Emerging Strategies for the Treatment of Tumor Stem Cells in Central Nervous System Malignancies

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Abstract High-grade central nervous system (CNS) tumors are notorious for high rates of recurrence and poor outcomes. A small cohort of tumor cells, dubbed tumor stem cells (TSC), are now being recognized as an important subset of the tumor that is resistant to chemotherapy and radiotherapy and account for the high recurrence rates. Recent research is developing modalities to target TSCs specifically in a bid to improve the response of the tumor as a whole. The methods being employed to target TSCs include targeting TSC-specific pathways or receptors, TSC-sensitizing agents to chemotherapy and radiotherapy, immunotherapy, TSC-differentiating agents, and viral therapy. This chapter provides an overview of strategies that are expected to help develop new and more effective treatments for CNS tumors.

Keywords Glioma stem cells • Tumor stem cells • Cancer stem cells • Chemotherapy sensitization • Radiotherapy sensitization • Immunotherapy • Differentiation agents • Virotherapy • Gene therapy

Introduction

Central nervous system (CNS) tumors are notorious for including some of the most lethal tumors in humans. The most common intrinsic brain tumor, the glioblastoma multiforme (GBM), carries a uniformly poor prognosis with most patients not surviving up till 2 years after diagnosis. The standard management strategy for patients with GBM is based on the protocol described by Stupp and

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colleagues: specifically, maximal safe surgical excision followed by radiotherapy and temozolomide (TMZ) chemotherapy [1]. Unfortunately, in spite of these aggressive measures, recurrence almost always occurs. This therapeutic regimen has only been able to increase the median survival for GBM from 12.1 months to the current 14.6 months [1]. The current prognosis of the disease stresses the importance of developing novel treatment strategies and therapeutics targeting tumor stem cell (TSC) populations have recently received notable attention in this regard.

The TSC hypothesis is based upon the presence of a small subset of tumor cells with properties akin to stem cells. According to this premise, TSCs sit at the apex of all tumor cells and exhibit properties of multi-lineage capacity and self-renewal [2]. While self-renewal maintains the population of the TSCs, the process of differentiation produces downstream tumor progenitor cells that generate the genetically diverse progeny of the tumor mass.

An important property of TSCs is the ability to initiate tumors when xenografted in nude mice. The xenograft initiation efficiency is significantly higher than implantation of traditional GBM cell lines [3, 4]. Additionally, TSCs are generally more resistant to conventional cytotoxic therapy, leading to tumor repopulation via differentiation of unaffected TSCs after cytotoxic therapy. Therefore, TSCs are thought to be a major factor driving recurrence and therapeutic resistance in gliomas (Fig. 1).

Challenges with Current Treatment Strategies

Current therapeutic strategies advocate a uniform regimen for patients with CNS tumors. For chemotherapy in GBM, TMZ is considered an essential part of the treatment approach. TMZ causes cytotoxicity against GBM by the creation of O6-methylguanine (O6MeG) lesions—leading to DNA fragmentation and disruption of DNA replication. The resulting effects include tumor suppression and tumor cell apoptotic cell death [5].

While the addition of TMZ to the chemotherapy protocol is only able to improve the median survival to 14.6 months, Heigi and colleagues reported a specific patient cohort of long-term GBM survivors with a median survival of 21.7 months [6]. Further investigation of their cohort revealed an absence of tumor methylguanine-DNA methyltransferase (MGMT) expression in their patients [6]. By removing the methyl groups added on by TMZ, MGMT prevents tumor cell death. However, methylation of its promoter leads to absent or reduced expression of the MGMT and increases the cytotoxic efficacy of TMZ. The overall effect is that of increased tumor cell death, translating into improved patient survival.

The effect of MGMT status on the response to treatment points towards the importance of understanding the differences within the tumor cell cohort that

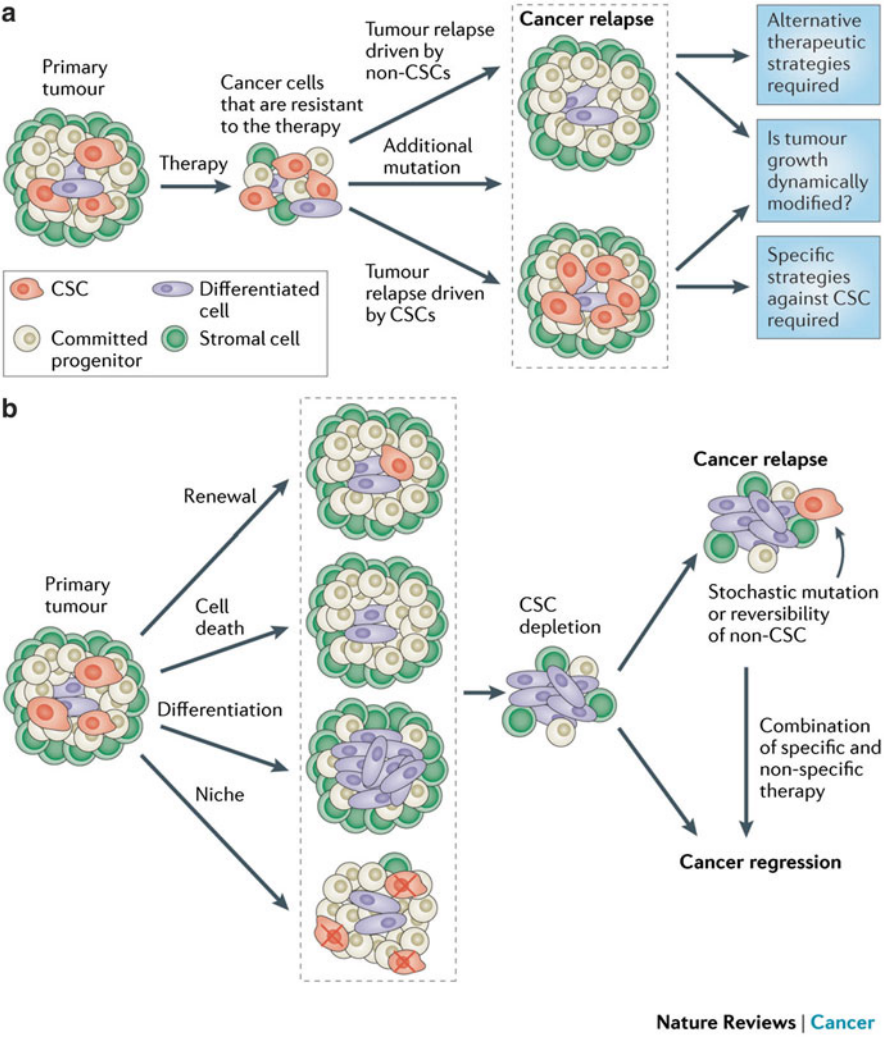

Fig. 1 Implication of cancer stem cells (CSCs) in cancer therapies and tumor relapse. (**a**) Anticancer therapies may not kill all tumor cells equally. CSCs that sustain tumor growth or another population of more slowly cycling tumor cells may be responsible for tumor resistance to therapies and tumor relapse. Depending on the population responsible for tumor relapse, new strategies should be designed to eradicate all tumor cells. (**b**) The CSC model suggests that inhibiting CSC renewal or promoting their differentiation should induce tumor regression. Drugs could impair CSC self-renewal, induce their specific cell death, induce their differentiation, or target their niche. All of these strategies would lead to the depletion of the pool of CSCs and subsequent tumor regression. However, if the CSC potential is reversible, or if newly acquired mutations confer resistance to therapy, then tumor regression would only be transient, leading to cancer relapse (reprinted with permission from Beck B, Blanpain C, Nat Rev Cancer. 2013 Oct;13(10):727–38. Unraveling cancer stem cell potential)

dictates the ultimate response to treatment. For treatment purposes the TSC fraction is increasingly being recognized as an important, and in some ways fundamentally different, part of the tumor. Liu and colleagues reported that CD133+ cells depicted a multifold higher activity of MGMT compared to CD133− cells, which translates into improved DNA repair and increased resistance to TMZ [7, 8]. Another reason for the increased resistance to TMZ may be the downregulation of autophagy-related proteins in the TSCs [9]. TSCs have also shown to possess stronger drug resistance to other conventional anticancer drugs, such as doxorubicin (Dox), etoposide (VP-16), carboplatin, and BCNU due to an enhanced expression of multidrug resistance (MDR) 1 [10]. Thus, increasing evidence points towards the relatively refractory nature of TSCs to conventional chemotherapy.

While Beier and colleagues were able to show that TMZ induced a dose- and time-dependent decline of brain TSCs in a cell culture study, TMZ needed clinically unreachable levels to be effective [11]. Glioma TSCs also show an upregulation of mRNAs of FAS-associating death domain (FADD)-like antiapoptotic molecule (FLIP), B-cell CLL/lymphoma 2 (Bcl-2), Bcl-X, and some inhibitor of apoptosis (IAP) family members [12–14]. Other factors that confer a protective advantage to TSCs include a higher expression of breakpoint cluster region pseudogene 1 (BCRP1; drug-resistant gene) and antiapoptosis proteins and inhibitors [7].

The fraction of tumor cells expressing CD133 is also known to be enriched after radiation in gliomas [15]. CD133-expressing glioma cells survive ionizing radiation in increased proportions relative to most other tumor cells. This is because TSCs preferentially activate the DNA damage checkpoint in response to radiation, and repair radiation-induced DNA damage more effectively than CD133-negative tumor cells. With exposure to conventional radiation, CD133+ cells exhibit enhanced activation of three key mediators of cell cycle check points: Rad17, Chk1, and Chk2 [16, 17]. Interestingly, if administered specific inhibitors of the Chk1 and Chk2 checkpoint kinases TSCs become more radiosensitive, akin to CD133− tumor cells [16].

Due to their inherent resistant nature, TSCs are worthwhile targets for the development of specific treatment modalities to improve the overall response of tumors to treatment [18]. Targeting a specific molecular protein signal pathway of TSCs with a therapeutic target is one of the ways investigators are aiming to eradicate these cells. Other strategies include virotherapy, increasing TSC chemosensitivity and radiosensitivity by using hypersensitivity agents [19, 20], immunotherapy using autologous dendritic cells, and using differentiation agents in a bid to promote differentiation of TSCs [21]. Improving knowledge of the unique characteristics of TSCs is driving the development of TSC-specific therapeutics. Based on the suggested pivotal role of TSCs in the origin, development, and maintenance of tumors, future therapies will aim to effectively eradicate them to improve the response rates in tumors and decrease recurrences. We will now review some of the basic strategies being employed to target TSCs that are expected to help engineer more effective treatment strategies in the future.

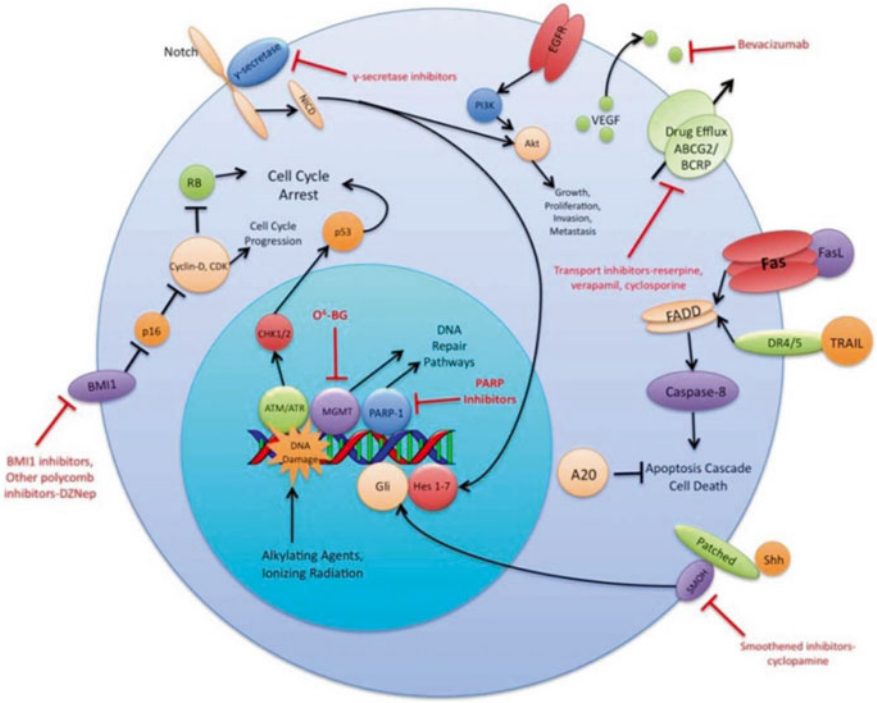

Fig. 2 Mediators of TSC treatment resistance. Depicted are the various treatment resistance mechanisms and pathways differentially expressed or regulated in TSC versus their differentiated cell counterparts. *Blocked red lines* indicate ways to inhibit or block these mediators (from Schmalz PG1, Shen MJ, Park JK. Cancers (Basel). 2011 Feb 10;3(1):621–35. Treatment resistance mechanisms of malignant glioma tumor stem cells (open access))

Targeting TSC-Specific Pathways and Receptors

One of the major methods to target TSCs is to identify pathways and/or receptors that are specific for TSCs (Fig. 2). These pathways can then be exploited to decrease the number of TSCs while combining with conventional therapeutics will treat the overall tumor mass. Some of the major targets of interest are summarized below.

Notch ligands, receptors, and targets have been found in a wide range of neoplasms, including, but not limited to, lung, breast, cervix, renal, pancreas, medulloblastoma (MB), and GBM [22–31]. Additionally, in many of these tumors increased Notch activity has been shown to promote tumor growth, with studies showing that Notch pathway blockade inhibits proliferation of tumor cells. In the CNS, Notch signaling pathway regulates neural stem cells (NSCs). Studies have also demonstrated higher Notch activity in CNS TSCs [32].

The Notch pathway blockade by gamma-secretase inhibitors is another important pathway that depletes glioma TSCs through reduced proliferation and increased apoptosis associated with decreased AKT and STAT3 phosphorylation [33]. Using a three-dimensional organotypic explant system of surgical GBM specimens, Hovinga and colleagues inhibited Notch signaling and reported not only decreased proliferation and self-renewal of tumor cells, but also a decrease in endothelial cells [25]. These findings suggest that the Notch pathway plays a critical role in linking angiogenesis and TSC renewal. A more recent study suggested that the brain microvascular endothelial cells are the source of Notch ligands that lead to TSC sustenance and renewal [31]. A Notch signaling pathway inhibitor RO4929097 is currently being evaluated in clinical trials for recurrent and progressive GBMs (NCT01122901).

The hedgehog (Hh) pathway is another significant pathway that plays an essential role in development of the cerebellum [34, 35]. MB, a primitive neuroectodermal tumor, is thought to arise from immature neural progenitors in the cerebellum [36]. Additionally, Michael and colleagues showed that genomic alterations in components of the Hh signaling pathway were present up to 25 % of human MBs [37]. Additional work using knockdown experiments of Bmi1 demonstrated that Hh signaling drives Bmi1 expression, which is a key TSC regulatory gene implicated in the pathogenesis of MB [38].

The Hh pathway is similarly important in the pathogenesis of gliomas. Gli, a component of the Hh signaling pathway, is amplified in gliomas [39]. Bar et al. reported that cyclopamine blocks the Hh pathway causing a depletion of TSC in GBM [40]. Likewise, Clement and colleagues reported that interference of Hh-Gli signaling with cyclopamine or through lentiviral mediated silencing resulted in decreased self-renewal and tumorigenicity of TSCs [41]. SANT-1 inhibition of Hh has also been shown to reduce proliferation of glioma TSCs [42].

Glioma TSCs have also shown a positive correlation with microvessel density and have multiple regulatory roles in endothelial cells [43]. They are thought to enhance the migration and proliferation of the endothelial cells by secretion of sonic hedgehog (Shh), leading to activation of the Hh pathway of the endothelial cells [44]. Consequently, GDC-0449 or vismodegib (a small-molecule antagonist of the Hh pathway) has recently garnered interest and is being tested in a clinical trial for recurrent GBM (NCT00980343) [45]. A case report of a patient with refractory metastatic MB managed with GDC-0449 has also been reported. This treatment resulted in rapid (although transient) regression of the tumor and reduction of symptoms [46]. GDC-0449 is also being evaluated in clinical trials for recurrent and recalcitrant MB (NCT00939484 and NCT00822458).

Bao and colleagues were the first to report the intimate relationship between glioma TSCs and the microvasculature. They reported that CD133+ cells produced high levels of VEGF that induced endothelial cell migration. Conversely, treatment of CD133+ GBM cells with bevacizumab blocked the tumor cells' ability to induce endothelial cell migration and initiate tumors in vivo [47]. Similarly, Calabrese et al. demonstrated that treatment of GBM with bevacizumab depleted tumor blood vessels and caused a significant reduction in the number of GBM TSCs [48]. Due to the efficacy of antiangiogenic agents in preclinical studies, they have been tested in clinical trials. Unfortunately,

bevacizumab has failed to show improvement in the overall survival of patients with newly diagnosed GBM [49]. Similarly, cediranib did not prolong progression-free survival in patients with recurrent GBM, either as monotherapy or in combination with lomustine, compared to patients who were treated with lomustine alone [50].

Amplification and/or mutation of receptor tyrosine kinases, such as epidermal growth factor receptor (EGFR), is another common genetic alteration in GBM [51, 52]. Recent studies have demonstrated the presence of a constitutively active EGFR mutant (EGFRvIII) associated with glioma TSCs. This pathway potentiates tumor growth and heterogeneity through IL-6-mediated Notch signaling [53, 54], and Src family kinase (SFK)-dependent phosphorylation of Dock180 [55, 56]. Clinical trails investigating the efficacy of EGFR inhibitors however have yielded disappointing results [57–59].

Aberrant Wnt signaling is molecularly linked to many human cancers, including colorectal, breast, ovarian, and hepatocellular carcinoma, neuroectodermal tumors, and glioma [60–63]. Dysregulation of the Wnt-pathway has also been documented in glioma TSCs [64, 65]. Investigators have also identified a role for this pathway in MBs [66, 67]. Other similar targets currently being investigated to treat CNS TSCs include the homeobox (HOx) family [7], phosphatase and tensin (PTEN) [68], telomerase [69], efflux transporters [70, 71], and microRNA [72–74].

Chemotherapy and Radiotherapy Sensitizers

Increased resistance to chemotherapy is a great challenge when treating TSCs. However investigators have reported several ways to potentiate the cytotoxicity of chemotherapeutic agents. These include cell-cycle checkpoint abrogation [75, 76], depletion in the expression of antiapoptosis proteins [77], and DNA repair enzymes [78].

A molecular chaperone, 90-kDa heat-shock protein (hsp90), has recently been described as a chemotherapy sensitizer because it is expressed at 2–10-fold higher levels in tumors compared to normal tissues [79]. Ohba and colleagues reported that inhibition of hsp90 potentiated the cytotoxicity of chemotherapeutic agents in human glioma cell lines [80]. On the other hand, Sauvageot and colleagues reported that while 17-AAG (inhibitor of hsp90) inhibited the growth of glioma cells and although it has a synergistic effect with radiation, it was not found to synergize with TMZ [81].

GPI 15427, a novel poly(ADP-ribose) polymerase-1 (PARP-1) inhibitor, significantly increases the life span of its tumor-bearing mice when it is administered systemically shortly before TMZ [82]. The same group later used the oral route to administer GPI 15427 and found it to be efficacious as a chemosensitizer as well [83, 84].

More recently, the effect of secreted frizzled-related protein 4 (sFRP4), a Wnt signaling antagonist, in chemosensitizing glioma TSCs was examined. The results indicated that sFRP4 was able to significantly sensitize glioma TSCs to doxorubicin or cisplatin [85]. Similarly, another study used proteasome inhibitor bortezomib and

revealed that combination therapies based on bortezomib and bevacizumab offered an increased benefit when the two agents are used in combination [86]. Xu et al. targeted CD44, which is upregulated in GBM, and reported that its depletion impeded the growth of GBM and sensitized the tumor cells to cytotoxic drugs in vivo [87]. Tyrosine kinase inhibitors have also been experimented for sensitization of the tumor. Wachsberger and colleagues used cediranib, a potent receptor tyrosine kinase inhibitor that inhibits all three VEGF receptors. They reported that while cediranib did not radiosensitize the glioma cells, it did enhance the effectiveness of TMZ [88].

1,3-Bis(2-chloroethyl)-1-nitrosourea (BCNU) is one of the most commonly used chemotherapeutic agents in the treatment of GBM but it often fails to eradicate TSCs. Research has uncovered an overexpression of multiple ion channel genes that are related to drug efflux. However when a chloride channel blocker, 4,4′-diisothiocyanostilbene-2,2′-disulfonic acid, is used in combination with BCNU, the effect of BCNU is seen to synergistically increase [89].

Some investigators have aimed to disrupt the TSC niche by administering antiangiogenic agents with the intention of disrupting the stemness of the tumor cells. By losing the stem-cell characteristics the tumor cells may become more sensitized to chemotherapy. This technique has been used by researchers to show that combined antiangiogenic and cytotoxic drugs can result in a significant reduction in the number of glioma TSCs [90].

There has also been a concentrated effort to understand the biology of TSC radioresistance and develop approaches to sensitize the tumor cells to ionizing radiation. As TGF-β is a modifier of radiation responses, TGF-β receptor (TGFβR) I kinase inhibitor (LY2109761) has been used in combination with radiotherapy as an approach to increase the radiosensitivity of glioma cell lines including in TSCs [91]. Similarly, LY364947, another small-molecule inhibitor of TGF-β type I receptor kinase, was used by investigators to show improved tumor response when it was administered prior to radiotherapy [92]. A TGF-β inhibitor, LY2157299, alongside TMZ-based treatment regimen is also being evaluated in an ongoing clinical trial (NCT01220271).

EGFR activation has also been implicated in the radioresistance of many cancers, including brain tumors. Combining EGFR targeting with radiotherapy is an appealing option to increase the cytotoxic effect of radiation. To test this strategy, Geoerger et al. used gefitinib (tyrosine kinase inhibitor) in two xenograft models: an EGFR-amplified glioma and an EGFR-expressing ependymoma. For both the models, there was a positive trend towards superior antitumor activity when combined therapy was administered (gefitinib + radiation) [93].

Kang and colleagues further investigated the effect of gefitinib in glioma TSCs and found that it enhanced radiosensitivity of TSCs by reducing EGFR-Akt activation and DNA-PKcs expression. This was accompanied by enhanced irradiation-induced DNA double-strand breaks and inhibition of its repair [94]. Likewise,

another group investigated the efficacy of ZD1839 (Iressa), a selective EGFR tyrosine kinase inhibitor, on the radiation sensitivity of the U251 GBM cell line. In their radiation survival experiments, ZD1839 had a significant radiosensitizing effect and increased tumor cell death [95]. In the clinical domain, a phase 1/2 study of radiation therapy with concurrent gefitinib for newly diagnosed GBM showed good tolerance of the drug but no benefit in survival [96]. Other tyrosine kinase inhibitors investigated as radiosensitizers for GBM include erlotinib [57] and vandetanib [97].

Signal transducer and activator of transcription (STAT) 3 is a member of a family of DNA-binding molecules, and the aberrant activity of the JAK2/STAT3 pathway is associated with glioma TSCs. Inhibition of this pathway leads to decreased proliferation of glioma TSCs [98, 99]. Yang and colleagues reported that resveratrol (inhibitor of the STAT3 axis) therapy significantly improved the survival rate in their xenotransplant model in part by synergistically enhancing the radiosensitivity of radiation-treated GBM TSCs [100].

STAT3 pathway also plays a key role in mediating CSC properties in MB-derived CD133(+) cells [101]. Celecoxib is a selective COX-2 inhibitor and has been shown to potentially reduce STAT3 phosphorylation [102, 103]. Incubation of MB TSCs with celecoxib has shown to dose-dependently suppress the TSC properties of the tumor cells and enhance the radiotherapy effect on the induction of apoptosis [104]. Similarly, inhibition of phosphorylated STAT3 by cucurbitacin I has also demonstrated enhancement of the chemoradiosensitivity of MB TSCs [101].

Valproic acid (VPA) is a commonly prescribed antiepileptic drug used for the management of seizures in brain tumor patients. Besides its antiseizure property, VPA is an effective inhibitor of histone deacetylase and is involved in modulating chromatin structure and gene expression [105–107]. Interaction between VPA and TMZ has been studied to depict enhanced cytotoxicity in TMZ-sensitive cell line (D384) and the TMZ-resistant cell line (T98). The enhancement of TMZ-induced apoptosis is associated with increased reactive oxygen species production and glutathione depletion. Pretreatment with *N*-acetylcysteine can partially recover the apoptotic effect of the TMZ/VPA combination treatment [108]. Furthermore, the combination of VPA and TMZ also causes significant radiation enhancement in the glioma cell lines [109].

Another approach to make glioma TSCs more radiosensitive is to inhibit the DNA damage responses (DDR) that follow radiotherapy [110]. A dual phosphoinositide 3-kinase/mTOR inhibitor NVP-BEZ235 can potently inhibit two central DDR kinases, DNA-dependent protein kinase catalytic subunit (DNA-PKcs) and ataxia-telangiectasia mutated (ATM), and has been shown to potentiate the damage caused by ionizing radiation in glioma cells [111, 112]. The recognition of various pathways and receptors that can be modulated to increase the chemoradiosensitivity of CNS TSCs is an area of intense research that promises to identify specific clinical targets that may be exploited.

Immunotherapy

GBMs secrete multiple immunosuppressive factors, including transforming growth factor-β (TGF-β) and prostaglandin E2 (PGE2), which lead to a profound immunosuppressive effect both locally and systemically [113, 114]. TGF-β expands the pool of immunosuppressive regulatory T cells, resulting in suppression of T cell proliferation. Additionally, TGF-β and PGE2 downregulate the expression of major histocompatibility complex (MHC) class II, as well as the antigen processing of dendritic cells (DCs) [115]. Disruption of the immunosuppressive environment represents a promising immunological target to treat tumor cells.

Glioma cells also express certain antigens that are not expressed elsewhere in the brain. These antigens can be recognized by T cells, which can play an important role in tumor rejection. Some of the major glioma antigens include MAGE-1 [116], SOX6 [117], gp100, TRP-2 [118, 119], EGFRvIII [120], L13Ra2 [121], HER-2 [118], WT1 [122], SART-3 [123], and SOX11 [124, 125]. In general, immunotherapies consist of antibody-mediated immunotherapy, active immunotherapy that induces antitumor immunity in patients via a cancer vaccine, and adoptive or passive immunotherapy whereby tumor antigen-activated T cells are prepared ex vivo and administered to patients [114].

Tenascin is a well-known antigen associated with glioma and is an extracellular matrix molecule that is prominently expressed in the fibrillary matrix and perivascular patterns of gliomas [126, 127]. Multiple monoclonal antibodies (mAb) specific for human tenascin have also been generated [128, 129]. As EGFR is highly expressed by glioma cells, a chimeric mAb (cetuximab) has also been used in clinical trials but showed disappointing results [130]. More recently, a chimeric form of mAb ch806 administered to a patient with anaplastic astrocytoma showed good localization of the mAb at the tumor [131]. Various clinical trials have also studied the efficacy of various mAb to EGFR [114].

HER2-specific T cells against CD133+ cells generated by transduction with a retroviral vector encoding a HER2-specific chimeric antigen receptor have been used by investigators to show sustained regression of autologous GBM xenografts [132]. The same group also reported regression of experimental MB following transfer of HER2-specific T cells [133]. Similarly, IL-13 receptor alpha2 (IL13Ralpha2) is a glioma-restricted cell-surface epitope that is not otherwise detected within the CNS. Numerous preclinical studies have demonstrated the ability of L13-zetakine-redirected T cells to cause regression of GBM and GBM TSCs, as well as MB TSCs [134–137].

Dendritic cells (DCs) are the most potent antigen-presenting cells and have the ability to prime naïve T cells. A variety of tumor-associated antigens (specific tumor-associated peptides, tumor RNA and cDNA, tumor cell lysate, or apoptotic tumor cells) have been tested in numerous studies [138, 139]. Initial clinical trials using DC vaccines have shown to have strong systemic and intracranial T cell response and robust infiltration with T cells along with positive clinical outcomes [140–142]. Some studies have also suggested that eliminating the regulatory T cells would lead to improved anti-glioma immunity [143, 144].

TSC Differentiation

Due to their nature, TSCs play an important role in the tumorigenicity and maintenance of CNS tumors. While other agents aim to decrease the number of TSCs via specific targeting, differentiation agents aim to preferentially route the TSC into differentiating into progenitor cells. The strategy helps in decreasing the number of TSCs and gives rise to downstream tumor stem cells that are much more likely to be vulnerable to established therapeutics.

One of the first agents to be used as a differentiating agent for GBM TSCs was bone morphogenetic protein (BMP) 4 [145]. BMPs have an instructive role in the adult brain stem cell niche and favor the acquisition of an astroglial fate [146, 147]. Piccirillo and colleagues demonstrated that BMPs trigger the Smad signaling cascade in GBM cells. This was followed by a decrease in the size of CD133+ population and a decrease in their clonogenic ability [145].

A closer look at the oncogene BMI1 that regulates gene expression by modifying chromatin organization demonstrated that BMI1 was highly expressed in CD133+ cells. Knockdown of this gene using short hairpin RNA-expressing lentiviruses resulted in the inhibition of clonogenic potential in vitro and of brain tumor formation in vivo [148]. More recent research has shown the importance of BMI1 to self-renewal in CD133+ populations as well [149].

Metformin, a first-line drug for type II diabetes, was recently reported to possess anticancer properties affecting the survival TSCs in breast cancer models [150–152]. Würth and colleagues investigated the effect of metformin on glioma cells and reported a TSC-specific inhibition of Akt-dependent cell survival pathway that affected the self-renewal mechanisms [153]. Clinical trials using metformin for treatment of GBM are being conducted in the light of these promising results (NCT02149459 and NCT01430351).

Induction of autophagy has also shown to promote differentiation in glioma TSCs. Drugs such as rapamycin [154] and curcumin [155] trigger the differentiation cascade in TSCs by activating autophagy. Other differentiating targets include girdin, an actin-binding protein [156], and the vanilloid-2 cation channel [157]. Cannabinoids and sorafenib have also been documented to induce glioma TSC differentiation and deplete GBM TSCs [158].

Virotherapy and Gene Therapy

Among the emerging therapeutic options for CNS TSCs, virotherapy has shown noteworthy promise in terms of targeting glioma TSCs [159] (Fig. 3). Fueyo and colleagues constructed a tumor-selective adenovirus (Delta24) that carried a 24-bp deletion in the E1A region responsible for binding Rb protein. In vivo and in vitro results from their study demonstrated a potent lytic effect of glioma cells [160]. Later another group used a second-generation Delta24 (Delta24-hyCD) and

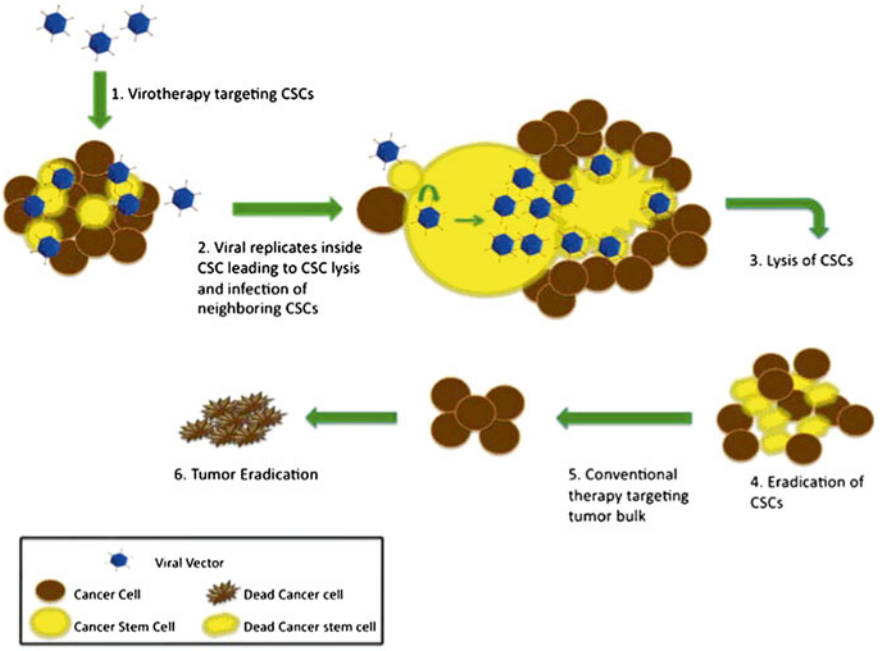

Fig. 3 Stem cell-targeted virotherapy. Adenoviral vectors are genetically modified to recognize and multiply only in cancer stem cells (CSCs). Viral replication in CSCs leads to destruction of CSCs and release of viral progeny, which in turn further infect neighboring stem cells. Repetition of this cycle leads to eradication of CSCs. Thus targeted therapy in addition to conventional therapy can lead to eradication of the tumor (reprinted with permission from Dey M et al. Stem Cell Rev. 2011 Mar;7(1):119–29. Cancer stem cells: the final frontier for glioma virotherapy)

exhibited significant chemosensitization and significant glioma control when 5-fluorocytosine was coupled with Delta24-hyCD [161].

In another study, a combination of adenoviral virotherapy and TMZ chemotherapy demonstrated a significant overexpression of autophagy markers, acidic vesicular organelles, and light-chain 3 (LC3) in vitro. In vivo studies showed significantly higher survival with combination therapy [162].

Gene silencing techniques can also be used to better understand the role of certain genes in the biology of TSCs and identify viable therapeutic targets. Bao and colleagues investigated the role of a neuronal cell adhesion molecule, L1CAM, in glioma TSCs using lentiviral mediated shRNA interference. They reported disrupted neurosphere formation, induced apoptosis, and inhibited growth of glioma TSCs [163]. Similarly, Wang and colleagues interrogated the significance of c-Myc expression in glioma TSCs using shRNA interference and showed that decreased expression of the target decreased proliferation and survival of TSCs [164].

Conclusion

Promising results from preclinical research using TSC-directed therapy have led to hopes for significant improvement in outcomes with high-grade CNS tumors. In this regard, the combination of conventional surgery, chemotherapy, and radiotherapy with TSC-targeted therapy may provide a new treatment approach to improve the response of CNS tumors. The potential efficacy of these therapeutic measures is being tested in various clinical trials and may direct future therapeutic interventions for CNS malignancies.

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