez-Manzano C, Alemany R, Lee PS, McDonnell TJ, Mitlianga P, et al: A mutant oncolytic adenovirus targeting the Rb pathway produces anti-glioma effect in vivo. **Oncogene** **19**:2–12, 2000
40. Galli R, Binda E, Orfanelli U, Cipelletti B, Gritti A, De Vitis S, et al: Isolation and characterization of tumorigenic, stem-like neural precursors from human glioblastoma. **Cancer Res** **64**:7011–7021, 2004
41. Georger B, Gaspar N, Opolon P, Morizet J, Devanz P, Lecluse Y, et al: EGFR tyrosine kinase inhibition radiosensitizes and induces apoptosis in malignant glioma and childhood ependymoma xenografts. **Int J Cancer** **123**:209–216, 2008
42. Gil del Alcazar CR, Hardebeck MC, Mukherjee B, Tomimatsu N, Gao X, Yan J, et al: Inhibition of DNA double-strand break repair by the dual PI3K/mTOR inhibitor NVP-BEZ235 as a strategy for radiosensitization of glioblastoma. **Clin Cancer Res** **20**:1235–1248, 2014
43. Gilbert MR, Dignam JJ, Armstrong TS, Wefel JS, Blumenthal DT, Vogelbaum MA, et al: A randomized trial of bevacizumab for newly diagnosed glioblastoma. **N Engl J Med** **370**:699–708, 2014
44. Göttlicher M, Minucci S, Zhu P, Krämer OH, Schimpf A, Giavarra S, et al: Valproic acid defines a novel class of HDAC inhibitors inducing differentiation of transformed cells. **EMBO J** **20**:6969–6978, 2001
45. Grauer OM, Suttmüller RPM, van Maren W, Jacobs JFM, Benink E, Toonen LWJ, et al: Elimination of regulatory T cells is essential for an effective vaccination with tumor lysate-pulsed dendritic cells in a murine glioma model. **Int J Cancer** **122**:1794–1802, 2008
46. Guessous F, Zhang Y, Kofman A, Catania A, Li Y, Schiff D, et al: microRNA-34a is tumor suppressive in brain tumors and glioma stem cells. **Cell Cycle** **9**:1031–1036, 2010
47. Guo Q, Guo P, Mao Q, Lan J, Lin Y, Jiang J, et al: ID1 affects the efficacy of radiotherapy in glioblastoma through inhibition of DNA repair pathways. **Med Oncol** **30**:325, 2013
48. Hardee ME, Marciscano AE, Medina-Ramirez CM, Zagzag D, Narayana A, Lonning SM, et al: Resistance of glioblastoma-initiating cells to radiation mediated by the tumor microenvironment can be abolished by inhibiting transforming growth factor-β. **Cancer Res** **72**:4119–4129, 2012
49. Harris PS, Venkataraman S, Alimova I, Birks DK, Donson AM, Knipstein J, et al: Polo-like kinase 1 (PLK1) inhibition suppresses cell growth and enhances radiation sensitivity in medulloblastoma cells. **BMC Cancer** **12**:80, 2012
50. Hasselbalch B, Eriksen JG, Broholm H, Christensen IJ, Grunnet K, Horsman MR, et al: Prospective evaluation of angiogenic, hypoxic and EGFR-related biomarkers in recurrent glioblastoma multiforme treated with cetuximab, bevacizumab and irinotecan. **APMIS** **118**:585–594, 2010
51. Hegi ME, Diserens AC, Bady P, Kamoshima Y, Kouwenhoven MCM, Delorenzi M, et al: Pathway analysis of glioblastoma tissue after preoperative treatment with the EGFR tyrosine kinase inhibitor gefitinib—a phase II trial. **Mol Cancer Ther** **10**:1102–1112, 2011
52. Hegi ME, Diserens AC, Gorlia T, Hamou MF, de Tribolet N, Weller M, et al: MGMT gene silencing and benefit from temozolomide in glioblastoma. **N Engl J Med** **352**:997–1003, 2005

53. Hermann A, Maisel M, Liebau S, Gerlach M, Kleger A, Schwarz J, et al: Mesodermal cell types induce neurogenesis from adult human hippocampal progenitor cells. **J Neurochem** **98**:629–640, 2006
54. Hirose Y, Berger MS, Pieper RO: Abrogation of the Chk1-mediated G(2) checkpoint pathway potentiates temozolomide-induced toxicity in a p53-independent manner in human glioblastoma cells. **Cancer Res** **61**:5843–5849, 2001
55. Hirose Y, Katayama M, Stokoe D, Haas-Kogan DA, Berger MS, Pieper RO: The p38 mitogen-activated protein kinase pathway links the DNA mismatch repair system to the G2 checkpoint and to resistance to chemotherapeutic DNA-methylating agents. **Mol Cell Biol** **23**:8306–8315, 2003
56. Hirose Y, Kreklau EL, Erickson LC, Berger MS, Pieper RO: Delayed depletion of O6-methylguanine-DNA methyltransferase resulting in failure to protect the human glioblastoma cell line SF767 from temozolomide-induced cytotoxicity. **J Neurosurg** **98**:591–598, 2003
57. Hirsch HA, Iliopoulos D, Tschlis PN, Struhl K: Metformin selectively targets cancer stem cells, and acts together with chemotherapy to block tumor growth and prolong remission. **Cancer Res** **69**:7507–7511, 2009
58. Hockfield S, McKay RD: Identification of major cell classes in the developing mammalian nervous system. **J Neurosci** **5**:3310–3328, 1985
59. Holyoake T, Jiang X, Eaves C, Eaves A: Isolation of a highly quiescent subpopulation of primitive leukemic cells in chronic myeloid leukemia. **Blood** **94**:2056–2064, 1999
60. Horn PA, Tesch H, Staib P, Kube D, Diehl V, Voliotis D: Expression of AC133, a novel hematopoietic precursor antigen, on acute myeloid leukemia cells. **Blood** **93**:1435–1437, 1999
61. Houde C, Li Y, Song L, Barton K, Zhang Q, Godwin J, et al: Overexpression of the NOTCH ligand JAG2 in malignant plasma cells from multiple myeloma patients and cell lines. **Blood** **104**:3697–3704, 2004
62. Hovinga KE, Shimizu F, Wang R, Panagiotakos G, Van Der Heijden M, Moayedpardazi H, et al: Inhibition of notch signaling in glioblastoma targets cancer stem cells via an endothelial cell intermediate. **Stem Cells** **28**:1019–1029, 2010
63. Inda MM, Bonavia R, Mukasa A, Narita Y, Sah DWY, Vandenberg S, et al: Tumor heterogeneity is an active process maintained by a mutant EGFR-induced cytokine circuit in glioblastoma. **Genes Dev** **24**:1731–1745, 2010
64. Jin X, Yin J, Kim SH, Sohn YW, Beck S, Lim YC, et al: EGFR-AKT-Smad signaling promotes formation of glioma stem-like cells and tumor angiogenesis by ID3-driven cytokine induction. **Cancer Res** **71**:7125–7134, 2011
65. Jung JW, Park SB, Lee SJ, Seo MS, Trosko JE, Kang KS: Metformin represses self-renewal of the human breast carcinoma stem cells via inhibition of estrogen receptor-mediated OCT4 expression. **PLoS One** **6**:e28068, 2011
66. Kahlon KS, Brown C, Cooper LJN, Raubitschek A, Forman SJ, Jensen MC: Specific recognition and killing of glioblastoma multiforme by interleukin 13-zetakine redirected cytolytic T cells. **Cancer Res** **64**:9160–9166, 2004
67. Kai K, D'Costa S, Yoon BI, Brody AR, Sills RC, Kim Y: Characterization of side population cells in human malignant mesothelioma cell lines. **Lung Cancer** **70**:146–151, 2010
68. Kang KB, Zhu C, Wong YL, Gao Q, Ty A, Wong MC: Gefitinib radiosensitizes stem-like glioma cells: inhibition of epidermal growth factor receptor-Akt-DNA-PK signaling, accompanied by inhibition of DNA double-strand break repair. **Int J Radiat Oncol Biol Phys** **83**:e43–e52, 2012
69. Kang MK, Kang SK: Pharmacologic blockade of chloride channel synergistically enhances apoptosis of chemotherapeutic drug-resistant cancer stem cells. **Biochem Biophys Res Commun** **373**:539–544, 2008
70. Kelly JJ, Stechishin O, Chojnacki A, Lun X, Sun B, Senger DL, et al: Proliferation of human glioblastoma stem cells occurs independently of exogenous mitogens. **Stem Cells** **27**:1722–1733, 2009
71. Kim KH, Seol HJ, Kim EH, Rhee Y, Jin HJ, Lee Y, et al: Wnt/ β -catenin signaling is a key downstream mediator of MET signaling in glioblastoma stem cells. **Neuro Oncol** **15**:161–171, 2013
72. Kim SS, Rait A, Kim E, Pirollo KF, Nishida M, Farkas N, et al: A nanoparticle carrying the p53 gene targets tumors including cancer stem cells, sensitizes glioblastoma to chemotherapy and improves survival. **ACS Nano** **8**:5494–5514, 2014
73. Kinzler KW, Bigner SH, Bigner DD, Trent JM, Law ML, O'Brien SJ, et al: Identification of an amplified, highly expressed gene in a human glioma. **Science** **236**:70–73, 1987
74. Kong S, Sengupta S, Tyler B, Bais AJ, Ma Q, Doucette S, et al: Suppression of human glioma xenografts with second-generation IL13R-specific chimeric antigen receptor-modified T cells. **Clin Cancer Res** **18**:5949–5960, 2012
75. Kostrouchová M, Kostrouch Z, Kostrouchová M: Valproic acid, a molecular lead to multiple regulatory pathways. **Folia Biol (Praha)** **53**:37–49, 2007
76. Krafft C, Sobottka SB, Geiger KD, Schackert G, Salzer R: Classification of malignant gliomas by infrared spectroscopic imaging and linear discriminant analysis. **Anal Bioanal Chem** **387**:1669–1677, 2007
77. Krafft C, Thümmel K, Sobottka SB, Schackert G, Salzer R: Classification of malignant gliomas by infrared spectroscopy and linear discriminant analysis. **Biopolymers** **82**:301–305, 2006
78. Lendahl U, Zimmerman LB, McKay RD: CNS stem cells express a new class of intermediate filament protein. **Cell** **60**:585–595, 1990
79. Li Y, Guessous F, Zhang Y, Dipierro C, Kefas B, Johnson E, et al: MicroRNA-34a inhibits glioblastoma growth by targeting multiple oncogenes. **Cancer Res** **69**:7569–7576, 2009
80. Li Z, Lee JW, Mukherjee D, Ji J, Jeswani SP, Black KL, et al: Immunotherapy targeting glioma stem cells—insights and perspectives. **Expert Opin Biol Ther** **12**:165–178, 2012
81. Liau LM, Prins RM, Kierstschner SM, Odesa SK, Kremen TJ, Giovannone AJ, et al: Dendritic cell vaccination in glioblastoma patients induces systemic and intracranial T-cell responses modulated by the local central nervous system tumor microenvironment. **Clin Cancer Res** **11**:5515–5525, 2005
82. Lim DA, Tramontin AD, Trevejo JM, Herrera DG, García-Verdugo JM, Alvarez-Buylla A: Noggin antagonizes BMP signaling to create a niche for adult neurogenesis. **Neuron** **28**:713–726, 2000
83. Lindvall C, Bu W, Williams BO, Li Y: Wnt signaling, stem cells, and the cellular origin of breast cancer. **Stem Cell Rev** **3**:157–168, 2007
84. Liu G, Yuan X, Zeng Z, Tunici P, Ng H, Abdulkadir IR, et al: Analysis of gene expression and chemoresistance of CD133+ cancer stem cells in glioblastoma. **Mol Cancer** **5**:67, 2006
85. Lustig B, Behrens J: The Wnt signaling pathway and its role in tumor development. **J Cancer Res Clin Oncol** **129**:199–221, 2003
86. Ma HI, Chiou SH, Hueng DY, Tai LK, Huang PI, Kao CL, et al: Celecoxib and radioresistant glioblastoma-derived CD133+ cells: improvement in radiotherapeutic effects. Laboratory investigation. **J Neurosurg** **114**:651–662, 2011
87. Maes W, Rosas GG, Verbinen B, Boon L, De Vleeschouwer S, Ceuppens JL, et al: DC vaccination with anti-CD25 treatment leads to long-term immunity against experimental glioma. **Neuro Oncol** **11**:529–542, 2009
88. Marian CO, Cho SK, McEllin BM, Maher EA, Hatanpaa KJ, Madden CJ, et al: The telomerase antagonist, imetelstat, efficiently targets glioblastoma tumor-initiating cells leading to decreased proliferation and tumor growth. **Clin Cancer Res** **16**:154–163, 2010

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89. Martinez R, Schackert G, Yaya-Tur R, Rojas-Marcos I, Herman JG, Esteller M: Frequent hypermethylation of the DNA repair gene MGMT in long-term survivors of glioblastoma multiforme. **J Neurooncol** **83**:91–93, 2007
90. Meiss F, Zeiser R: Vismodegib. **Recent Results Cancer Res** **201**:405–417, 2014
91. Michelakis ED, Sutendra G, Dromparis P, Webster L, Haromy A, Niven E, et al: Metabolic modulation of glioblastoma with dichloroacetate. **Sci Transl Med** **2**:31ra34, 2010
92. Miyamoto Y, Maitra A, Ghosh B, Zechner U, Argani P, Iacobuzio-Donahue CA, et al: Notch mediates TGF alpha-induced changes in epithelial differentiation during pancreatic tumorigenesis. **Cancer Cell** **3**:565–576, 2003
93. Mizrak D, Brittan M, Alison M: CD133: molecule of the moment. **J Pathol** **214**:3–9, 2008
94. Morelli MB, Nabissi M, Amantini C, Farfariello V, Ricci-Vitiani L, di Martino S, et al: The transient receptor potential vanilloid-2 cation channel impairs glioblastoma stem-like cell proliferation and promotes differentiation. **Int J Cancer** **131**:E1067–E1077, 2012
95. Mukherjee B, Tomimatsu N, Amancerla K, Camacho CV, Pichamoorthy N, Burma S: The dual PI3K/mTOR inhibitor NVP-BEZ235 is a potent inhibitor of ATM- and DNA-PKCs-mediated DNA damage responses. **Neoplasia** **14**:34–43, 2012
96. Nakai E, Park K, Yawata T, Chihara T, Kumazawa A, Nakabayashi H, et al: Enhanced MDR1 expression and chemoresistance of cancer stem cells derived from glioblastoma. **Cancer Invest** **27**:901–908, 2009
97. Natsume A, Kato T, Kinjo S, Enomoto A, Toda H, Shimato S, et al: Girdin maintains the stemness of glioblastoma stem cells. **Oncogene** **31**:2715–2724, 2012
98. Neuzil J, Stantic M, Zobalova R, Chladova J, Wang X, Prochazka L, et al: Tumour-initiating cells vs. cancer ‘stem’ cells and CD133: what’s in the name? **Biochem Biophys Res Commun** **355**:855–859, 2007
99. Nickoloff BJ, Osborne BA, Miele L: Notch signaling as a therapeutic target in cancer: a new approach to the development of cell fate modifying agents. **Oncogene** **22**:6598–6608, 2003
100. Nicolis SK: Cancer stem cells and “stemness” genes in neuro-oncology. **Neurobiol Dis** **25**:217–229, 2007
101. Ohba S, Hirose Y, Yoshida K, Yazaki T, Kawase T: Inhibition of 90-kD heat shock protein potentiates the cytotoxicity of chemotherapeutic agents in human glioma cells. Laboratory investigation. **J Neurosurg** **112**:33–42, 2010
102. Oka N, Soeda A, Noda S, Iwama T: Brain tumor stem cells from an adenoid glioblastoma multiforme. **Neurol Med Chir (Tokyo)** **49**:146–151, 2009
103. Osuka S, Sampetean O, Shimizu T, Saga I, Onishi N, Sugihara E, et al: IGF1 receptor signaling regulates adaptive radioprotection in glioma stem cells. **Stem Cells** **31**:627–640, 2013
104. Panchision DM, McKay RDG: The control of neural stem cells by morphogenic signals. **Curr Opin Genet Dev** **12**:478–487, 2002
105. Parr C, Watkins G, Jiang WG: The possible correlation of Notch-1 and Notch-2 with clinical outcome and tumour clinicopathological parameters in human breast cancer. **Int J Mol Med** **14**:779–786, 2004
106. Parsons DW, Jones S, Zhang X, Lin JCH, Leary RJ, Angenendt P, et al: An integrated genomic analysis of human glioblastoma multiforme. **Science** **321**:1807–1812, 2008
107. Pece S, Serresi M, Santolini E, Capra M, Hulleman E, Galimberti V, et al: Loss of negative regulation by Numb over Notch is relevant to human breast carcinogenesis. **J Cell Biol** **167**:215–221, 2004
108. Pérez Castillo A, Aguilar-Morante D, Morales-García JA, Dorado J: Cancer stem cells and brain tumors. **Clin Transl Oncol** **10**:262–267, 2008
109. Phiel CJ, Zhang F, Huang EY, Guenther MG, Lazar MA, Klein PS: Histone deacetylase is a direct target of valproic acid, a potent anticonvulsant, mood stabilizer, and teratogen. **J Biol Chem** **276**:36734–36741, 2001
110. Piccirillo SGM, Reynolds BA, Zanetti N, Lamorte G, Binda E, Broggi G, et al: Bone morphogenetic proteins inhibit the tumorigenic potential of human brain tumour-initiating cells. **Nature** **444**:761–765, 2006 (Letter)
111. Polakis P: The many ways of Wnt in cancer. **Curr Opin Genet Dev** **17**:45–51, 2007
112. Purow BW, Haque RM, Noel MW, Su Q, Burdick MJ, Lee J, et al: Expression of Notch-1 and its ligands, Delta-like-1 and Jagged-1, is critical for glioma cell survival and proliferation. **Cancer Res** **65**:2353–2363, 2005
113. Raizer JJ, Abrey LE, Lassman AB, Chang SM, Lamborn KR, Kuhn JG, et al: A phase II trial of erlotinib in patients with recurrent malignant gliomas and nonprogressive glioblastoma multiforme postradiation therapy. **Neuro Oncol** **12**:95–103, 2010
114. Rasper M, Schäfer A, Piontek G, Teufel J, Brockhoff G, Ringel F, et al: Aldehyde dehydrogenase 1 positive glioblastoma cells show brain tumor stem cell capacity. **Neuro Oncol** **12**:1024–1033, 2010
115. Rich JN, Eyster CE: Cancer stem cells in brain tumor biology. **Cold Spring Harb Symp Quant Biol** **73**:411–420, 2008
116. Ropolo M, Daga A, Griffero F, Foresta M, Casartelli G, Zunino A, et al: Comparative analysis of DNA repair in stem and non-stem glioma cell cultures. **Mol Cancer Res** **7**:383–392, 2009
117. Sagar D, Foss C, El Baz R, Pomper MG, Khan ZK, Jain P: Mechanisms of dendritic cell trafficking across the blood-brain barrier. **J Neuroimmune Pharmacol** **7**:74–94, 2012
118. Sakariassen PØ, Immervoll H, Chekenya M: Cancer stem cells as mediators of treatment resistance in brain tumors: status and controversies. **Neoplasia** **9**:882–892, 2007
119. Sandberg CJ, Altschuler G, Jeong J, Strømme KK, Stangeland B, Murrell W, et al: Comparison of glioma stem cells to neural stem cells from the adult human brain identifies dysregulated Wnt-signaling and a fingerprint associated with clinical outcome. **Exp Cell Res** **319**:2230–2243, 2013
120. Schmalz PGR, Shen MJ, Park JK: Treatment resistance mechanisms of malignant glioma tumor stem cells. **Cancers (Basel)** **3**:621–635, 2011
121. Sharma V, Dixit D, Ghosh S, Sen E: COX-2 regulates the proliferation of glioma stem like cells. **Neurochem Int** **59**:567–571, 2011
122. Shen G, Shen F, Shi Z, Liu W, Hu W, Zheng X, et al: Identification of cancer stem-like cells in the C6 glioma cell line and the limitation of current identification methods. **In Vitro Cell Dev Biol Anim** **44**:280–289, 2008
123. Singh SK, Clarke ID, Terasaki M, Bonn VE, Hawkins C, Squire J, et al: Identification of a cancer stem cell in human brain tumors. **Cancer Res** **63**:5821–5828, 2003
124. Singh SK, Hawkins C, Clarke ID, Squire JA, Bayani J, Hide T, et al: Identification of human brain tumour initiating cells. **Nature** **432**:396–401, 2004
125. Solit DB, Basso AD, Olshen AB, Scher HI, Rosen N: Inhibition of heat shock protein 90 function down-regulates Akt kinase and sensitizes tumors to Taxol. **Cancer Res** **63**:2139–2144, 2003
126. Song CW, Lee H, Dings RPM, Williams B, Powers J, Santos TD, et al: Metformin kills and radiosensitizes cancer cells and preferentially kills cancer stem cells. **Sci Rep** **2**:362, 2012
127. Srivastava VK, Nalbantoglu J: Flow cytometric characterization of the DAOY medulloblastoma cell line for the cancer stem-like phenotype. **Cytometry A** **73**:940–948, 2008
128. Stea B, Falsey R, Kislin K, Patel J, Glanzberg H, Carey S, et al: Time and dose-dependent radiosensitization of the glioblastoma multiforme U251 cells by the EGF receptor tyrosine kinase inhibitor ZD1839 (‘Iressa’). **Cancer Lett** **202**:43–51, 2003
129. Stechishin OD, Luchman HA, Ruan Y, Blough MD, Nguyen SA, Kelly JJ, et al: On-target JAK2/STAT3 inhibition slows disease progression in orthotopic xenografts of human glioblastoma brain tumor stem cells. **Neuro Oncol** **15**:198–207, 2013

130. Steiner G, Küchler S, Hermann A, Koch E, Salzer R, Schackert G, et al: Rapid and label-free classification of human glioma cells by infrared spectroscopic imaging. **Cytometry A** **73A**:1158–1164, 2008
131. Stupp R, Mason WP, van den Bent MJ, Weller M, Fisher B, Taphoorn MJB, et al: Radiotherapy plus concomitant and adjuvant temozolomide for glioblastoma. **N Engl J Med** **352**:987–996, 2005
132. Tentori L, Leonetti C, Scarsella M, D'Amati G, Vergati M, Portarena I, et al: Systemic administration of GPI 15427, a novel poly(ADP-ribose) polymerase-1 inhibitor, increases the antitumor activity of temozolomide against intracranial melanoma, glioma, lymphoma. **Clin Cancer Res** **9**:5370–5379, 2003
133. Tentori L, Leonetti C, Scarsella M, Muzi A, Vergati M, Forini O, et al: Poly(ADP-ribose) glycohydrolase inhibitor as chemosensitizer of malignant melanoma for temozolomide. **Eur J Cancer** **41**:2948–2957, 2005
134. Tentori L, Leonetti C, Scarsella M, Vergati M, Xu W, Calvin D, et al: Brain distribution and efficacy as chemosensitizer of an oral formulation of PARP-1 inhibitor GPI 15427 in experimental models of CNS tumors. **Int J Oncol** **26**:415–422, 2005
135. Ulasov IV, Sonabend AM, Nandi S, Khramtsov A, Han Y, Lesniak MS: Combination of adenoviral virotherapy and temozolomide chemotherapy eradicates malignant glioma through autophagic and apoptotic cell death in vivo. **Br J Cancer** **100**:1154–1164, 2009
136. Van Niftrik KA, Van den Berg J, Slotman BJ, Lafleur MVM, Sminia P, Stalpers LJA: Valproic acid sensitizes human glioma cells for temozolomide and γ -radiation. **J Neurooncol** **107**:61–67, 2012
137. Venugopal C, Li N, Wang X, Manoranjan B, Hawkins C, Gunnarsson T, et al: Bmi1 marks intermediate precursors during differentiation of human brain tumor initiating cells. **Stem Cell Res (Amst)** **8**:141–153, 2012
138. Vescovi AL, Galli R, Reynolds BA: Brain tumour stem cells. **Nat Rev Cancer** **6**:425–436, 2006
139. Wachsberger PR, Lawrence RY, Liu Y, Xia X, Andersen B, Dicker AP: Cediranib enhances control of wild type EGFR and EGFRvIII-expressing gliomas through potentiating temozolomide, but not through radiosensitization: implications for the clinic. **J Neurooncol** **105**:181–190, 2011
140. Wakimoto H, Mohapatra G, Kanai R, Curry WT Jr, Yip S, Nitta M, et al: Maintenance of primary tumor phenotype and genotype in glioblastoma stem cells. **Neuro Oncol** **14**:132–144, 2012
141. Waldmann TA: Immunotherapy: past, present and future. **Nat Med** **9**:269–277, 2003
142. Wang CH, Chiou SH, Chou CP, Chen YC, Huang YJ, Peng CA: Photothermolysis of glioblastoma stem-like cells targeted by carbon nanotubes conjugated with CD133 monoclonal antibody. **Nanomedicine (Lond Print)** **7**:69–79, 2011
143. Wang J, Wang H, Li Z, Wu Q, Lathia JD, McLendon RE, et al: c-Myc is required for maintenance of glioma cancer stem cells. **PLoS One** **3**:e3769, 2008
144. Wang WJ, Long LM, Yang N, Zhang QQ, Ji WJ, Zhao JH, et al: NVP-BEZ235, a novel dual PI3K/mTOR inhibitor, enhances the radiosensitivity of human glioma stem cells in vitro. **Acta Pharmacol Sin** **34**:681–690, 2013
145. Warrier S, Balu SK, Kumar AP, Millward M, Dharmarajan A: Wnt antagonist, secreted frizzled-related protein 4 (sFRP4), increases chemotherapeutic response of glioma stem-like cells. **Oncol Res** **21**:93–102, 2013
146. Wechsler-Reya RJ, Scott MP: Control of neuronal precursor proliferation in the cerebellum by Sonic Hedgehog. **Neuron** **22**:103–114, 1999
147. Whiteway SL, Harris PS, Venkataraman S, Alimova I, Birks DK, Donson AM, et al: Inhibition of cyclin-dependent kinase 6 suppresses cell proliferation and enhances radiation sensitivity in medulloblastoma cells. **J Neurooncol** **111**:113–121, 2013
148. Würth R, Pattarozzi A, Gatti M, Bajetto A, Corsaro A, Parodi A, et al: Metformin selectively affects human glioblastoma tumor-initiating cell viability: a role for metformin-induced inhibition of Akt. **Cell Cycle** **12**:145–156, 2013
149. Xu Y, Stamenkovic I, Yu Q: CD44 attenuates activation of the hippo signaling pathway and is a prime therapeutic target for glioblastoma. **Cancer Res** **70**:2455–2464, 2010
150. Yan GN, Lv YF, Yang L, Yao XH, Cui YH, Guo DY: Glioma stem cells enhance endothelial cell migration and proliferation via the Hedgehog pathway. **Oncol Lett** **6**:1524–1530, 2013
151. Yang W, Wei J, Guo T, Shen Y, Liu F: Knockdown of miR-210 decreases hypoxic glioma stem cells stemness and radioresistance. **Exp Cell Res** **326**:22–35, 2014
152. Yang YP, Chang YL, Huang PI, Chiou GY, Tseng LM, Chiou SH, et al: Resveratrol suppresses tumorigenicity and enhances radiosensitivity in primary glioblastoma tumor initiating cells by inhibiting the STAT3 axis. **J Cell Physiol** **227**:976–993, 2012
153. Yu JS, Liu G, Ying H, Yong WH, Black KL, Wheeler CJ: Vaccination with tumor lysate-pulsed dendritic cells elicits antigen-specific, cytotoxic T-cells in patients with malignant glioma. **Cancer Res** **64**:4973–4979, 2004
154. Yu JS, Wheeler CJ, Zeltzer PM, Ying H, Finger DN, Lee PK, et al: Vaccination of malignant glioma patients with peptide-pulsed dendritic cells elicits systemic cytotoxicity and intracranial T-cell infiltration. **Cancer Res** **61**:842–847, 2001
155. Zhang J, Stevens MFG, Bradshaw TD: Temozolomide: mechanisms of action, repair and resistance. **Curr Mol Pharmacol** **5**:102–114, 2012
156. Zhang M, Kleber S, Röhrich M, Timke C, Han N, Tuettenberg J, et al: Blockade of TGF- β signaling by the TGF β R-I kinase inhibitor LY2109761 enhances radiation response and prolongs survival in glioblastoma. **Cancer Res** **71**:7155–7167, 2011
157. Zhu TS, Costello MA, Talsma CE, Flack CG, Crowley JG, Hamm LL, et al: Endothelial cells create a stem cell niche in glioblastoma by providing NOTCH ligands that nurture self-renewal of cancer stem-like cells. **Cancer Res** **71**:6061–6072, 2011
158. Zhuang W, Li B, Long L, Chen L, Huang Q, Liang Z: Induction of autophagy promotes differentiation of glioma-initiating cells and their radiosensitivity. **Int J Cancer** **129**:2720–2731, 2011
159. Zhuang W, Long L, Zheng B, Ji W, Yang N, Zhang Q, et al: Curcumin promotes differentiation of glioma-initiating cells by inducing autophagy. **Cancer Sci** **103**:684–690, 2012

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