

Brain Tumor Treatment: 2017 Update

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Abstract

Malignant brain tumors are a heterogeneous group of diseases arising from different cell types that affect both adults and children. The high recurrence rate of malignant brain tumors typically is due to reappearance of focal masses, indicating that a sub population of tumor cells are insensitive to current therapies and may be responsible for reinitiating tumor growth. It is generally agreed that the resistant tumor cells are comprised of cancer stem cells or tumor-initiating cells. While brain tumor stem cells (BTSCs) were first isolated within the last decade, much of the early research has been focused on identifying the BTSC markers and therapeutic targets. The challenge however, is to translate this knowledge to therapeutics. In the current review, we survey the remedial strategies to target BTSCs, which includes diagnostic, pharmacologic, immunologic, viral, and post-transcriptional approaches.

Keywords: cancer stem cells, brain neoplasms, glioma, therapeutics, cancer

1. Introduction

1.1 Introduction to BTSC Therapeutics

Malignant brain tumor is a leading cause of cancer-related death in children. In 2004, an estimated 7.2 per 100,000 people in the US developed malignant brain tumors, with a 5-year survival rate of only 28% (Porter, McCarthy et al. 2010). The location of brain tumors is within vital areas of the central nervous system, thus making it difficult for conventional therapeutic interventions including surgery and chemotherapy. The recurrence rate of brain tumor is high and typically manifests as focal masses, indicating that a sub population of tumor cells, able to escape current treatments, may be responsible for reinitiating tumor growth. Therefore, new targeted alternative therapies are vitally needed to treat brain tumor as the current therapeutic options are limited (Pardal, Clarke et al. 2003).

It is generally agreed that a high rate of recurrence in brain tumor is due to the resistance of a sub-population of cancer stem cells (CSCs) or tumor-initiating cells (Clarke, Dick et al. 2006). The involvement of CSCs in tumor formation was originally reported in malignant brain tumors within the last decade (Hemmati, Nakano et al. 2003, Singh, Clarke et al. 2003). Subsequently, brain tumor stem cells (BTSCs) have been reported to show resistance against radiation (Bao, Wu et al. 2006, Kang, Hur et al. 2008, Tomuleasa, Soritau et al. 2010) and numerous chemotherapeutic agents, including temozolomide and paclitaxel (Kong, Kim et al. 2008). BTSCs have also been associated with adult brain and pediatric brain tumors (Thirant, Bessette et al. 2011) expressing a shorter survival (Beier, Wischhusen et al. 2008) progressive phenotype (Kong, Kim et al. 2008), leading to a higher mortality rate (Pallini, Ricci-Vitiani et al. 2008, Laks, Masterman-Smith et al. 2009). Moreover, a retrospective analysis found that targeting the BTSC niche (distinct from the neural stem cell population) – with radiotherapy - led to a significantly decreased risk of the disease progression and dose-dependent improvement in patient survival (Evers, Lee et al. 2010). These recent studies suggest that BTSCs may have clinical relevance in malignant brain tumors.

Most of the research on BTSCs focuses upon the pursuit of relevant BTSC markers or therapeutic targets. However, these studies which identify the potential BTSC markers, do not focus upon them being a potential remedial target, which could hold more direct clinical promise. Therefore, in this review, we summarize the

recent and ongoing therapeutic approaches and strategies to target BTSCs.

1.2 Diagnostic Approach to Identify BTSCs

The concept of the existence of BTSCs originally gained prominence when researchers started identifying cancer stem cells (CSCs) amongst human astrocytomas (glioma) (Altaner 2008), neuroblastoma (Walton, Kattan et al. 2004), meningioma (Hueng, Sytwu et al. 2011), schwannoma (Hodges, Karikari et al. 2011) and medulloblastoma (Lasky, Choe et al. 2009) sub-population. Characterizing BTSCs based on their source of origin or prognostic stages of growth is still a challenge as these tumor cells shared a similar marker attribute. Furthermore, identification of precursor or early stage detection markers of these cell populations becomes an extreme challenge. Table 1 illustrates some of the early precursor markers associated with different stages of cancer stem cell populations.

Recently, Beck and colleagues (Beck, Jin et al. 2011) used phage display to screen peptide libraries for ligands that preferentially bind to their targets in the gliomas (Pasqualini and Ruoslahti 1996). They identified a stem cell-targeting (GSCT) 'AQYLNPS' peptide that selectively binds to the N-terminal isotype(s) of nestin, an intermediate filament protein found exclusively in neural precursor cells (Lendahl, Zimmerman et al. 1990) and surprisingly expressed in undifferentiated glioblastoma BTSCs (Beck, Jin et al. 2011). Interestingly, the GSCT peptide effectively penetrated glioblastoma tissue to target nestin+ BTSCs *in vitro* (Beck, Jin et al. 2011). However, given the fact that nestin is not universally expressed in BTSCs, the GSCT peptide may only be targeting a subpopulation of BTSCs that express nestin. Nonetheless, these significant findings suggest that the GSCT peptide may have utility for therapeutic screening of BTSCs. Alternatively, CSCs were identified in a murine brain tumor population by expressing EGFP under the control of nucleostemin promoter (a p53 mediate cell cycle modulator often expressed by highly proliferative cells) (Tamase, Muraguchi et al. 2009). Furthermore, screening of BTSCs using flow-cytometry and Elisa have identified CD133, MMP-9, nestin, Pax6, Math-1, musashi-1, BMI-1, CXCR4, CX3CR1, c-Myc and Sox2 expressing BTSCs from both gliomas and medulloblastomas (Tamase, Muraguchi et al. 2009, Cox, Wilder et al. 2013, Sullivan, Nahed et al. 2014).

Table 1. Characterization of Tumors based on markers

Tumor Type	Cell markers	Specificity
Grade-IVAstrocytoma: Glioblastoma (GBM)	1) Oct-4 2) CD-90(-ve)/VEGF (+)/PGE2 (+) 3)IL-17RA/CD 133/SOX-2/Nestin Eva1- (epithelial V-like antigen 1) CD133, CD15, CD49f	1) High expression acts as an independent prognosis marker for malignant gliomas (Krogh Petersen, Jensen et al. 2016). 2) Marker for Mesenchymal stromal cell in malignant gliomas (Svensson, Ramos-Moreno et al. 2016). 3) Markers for 2-19% Glial stem cell precursors (Parajuli, Anand et al. 2016) (Ohtsu, Nakatani et al. 2016).
Grade II-III Low grade/ Anaplastic astrocytoma	1) Netrin-1 2) P2RY12 3) Testin 4) B7-H6	1) gain of stemness marker (Ylivinkka, Sihto et al. 2017). 2) microglial astrocytoma marker (Zhu, Kros et al. 2017). 3) prognostic marker (Steponaitis, Kazlauskas et al. 2016). 4) latent grade III astrocytoma marker (Guo, Guo et al. 2016).
Glioma	1) Nos-1 2) CDA-1	1) Glioma stemcell maerker
Schwannoma	1) PDGFR 2) Oct-4/ Nestin 3) Cytokeratin 4) Calretinin 5) CD56 6) CD34 7) S-100	1/2) Stem cell markers 3) Retroperitoneal Schwannoma marker 4/5/6/7) Differentiation markers
Neuroblastoma	1) LGR5 2) CD133 3) BMI1	1) Neuroblastoma proliferation 2) Reduction of stemness of NB
Meningioma	1) CD133 2) ABCG mRNA 3) Nestin 4) miR21	1/2) stem cell marker 3/4) Anaplastic meningioma
Medulloblastoma	1) CD133 2) SOX-2 3) Mushashi1 4) BMI1	1) MB-stem cell markers

Note: Cell Markers: Surface, Specificity: Functionality

2. Pharmacologic

2.1 Differentiation

While the induction of differentiation of cancer cells is a rational therapeutic strategy for anticancer treatment, only a few agents, including retinoic acid, bone morphogenetic proteins (BMPs), and histone deacetylase (HDAC) inhibitors (Massard, Deutsch et al. 2006, Piccirillo, Reynolds et al. 2006) currently target cancer cell differentiation. All-*trans* retinoic acid (vitamin A) modulates cell differentiation and proliferation and is used as adjuvant therapy for acute promyelocytic leukemia (Massard, Deutsch et al. 2006). Treatment with retinoic acid induced differentiation of glioma BTSCs *ex vivo* as well as growth-inhibiting, antimigratory, and anti-angiogenic effects in mice transplanted with undifferentiated glioma BTSCs (Campos, Wan et al. 2010).

BMPs are a known modulators of neural stem cells (Panchision and McKay 2002). Treatment with BMP4 has been shown to eliminate the tumorigenicity of transplanted glioma cells, and more importantly stops tumor growth and prevents death in mice bearing glioma transplants. BMPs have also been shown to induce formation of differentiated astrocytes from GBMs. However, enriched SOX transcription factor-binding motifs in these astrocytes raises questions about the differential commitment of the GBM cells and possible tumorigenic property of re-activation (Car n, Stricker et al.).

However, BMPs have been shown to reduce the BTSC subpopulation by activating their cognate receptors and triggering Smad protein signaling (Piccirillo, Reynolds et al. 2006). A second study has also shown that the

neural precursor cells release BMP7, a paracrine tumor suppressor of glioma BTSCs that acts by activating Smad signaling (Chirasani, Sternjak et al. 2010). The observed effects of HDAC inhibitors in cancer treatment (Marks and Dokmanovic 2005) is mimicked by glioma neurospheres, which upon the treatment with HDAC inhibitors, induced differentiation *in vitro* through non-canonical Notch signaling and reduced the tumorigenicity of neurosphere xenografts in mice (Sun, Xia et al. 2009). However, a recent study has found that the treatment with HDAC inhibitors led to the accumulation of histone methylation. Combination treatment with HDAC and lysine-specific histone demethylase (LSD)-1 inhibitors resulted in synergistic apoptosis in glioblastoma cells but not normal astrocytes (Singh, Manton et al. 2011). Alternatively, drugs preventing L-MYC (Li, Oganessian et al. 2016), HMGA-2 (Zhong, Liu et al. 2016), fatty acid synthase (FASN) (Yasumoto, Miyazaki et al. 2016), astrocyte elevated gene-1 (AEG-1) (Hu, Emdad et al. 2016), and lysine demethylase KDM1A (Sareddy, Viswanadhapalli et al. 2016) activity and promoting PAX6/DLX5 transcriptional activation of WNT signaling (Hu, Wang et al. 2016) prevented glioma stem cell morphology and induced astrocytic differentiation. These preclinical studies suggest that eliminating the glioblastoma stem cell (GBSc) population and inducing astrocytic differentiation of the glioma cells may be a viable selective treatment strategy to explore for BTSCs, although further testing is needed in an *in vivo* systems.

2.2 Tumor Microenvironment

The tumor microenvironment thrives under hypoxic conditions upon the up-regulation of hypoxia inducible factor: HIF-1 α and HIF-2 α , which enhances the expression of stem-cell markers, chemo-resistance, and clonogenicity of BTSCs (McCord, Jamal et al. 2009, Bar, Lin et al. 2010, Pistollato, Abbadì et al. 2010, Pistollato, Rampazzo et al. 2010, Kolenda, Jensen et al. 2011). In contrast to the mitochondrial glucose oxidation phenotype of normal tissues (Kim, Tchernyshyov et al. 2006), HIF-1 α amplifies the expression of pyruvate dehydrogenase kinase (PDK), thereby contributing to the cytoplasmic glycolytic phenotype maintained in glioma. Dichloroacetate is an orphan small-molecule oral PDK inhibitor that has been shown to selectively improve mitochondrial function in cancer cells and decrease tumor growth and proliferation (Bonnet, Archer et al. 2007). Treatment with dichloroacetate improved mitochondrial function in freshly dissociated glioma tissue but not normal brain tissue. More importantly, administration of dichloroacetate for 15 months in 5 patients with glioblastoma resulted in some tumor regression and the patients demonstrated a good safety profile (Michelakis, Sutendra et al. 2010). These findings suggest that dichloroacetate may be an effective metabolic modulator for anticancer therapy and further clinical testing is warranted.

Accumulating evidence suggests that BTSCs, like normal stem cells, reside within vascular niches that help BTSCs to maintain their stem cell-like properties (Calabrese, Poppleton et al. 2007, Gilbertson and Rich 2007). Treatment with Interferon- β in glioma xenografts disrupted these vascular niches by promoting perivascular cell growth forming a barrier between the glioma BTSCs and endothelial cells. This, indirectly reduced the tumor growth and the BTSC population (Williams, Sims et al. 2010). Although these results suggest that interferon- β has anti-BTSC activity, the clinical use of interferon- β may be limited by its known systemic toxicity and short half-life (Williams, Sims et al. 2010).

2.3 Telomerase Antagonism

A hallmark of cancer cells is the re-activation of telomerase, a riboprotein enzyme that counteracts the shortening of telomeres resulting from repeated DNA replication, which enables uncontrolled cell expansion (Shay and Wright 2002). In brain tumors, a correlation has been reported between telomerase activity and histologic grade (Falchetti, Larocca et al. 2002). Gliomas are often associated with mutations in the telomerase reverse transcriptase gene (TERT) promoter sites (Simon, Hosen et al. 2015). Previous studies have successfully used RNA interference to down-regulate the hTERT gene in the gliomas and LN18 cells (Wang, Xue et al. 2012, Lavanya, Sibin et al. 2016), inducing apoptotic cell death. Treatment of glioblastoma cells with the telomerase inhibitor imetelstat alone, or in combination with temozolomide and radiation, progressively shortened the length of telomeres, induces slowed cell proliferation and leads to eventual cell death (Marian, Cho et al. 2010, Barszczyk, Buczkowicz et al. 2014). More importantly, imetelstat treatment in mice bearing glioblastoma xenografts led to a significant inhibition of tumor growth, suggesting that telomerase antagonism may be a beneficial therapeutic strategy to explore in glioma (Marian, Cho et al. 2010). Another drug, MST-312 has been successfully shown to shorten telomerase end extension and induce cell cycle arrest in the brain tumor cells (Gurung, Lim et al. 2014).

2.4 Apoptosis

Arsenic trioxide (As₂O₃) is an inorganic compound that has been used for centuries in various medicinal applications. While its mechanism of action is not well understood, it is thought that it acts via the induction of

apoptosis (Emadi and Gore 2010). Recent studies reported that treatment with arsenic trioxide alone or with berberine or radiation reduces glioma cell growth and induces apoptosis or autophagy (Kim, Yoon et al. 2008, Lin, Kuo et al. 2008, Chiu, Ho et al. 2009). More recently, arsenic trioxide treatment has been shown to deplete the glioma BTSC subpopulation *in vitro* and *in vivo* by inducing apoptosis through the downregulation of Sox2 (Sun and Zhang 2011) and/or Notch1 and Hes1 (Zhen, Zhao et al. 2010). Furthermore, pretreatment with arsenic trioxide before ionizing radiation, temozolomide, and bevacizumab resulted in increased sensitivity to these treatments in glioblastoma multiforme BTSCs (Tomuleasa, Soritau et al. 2010). Arsenic trioxide also induces telomerase translocation from the nucleus of the cell to the cytoplasm, phosphorylates the telomeres and activates p53/p21 mediated apoptotic signaling pathways activation/ cell cycle arrests (Cheng, Li et al. 2016). These findings suggest that further study of arsenic trioxide in the treatment of brain tumors is warranted.

Furthermore, currently, the efficacy of drugs such as KIF20A inhibitor and pterostilbene are also being tested for potential glioma apoptotic inducers (Yu, Zhong et al. 2016, Saito, Ohta et al. 2017).

2.5 Nuclear Hormone Receptors

Nuclear hormone receptors are transcriptional factors known to regulate cell growth and differentiation. Constitutive androstane receptor (CAR) is an orphan nuclear hormone receptor that inhibits apoptosis and is critical for tumorigenesis (Qatanani and Moore 2005). Expression of CAR was found to be lower in BTSCs than in bulk glioma cells but increased *in vitro* by treatment with 6-(4-chlorophenyl)imidazo[2,1-b][1,3]thiazole-5-carbaldehyde O-(3,4-dichlorobenzyl)oxime (CITCO), a selective CAR agonist (Chakraborty, Kanakasabai et al. 2011). CITCO dose-dependently inhibited CD133+ BTSC growth and expansion via cell cycle arrest and apoptosis *in vitro* and also inhibited BTSC tumorigenicity in mice bearing BTSC xenografts (Chakraborty, Kanakasabai et al. 2011). These effects were not observed in normal astrocytes (Chakraborty, Kanakasabai et al. 2011), suggesting that CITCO should be further explored as a selective targeted agent against BTSCs.

Alternatively, treatment of the gliomas with Estrogen receptor β inhibitor: MF101, DPN and liquiritigenin and thyroid receptor inhibitor has been shown to induce decreased cell proliferation and prevent re-differentiation of the glioma cells (Alexandros, Iordanis et al. 2011, Sareddy, Nair et al. 2012).

2.6 Autophagy

Autophagy is a self-degradation process in which cytosomal components within a cell are removed via the lysosomal compartment. Although the goal of ionizing radiation is to induce apoptosis, irradiated glioma cells have been observed to undergo autophagy instead of apoptosis (Yao, Komata et al. 2003). Inactivation of the protein kinase mammalian target of rapamycin (mTOR) was found to induce autophagy in glioma cells (Takeuchi, Kondo et al. 2005). In a phase 1 trial, administration of the mTOR inhibitor rapamycin reduced tumor cell proliferation in association with mTOR inhibition in 7 of the 14 patients with PTEN-deficient recurrent glioblastoma (Cloughesy, Yoshimoto et al. 2008). Combined treatment with rapamycin and radiation increased autophagy in glioma BTSCs *in vitro* with subsequently decreased tumorigenicity in mice bearing BTSC transplants, and more importantly, limited tumor size and prolonged survival in xenografted mice with favorable safety (Zhuang, Li et al. 2011). mTOR inhibitors are currently in clinical testing for the treatment of glioma (U.S. National Institutes of Health, U.S. National Institutes of Health, U.S. National Institutes of Health, U.S. National Institutes of Health, Georger, Kieran et al. 2012, Yalon, Rood et al. 2012). Pretreatment with cilengitide, an orphan peptide inhibitor of integrins $\alpha\beta3$ and $\alpha\beta5$, increased autophagy and decreased cell self-renewal in glioma BTSCs and increased their sensitivity to γ -radiation, resulting in significantly shortened survival (Lomonaco, Finniss et al. 2011). A phase 2 study of cilengitide monotherapy showed modest efficacy in patients with recurrent glioblastoma (Gilbert, Kuhn et al. 2012). A pivotal phase 3 trial of cilengitide in combination with temozolomide and radiotherapy in patients with newly diagnosed glioblastoma is also currently being undertaken (U.S. National Institutes of Health).

3. Treatment Optimization

3.1 Chemotherapy

Although temozolomide is the standard chemotherapy for the treatment of glioma, drug resistance is common (Hegi, Diserens et al. 2005, Stupp, Mason et al. 2005). Topical findings have shown BTSCs interacting with astrocytes and up-regulate various survival genes, conferring the BTSCs chemoresistant (Kim, Kim et al. 2011). A recent study found that glioma cells expressing CD133 were more resistant to temozolomide than unsorted glioma cells and showed enhanced transcriptional activity of the Notch and Sonic hedgehog pathways post-treatment *in vitro* (Ulasov, Nandi et al. 2011). Inhibition of Notch and Sonic signaling by co-administration

of γ -secretase inhibitor-1 and cyclopamine with temozolomide resulted in significantly increased cell death in the CD133+ subpopulation *in vitro*, suggesting a potential additive benefit of combination therapy that should be further explored (Ulasov, Nandi et al. 2011).

The effect of temozolomide and other chemotherapeutic agents for BTSCs versus neural stem/progenitor cells (NSCs) was compared in a recent study (Gong, Schwartz et al. 2011). Temozolomide and cisplatin both demonstrated unfavorable cytotoxicity by being more effective at reducing the number of NSCs than BTSCs *in vitro* (Gong, Schwartz et al. 2011). In contrast, single-agent treatment with the newer chemotherapeutic agents bortezomib or erlotinib resulted in a greater reduction of BTSCs than NSCs (Gong, Schwartz et al. 2011). These findings emphasize the importance of drug selectivity in optimizing treatment regimens for patients.

Although 1,3-bis(2-chloroethyl)-1-nitrosourea (BCNU) is a commonly used chemotherapy for patients with glioblastoma multiforme, a small population of glioma cells containing BTSCs are resistant to BCNU (Kang and Kang 2007). These resistant glioma BTSCs have been reported to over-express a number of ion channel genes related to drug efflux (Kang and Kang 2007), especially chloride intracellular channel 1 (Kang and Kang 2008). Combined treatment with the chloride channel blocker 4,4'-diisothiocyanostilbene-2,2'-disulfonic acid (DIDS) and BCNU increased sensitivity to BCNU *ex vivo* and enhanced apoptosis in BCNU-resistant BTSCs *in vitro* (Kang and Kang 2008). These results suggest that blocking drug efflux mechanisms should be explored in *in vivo* models as a potential therapeutic strategy against BTSCs.

3.2 Radiation

The primary aim of radiation therapy is to induce G2/M cell cycle arrest and create radio-sensitivity of the BTSCs. Recent studies implemented Temozolomide and radiation along with MMP14 knockout to prevent GBM proliferation by cell cycle inhibition and prevent angiogenesis (Ulasov, Thaci et al. 2013). Cyclooxygenase-2 (COX-2), another enzyme that converts arachidonic acid to prostaglandins are highly expressed in glioma cells (Joki, Heese et al. 2000) and CD133+ medulloblastoma cells than CD133- cells, suggesting that BTSCs may differentially express COX-2 (Chen, Hsu et al. 2010). Treatment with celecoxib, a widely used selective COX-2 inhibitor, effectively inhibited colony formation and cell proliferation *in vitro* and significantly increased radiosensitivity in mice bearing medulloblastoma xenotransplants (Chen, Hsu et al. 2010). Another study found high expression levels of COX-2 in CD133+ but not CD133- glioblastoma cells and similar antitumor and radiosensitization effects of celecoxib on CD133+ glioblastoma cells *in vitro*, as well as statistically significant decreases in tumor growth and increases in mean survival rate in mice bearing glioblastoma xenografts (Ma, Chiou et al. 2011). These studies suggest that adding celecoxib to radiotherapy for the treatment of brain tumors may warrant clinical study. Prevention of DNA strand break (DSB) repair post radiation treatment by targeted disruption of DSB repair protein activity in the gliomas can be also used to treat the disease. Some of the protein specifically targeted for such therapeutics are the disruption of RAD51 (King, Brend et al. 2017), DNA-dependent protein kinase catalytic subunit (DNA-PKcs), ataxia-telangiectasia mutated (ATM) (Gil del Alcazar, Hardebeck et al. 2014) and EGFRvIII (Mukherjee, McEllin et al. 2009) protein.

4. Immunological Approaches for Targeting BTSCs

The invasive nature of GBMs and MBs often localizes the tumor niche beyond the blood brain barrier along the inner pockets of brain parenchyma and mesenchyma beyond the reach of therapeutic agents (Cheng, Wu et al. 2011). This is also complimented by a decreased cellular and humoral immunity *in vivo* and the immunotherapies against glioma have not proven clinically efficacious (Parney, Hao et al. 2000). With recent advances in B-cell and dendritic cell vaccines, activating the body's innate immune response against treatment of gliomas remains a promising therapeutic strategy. An open-label study in 12 patients with glioblastoma multiforme, the most aggressive form of primary brain tumor, showed that single-agent treatment with a tumor B-cell vaccine of verified BTSC origin, achieved more favorable safety and longer-lasting survival benefit than mixed leukocyte culture (MLC) alone or in combination (Moviglia, Carrizo et al. 2008).

Dendritic cell vaccines are also some of the most promising immunotherapy strategies for glioma (Liau, Black et al. 2000, Liu, Khong et al. 2003, Liau, Prins et al. 2005, Liu, Akasaki et al. 2005). A dendritic cell vaccine prepared using BTSCs expressing CD133+, nestin+ tumor-associated antigens (EGFR, HER2, TRP2, MRP3, AIM2, SOX2, IL13R α 2) (Xu, Liu et al. 2009) could induce a T-cell response and resulted in prolonged survival of rats bearing glioma BTSCs. A second dendritic cell vaccine prepared using sorted CD133+ tumor-associated antigens was shown to be significantly more cytotoxic *in vitro* than a dendritic cell vaccine prepared using unsorted tumor-associated antigens (Hua, Yao et al. 2011), suggesting that BTSCs are a promising antigen source for dendritic cell vaccinations. A phase 1 trial of dendritic cell vaccine loaded with BTSCs for progressive malignant brain tumor is ongoing (U.S. National Institutes of Health).

Adoptive T-cell transfer is another immunotherapy strategy that involves *ex vivo* preparation of tumor-specific T-cells and subsequent transfer into subjects. Creation of D-270 MG and D-245 MG xenografts which mimic glioma phenotypes, are a promising approach towards creating an invasive tumor model (Bigner, Humphrey et al. 1990). Administration of HER2-specific T-cells, stimulated with HER2+ glioblastoma cells eliminated both CD133+ and CD133- HER2+ glioblastoma BTSCs *in vitro* (Ahmed, Salsman et al. 2010). Additionally, treatment with HER2-specific T cells led to measurable tumor response and prolonged survival in mice bearing glioblastoma xenografts (Ahmed, Salsman et al. 2010). Also mutated Epidermal growth factor receptor, (EGFRvIII) expressing invasive gliomas cells has been shown to be selectively targeted by T-cells expressing chimeric antigen receptors (CARs) (Miao, Choi et al. 2014). This treatment strategy warrants clinical exploration as a reproducible alternative to tumor cell or dendritic cell vaccines, which are limited by their inability to reproducibly produce tumor-specific T-cells (Fine 2004).

5. Oncolytic Viral Approaches for Targeting BTSCs

Oncolytic viruses preferentially infect cancer cells and can be used as a drug delivery system across the blood-brain barrier. Adenovirus 16p and chimpanzee adenovirus CV23 have been shown to infect both CD133+ and CD133- primary glioma cells (Skog, Edlund et al. 2007). Treatment with adenovirus Delta-24-Arg-Gly-Asp (RGD) (Jiang, Gomez-Manzano et al. 2007), the naturally occurring picornavirus Seneca Valley virus-001 (Yu, Baxter et al. 2011), or herpes simplex virus (HSV) vector G47 Δ (Wakimoto, Kesari et al. 2009) effectively killed BTSCs and significantly improved survival in mice bearing BTSC xenografts. Combining HSV vector G47 Δ with temozolomide increased sensitivity to temozolomide in killing BTSCs while sparing neurons and induced long-term remission in 4 of 8 treated xenograft-bearing mice (Kanai, Rabkin et al. 2012). Lymphocytic choriomeningitis virus glycoprotein and vesicular stomatitis virus glycoprotein-pseudotyped lentiviral vectors were both able to efficiently infect glioblastoma BTSCs and carry the suicide gene HSV thymidine kinase fused to eGFP to mediate complete radiologic remission and significant survival benefit in rats bearing glioblastoma BTSC xenografts (Huszthy, Giroglou et al. 2009). These studies suggest the therapeutic potential of oncolytic viruses against BTSCs; indeed, Delta-24-RGD is currently in phase 1 clinical testing for the treatment of patients with recurrent glioma (U.S. National Institutes of Health). Myxoma virus (MYXV), in combination with rapamycin has been successfully shown to promote anti-tumor activity of BTSCs in mice infected with glioblastoma multiformed and reduce CD133 expressing stem cells (Zemp, Lun et al. 2013).

6. Post-translational

MicroRNAs are a class of short noncoding RNAs that act as post-transcriptional regulators. MicroRNAs have been shown to promote, as well as inhibit BTSC manifestation (Teplyuk, Mollenhauer et al. 2012, Lee, Finnis et al. 2013, Tominaga, Kosaka et al. 2015). BTSCs have been shown to induce microRNAs (miR-181c) to evade the blood brain barrier by modulating their actin dynamics and initiate migration (Tominaga, Kosaka et al. 2015). Meanwhile, transfection of miR-124 or microR-137 promoted differentiation in CD133+ glioblastoma multiforme BTSCs and reduced glioblastoma multiforme cell proliferation *in vitro* (Silber, Lim et al. 2008). miR-124 induced down regulation of Nur77 has been shown to reduce glioma cell viability, proliferation and invasiveness (Tenga, Beard et al. 2016). miR-326 transfection has been shown to be cytotoxic to both normal glioma cells and glioma BTSCs, thus, inhibiting glioma tumorigenicity in mice bearing xenografts (Kefas, Comeau et al. 2009). Expression of miR-34a has been shown to initiate glioma BTSC differentiation and reduce glioma xenograft growth (Guessous, Zhang et al. 2010). Similarly, miR-130b has been shown as a prognostic marker for glioma cell migration and invasiveness (Sheng, Chen et al. 2015). miR-497 upregulation by glioma cells confers resistance to the cells to the temozolomide treatment (Zhu, Tu et al. 2017). Lastly, miR-9 was found to inhibit mesenchymal differentiation in glioma BTSCs (Kim, Huang et al. 2011). Together these findings suggest that microRNAs may play multiple regulatory roles for BTSCs and warrant further exploration. Recent studies focus on microRNA profiling of cerebrospinal fluid of patients with GBM. qRT-PCR data makes an effort to classify metastasis and remission stages of GBM by profiling miR-10b, miR-21, miR-141, miR-200a, miR-200b, miR-200c, miR-15b and miR-125b as potential biomarkers of BTSCs (Teplyuk, Mollenhauer et al. 2012).

7. Conclusion

The field of BTSC research has grown exponentially in the past decade. Much of the initial research was focused on isolating BTSCs in different types of brain tumors or on adding to our understanding of BTSC biology. These early foundational studies have enabled a number of more recent studies to shift their focus toward translational medicine. The specific therapeutic strategies summarized in this review suggest myriad possible modalities for targeting BTSCs. Some strategies aim to block the maintenance of stem-cell-like properties of BTSCs by

inducing differentiation or disrupting the CSC niche, while other strategies follow the traditional approach of inducing cell death via apoptosis, autophagy or radiochemotherapy. Provoking a tumor-specific immune response using tumor-associated antigens remains a promising therapeutic strategy for targeting both bulk tumor cells and BTSCs. Lastly, the relatively recent discoveries that oncolytic viruses and microRNAs may prove useful as targeted agents or as delivery systems for other targeted agents against BTSCs is encouraging. While these findings are at early research stages, the challenge is to translate these approaches to clinical developments.

It has been shown that serum-derived cell lines of malignant brain tumors are poor genotypic and phenotypic representatives of human brain tumors (Lee, Kotliarova et al. 2006). Accordingly, studies conducted using BTSCs derived from established cancer cell lines should be repeated using freshly dissociated tumor samples, freshly derived tumor cell lines grown in serum-free culture, or *in vivo* rodent models to confirm their findings. Another major challenge to the proposed therapeutic strategies is the requirement that they should selectively target BTSCs while sparing neural stem or progenitor cells. Considering the ongoing challenge faced by traditional cytotoxic agents to target cancer cells while sparing normal tissue cells, this requirement may be equally challenging with the BTSCs. Moreover, recent reports have questioned the value of the most frequently cited potential BTSC marker, CD133, given the findings of CD133⁻ BTSC populations (Beier, Hau et al. 2007, Joo, Kim et al. 2008, Ogden, Waziri et al. 2008, Yao, Wang et al. 2008, Clement, Dutoit et al. 2009, Chen, Nishimura et al. 2010, Schittenhelm, Simon et al. 2011) and variable CD133 expression depending on tumor grade (Rebetz, Tian et al. 2008), environmental conditions (i.e., hypoxia and mitochondrial dysfunction) (Griguer, Oliva et al. 2008), location (i.e., single BTSC versus BTSCs residing in niches) (Christensen, Schroder et al. 2011), and experimental assay techniques (Hermansen, Christensen et al. 2011). Whether this variability of expression extends to other putative BTSC markers will determine the clinical utility of therapeutic strategies targeting single BTSC markers, such as the aforementioned GCST peptide that selectively recognizes nestin⁺ BTSCs. Finally, while a few of the aforementioned targeted agents are currently in clinical testing, ultimately only the demonstration of safety and efficacy in pivotal clinical trials will provide definitive evidence of the translational relevance of these agents and help to inform the future direction of therapeutic strategies.

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