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Therapeutic Targeting of Malignant Glioma

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Abstract: Glioblastoma Multiforme (GMB) is the most aggressive primary brain tumor with poor survival rates and universal recurrence despite aggressive treatments. Recent research suggested that GBM has multiple glioma cell populations, some of which are organized in a stem cell hierarchical order with different stages of differentiation. Evidence indicated that recurrence is due to a development or persistence of a subpopulation of these tumor cells which are inherently resistant to treatment and these were defined as the glioma stem-like cells (GSC). It is hypothesized that GSC become highly malignant by accumulating mutations in oncogenic pathways. These cells present with specific surface markers which helps identify them. Targeting the surface markers as well as the signaling pathways of GSCs has been an ongoing research effort. This review focuses on summarizing the current treatment modalities used to glioblastoma treatments, evaluating their efficacy in controlling and eradicating the GSCs, discussing the mechanisms involved in GSC tumor proliferation and resistance to treatments in addition to proposing potential avenues to target GSCs in order to provide a potential cure for this cancer.

Keywords: chemotherapy, glioma stem cells, immunotherapy, malignant glioma, treatment resistance.

INTRODUCTION

Glioblastoma Multiforme (GBM) is the most common primary brain tumor with an incidence of approximately 10,000 new cases annually in the United States [1]. Despite gross total resection, and treatment with radiation and chemotherapy - temozolomide followed by bevacizumab at the time of recurrence -, the median survival has improved only to 18 months in the last decade [1,2]. Once the GBMs recur, they are universally fatal, with survival less than a few weeks, despite aggressive treatment [3].

The main cause for GBM recurrence is not precisely known, but recent research suggest that it involves the development (and/or persistence) of subpopulations of tumor cells with resistance to treatment [3]. GBM is characterized by the presence of multiple glioma cell populations [4,5] organized in a stem cell hierarchical order with different stages of differentiation [6]. The cell population situated at the apex of the cellular hierarchy - the glioma stem-like cells (GSC) have been the proposed reason for the recurrence of malignant gliomas after treatment [6-8].

It is hypothesized that glioma cancer stem cells (GSC) can arise either from neural stem or progenitor cells [9] after the accumulation of mutations in oncogenic pathways such as NF1, p53 and PTEN [10] (see Fig. 1). They have the ability to self-renew and to initiate brain tumors [9]. In addition, they express neural stem cell markers and are multipotent [9]. They constitute only a minor subpopulation of the entire tumor. The progeny of GSC are progenitor like cells and differentiated cells, which divide rapidly, and form the bulk of the gliomas [9]. Several surface markers associated with GSC are known [9]. These markers, which include CD133/prominin, Musashi homolog, nestin and A2B5 [3,9], are useful for the isolation and enrichment of GSC.

Previous experiments indicate that “non-stem” cells can produce tumors in orthotopic xenograft models only when implanted in very high numbers [11]. These experimental tumors lack the classical malignant glioma behaviors-namely, an invasive phenotype, vascular proliferation-, and a limited capacity of tumor initiation [11]. However, orthotopic implantation of a small number of stem-like cells in appropriate animal models generate aggressive growth of tumors [12,13] bringing further support to the stem cell hypothesis for glioma formation [14].

In this review we will first summarize the current treatment modalities used for glioblastoma treatments and evaluate their effectiveness in controlling and eradicating the GCS. We will review the mechanisms involved in GSC-driven tumor proliferation, invasion and resistance to treatment and consider potential therapeutic targets that might hinder or block these oncogenic pathways. Finally, we will discuss potential avenues to target GSC in order to decrease the tumor burden and potentially provide a cure for this type of cancer.

Current Therapeutic Modalities: Glioma Stem Cells Resist the Current, First-Line Treatments for Malignant Gliomas - Radiation and Temozolomide (TMZ)

The standard of care for malignant gliomas after resection is temozolomide (TMZ) plus radiation therapy. A phase III clinical study showed a survival benefit by adding TMZ to postoperative radiation treatment [2,15]. The upregulation of O'-methylguanine-DNA methyltransferase (MGMT) expression counteracts the effects of alkylating agents [16], such as TMZ. In vivo, the expression of MGMT correlated well with the resistance of malignant gliomas to TMZ treatment [17]. Ultimately, almost all the patients relapsed after treatment with radiation and temozolomide, with a progression free survival of zero at 5 years [18].

The main mechanism through which radiation damages and kills glioma cells is the induction of breakage of the hydrogen bonds within the DNA strands, altering the base pairs, inducing substitutes, destruction of sugars and forming dimers. However, GSC are resistant to radiation [13]. The mechanisms involved in this radio-resistance are the following:

1. Selective cellular growth arrest, or quiescence, is described as the primary means by which the GSC evade the radiation and TMZ chemotherapy induced cellular damage [9,19]. During quiescence, GSC are maintained in growth arrest by various cellular processes and signaling pathways. Once the radiation
and TMZ chemotherapy is completed, the arrested GSC can again become actively proliferating leading to fatal tumor recurrences [7,13,20,21].

2. Activation of checkpoint proteins is a major mechanism for radio-resistance of GBM. The most common checkpoint proteins overexpressed in GSC are ChK1 and ChK2 kinases [13]. These proteins are also expressed in high quantities in tumors isolated from population of cells enriched by radiation, such as CD133+ GSC populations [13]. Early analysis of tumor samples found that patients who had a high percentage of CD133+ GSC had a worse outcome than those patients with a lower percentage of CD133+ GSC [13]. Since the CD133+ GSC population was enriched after radiation suggested activation of DNA repair machinery in these cells. Further investigations showed that CD133+ cells were also able to repair the damage to their DNA more effectively than the CD133- GCS [13]. Treating CD133+ GSC with a specific ChK1 and ChK2 kinase inhibitors sensitized them to radiotherapy, suggesting that one of the main pathways for resistance to radiation therapy was the activation of the DNA damage checkpoint response in GSC [13].

Temozolomide (TMZ) is an alkylating agent that methylates the DNA thereby inhibiting DNA replication and cell proliferation. The GSC are resistant to TMZ, via multiple mechanisms of resistance [8].

1. GSC have higher levels of expression of O\textsuperscript{6} – methyl – guanine – DNA – methyltransferase (MGMT) [14,22]. MGMT is a DNA repair protein which reverses the alkylation in the O\textsuperscript{6} position of guanine and thus compensates for the DNA methylation effects [16,22-24]. \textit{In vivo}, the expression of MGMT correlates well with the resistance of malignant gliomas to TMZ treatment [17,23,24]. Bleau \textit{et al} showed that in a mouse model of glioma, treatment with TMZ increases the population of GSC [14]. However, GSC with normal levels of MGMT were resistant to TMZ [25], while only the MGMT negative GSC were killed by TMZ [26]. In the clinical setting, the patients who had methylation of the MGMT promoter – which leads to silencing of the promoter and prevents gene expression - benefited with the addition of TMZ to their treatment [15]. Chen \textit{et al} showed that a restricted cell population propagates glioblastoma growth after TMZ chemotherapy [8]. A relatively quiescent GCS population was identified after TMZ chemotherapy, that subsequently produced the highly proliferative cells [8].

2. Another mechanism of resistance to TMZ treatments is expression of multiple drug transporting proteins including ABC (ATP binding cassette) transporters which expel out of the cell the chemotherapeutics. Hirsmachmann –Jax \textit{et al} showed that a side population (SP) of cancer stem cells isolated from tumors and identified by flow cytometry contains these transporters and resists cytotoxic drug treatment. They also showed that ABCG2 and ABCA3 transporters were increased in GCS [27]. We showed that GSC have 10 times higher levels of ABCG2 than NSC [3]. Multidrug resistance 1 (MDR1) protein was also over-expressed in TMZ resistant CD133+ GSC [28]. CD133 was initially described as a marker which resisted the uptake of fluorescent markers, which coincidentally resemble chemotherapeutic drugs [22,29].

3. The evasion of the cell-death pathway is another important mechanism of resistance developed by GSC. By losing various proteins and overexpression of other proteins, GSC can modulate their response to chemotherapy and select the most resistant phenotype [9]. One mechanism of avoidance of cell-death pathways is the deletion of phosphatase and tensin homolog (PTEN) in glioma stem cells [14,30]. PTEN suppresses the Akt phosphorylation in this pathway, by reversing the PI3K phosphorylation, thus inhibiting cell proliferation [30]. Therefore, loss of PTEN leads to uncontrolled activation of Akt and tumor growth [14,30] Bleau \textit{et al} showed treatment with TMZ selects for GSC that lack the PTEN gene [14]. This loss leads to activation of Akt, resistance to apoptosis, tumor progression, and poor prognosis for the patient.
4. Several other mechanisms also contribute to the resistance to TMZ treatment. The apoptosis pathway is heavily exploited by GCS [31]. Anti-apoptotic genes, such as Bcl-2, were found to have higher expression in the GSC population which was resistant to TMZ [31]. The addition of XIAP inhibitors increases sensitivity to chemotherapy of the previously chemotherapy resistant GSC [32], thus suggesting that activation of antiapoptotic factors such as XIAPs, are involved in resistance of stem cells to TMZ chemotherapy.

5. Constitutive activation of the Notch pathway also increases the oncogenic potential of these cells and maintains their stem cell status [33]. Wang et al showed that blocking Notch pathway leads to increased sensitivity of GSC to radiation [34]. By upregulating PI3K/AKT levels and increasing the levels of Bcl-2 family proteins, the Notch pathway produces radioreistance in GSC. The knockdown Notch models increase the sensitivity of GSC to radiation [34].

6. Insulin like growth factor binding protein 2 (IGFBP2) can mediate the activation of AKT pathway leading to resistance to radiation and conventional chemotherapy [35]. IGFBP2 upregulates metalloproteinase-2 and CD24, which increases the ability of GSC to invade adjacent tissues [35]. In vitro inhibition of IGFBP2 in GSC lead to decreased AKT activation, increased GSC sensitivity to radiation and chemotherapy, as well as decreased stem cell gene expression [36].

Fig. (2). \textit{Signaling Mechanisms Important in Glioma Stem Cells and Potential Therapeutic Targeting (Part 1)}. Multiple stem cell and oncogenic pathways are involved in GSC-driven tumor proliferation, invasion and resistance to treatment. The most actively targeted by therapeutic agents are angiogenesis (VEGF inhibition) and differentiation (Notch inhibition). VEGF=Vascular endothelial growth factor, FT=Farnesyltransferase, RAS=Rat sarcoma gene family, MEK=Mitogen-activated protein kinase, ERK=Extracellular regulated kinase, gp60/80=60 kD/ 80 kD glycoprotein, JAK=Janus kinase, STAT3=Signal transducer and activator of transcription 3, BMP=Bone morphogenic proteins, SMAD=Homolog of both the Drosophila protein, mothers against decapentaplegic (MAD) and the Caenorhabditis elegans protein SMA (small body size), TGF\(\beta\)=Transforming growth factor beta, Sox=Sry-related HMG box, NICD=Intracellular domain of Notch, cMYC=Myelocytomatosis viral oncogene, Olig2/Olig4=Oligodendrocyte lineage transcription factor 2/4, TRRAP=Transformation/transcription domain-associated protein, NF-\(\kappa\)B= nuclear factor kappa-light-chain-enhancer of activated B cells.
Chemotherapy, but also induces new tumor formation and leads to resistance. Clinical studies of glioma patients with limited results [54].

Echinomycin and bortezomib were used in combination with shikonin derivatives, epidithiodiketopiperazines, and two representative polyamides, quinols and naphthoquinone spiroketal analogues, found to lead to an increase in oncogeneity of GSCs [47]. Therefore, animals [52]. In addition, expression of HIF-1α and HIF2α was reported to be expressed in GSC, but not in glioma tumor cells [52]. In embryonic stem cells, hypoxia maintains the self-renewal potential and prevents the differentiation of neural stem cells. Yoshida et al. demonstrated that hypoxia enhanced production of induced pluripotent stem cells (iPSC) [48]. GBM frequently display numerous GSC around the areas of necrosis [43]. CD133 is also a marker for hypoxic stress [49]. This may be the reason why some GBM GSC might be selected for in the hypoxic regions.

In addition, hypoxia increases the expression of GSC markers [39,47]. Previous work indicates that hypoxia increases CD133+ GSC [50,51]. In hypoxic conditions, GSC activate HIF-1α thus increasing their self-renewal ability and anti-differentiated status of GSC [52]. Notch signaling is important in hypoxia, as it maintains the cells in an undifferentiated state. Activation of Notch signaling pathway occurs by recruiting HIF-1α at Notch responsive promoters [53]. In addition, multiple HIF regulated genes were reported to be expressed in GSC, but not in glioma tumor cells [52]. Studies showed that losing factors such as HIF-2α for example, leads to a significant decrease in both GSC proliferation and self-renewal in cultures, and decrease in tumorigenic potential in animals [52]. In addition, expression of HIF-1α and HIF2α was found to lead to an increase in oncogeneity of GSCs [47]. Therefore, HIF-1α family proteins represent a solution for targeting GSC populations. There are a number of HIF1α targeted agents such as polyamides, quinols and naphthoquinone spiroketal analogues, shikonin derivatives, epidithiodiketopiperazines, and two representative drugs: echinomycin and bortezomib. Only bortezomib was tried in vivo.

**Hypoxia Inducible Factor HIF-1α Maintains the Tumorigenic Potential of Glioma Stem Cells and Increases Treatment Resistance**

Hypoxia is common in tumor growth, and for many years it was believed to inhibit tumor growth. However, recent studies indicate that hypoxia actually contributes to tumor growth and proliferation. In malignant gliomas, hypoxia was found to promote angiogenesis, tumor growth and radioresistance [47]. Hypoxic niches play an essential role in the maintenance of GSC [43,44,47]. These “hypoxic niches” were also involved in the maintenance of normal stem cells [9]. In embryonic stem cells, hypoxia maintains the self-renewal potential and prevents the differentiation of neural stem cells. Pathways that hypoxia regulates include the Notch, Hedgehog, PI3K/Akt, and STAT3 pathways. Inhibition of the Notch signaling pathway can target the endothelial cells in malignant gliomas [59]. Hovinga et al. showed that when the tumor endothelial cells were eliminated from a GBM explant, there was a simultaneous decrease in self-renewal of the tumor stem cells [59].

Combination treatments with Notch blockade and radiation therapy resulted in a decrease in proliferation and self-renewal of the tumor explants [59]. Thus, the Notch pathway links angiogenesis and GSC; this allows a dual targeting approach for future treatments. RO4929097 is a gamma secretase inhibitor of Notch signaling which has been used in Phase I trial in patients with advanced solid malignancies with promising results [60] and could have immediate relevance for GBM.

**The Hedgehog – GLI1 is Also Involved in Maintaining the Oncogenic Potential of Gliomas Stem Cells**

Hedgehog (HH) – GLI1 signaling pathway regulates self-renewal and tumor-forming abilities in CD133+ GSC [61]. Blockade of the HH pathway using cyclopalmine depletes GSC [25]. The homeobox gene, Nanog, was recently identified as a mediator for HH-GLI1 and contributes to the expansion of the CD133+ GSC population, and maintaining glioblastoma growth [61]. It has also been shown that a loss of p53 contributes to up-regulation of Nanog by activating HH pathway and by negatively regulating the activity and level of GLI1 [61]. GLI1 was also found to upregulate Notch and downregulate BMP signaling, which is a pre-differentiation action on stem cells [62]. GANT61 is a preclinical molecule targeting the GLI1 and GLI2 in the HH –GLI1 pathway causes apoptosis in myeloid leukemia cells [63].

**Epidermal Growth Factor Receptor (EGFR) Signaling Pathway is Involved in Maintaining the Oncogenic Potential of Gliomas Stem Cells**

Epidermal growth factor receptor (EGFR) signaling pathway is involved in maintenance of GSC with a malignant phenotype [64]. Approximately 40-60% of GBM tumors exhibit EGFR amplification along with a high EGFR protein expression levels [65]. The EGFR activation initiates the phosphatidylinositol-3-kinase (PI3K)/Akt pathway. PI3K are lipid kinases that phosphorylate lipid phosphatidylinositol (4, 5)-bisphosphate (PIP2) to generate phosphatidylinositol (3, 4, 5)-triphosphate (PIP3) [66,67]. PIP3 recruits AKT to the cell membrane, which enhances cell growth, survival, and proliferation of the cells. EGFR variant III (EGFR vIII) possesses a hierarchical model of expression, which was restructured to epigenetic mechanisms which are characteristic of GSC. Inhibition of the EGFR pathway via tyrosine kinase inhibitors induced apoptosis in CD133+ GSCs [69,70], while inhibition of AKT activity lead to suppression of self-renewal in GSC and CD133+ GSC apoptosis [71]. In vitro studies showed that it is possible to target only GSC and not NSC, due to lower levels of EGFR expression on the NSC surfaces [3]. Clinical trials targeting the EGFR variant III (EGFR vIII) have had promising results [72,73] in the GBM patient population.

**Transforming Growth Factor beta (TGFβ) Enhances the Tumor Formation Ability of Glioma Stem Cells**

Transforming growth factor beta (TGFβ) is a very powerful cytokine demonstrably involved in many cellular processes including embryonal development, cell growth, differentiation,
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When expressed within GSC, the expression of TGFβ is associated with self-renewal and tumorigenic potential of GSC. This occurs by the induction of LIF through Smad complex binding to LIF promoter via the activation of JAK-STAT pathway [75]. Therefore decreasing the secretion of TGFβ and inhibiting the JAK-STAT pathway can lead to a decrease in self-renewal and tumorigenic potential in GSC [75].

TGFβ signaling maintains the tumorigenic capacity of GSC via induction of SOX2 expression, which was promoted by induction of SOX4 [76]. SOX4 is a direct target gene of TGFβ signaling, and SOX4 associates with SOX2 enhancer region, promoting its expression [76]. Silencing SOX2 leads to decrease in oncogenicity and self-renewal in GSC [77]. The undifferentiated phenotype of GSC is one of the key criteria for retaining the tumorigenic potential of these cells [76, 77]. Cox et al showed that SOX2 overexpression can be correlated with either poor prognosis or increased tumorigenesis [78]. Annozovazzi et al also showed that SOX2 overexpression was correlated with highest malignancy grade [79]. Alonso et al also demonstrated that SOX2 overexpression induced invasion and migration in gliomas stem cells [80]. In addition, knockdown experiments showed that SOX2 was essential for maintaining stem cell phenotype in malignant cells [80].

Platelet Derived Growth Factor Receptor (PDGFR) Augments GSC Tumorigenic Capacity

PDGFR plays an important role in the developing and adult brain. This protein is also over expressed in GBM at the transcriptional level. In vivo studies correlated abnormal PDGF signaling with glioma formation [81]. In animal studies, PDGF blocked neuroblast generation and enhanced neural stem cell proliferation in the subventricular zone with formation of glioma like hyperplasia [82]. PDGFRβ was recently reported to be highly expressed in GSC [83]. Pharmacological inhibition of PDGFRβ decreases GSC self-renewal potential, survival, tumor growth, and invasion [83]. Imatinib, a tyrosine kinase inhibitor, which inhibits the PDGF signaling pathway, reduces the ability of GSC to differentiate [84].

The c-Myc Oncogene Maintains the Tumorigenic Potential in Glioma Stem Cells

The oncogenic transcription factor c-Myc, is involved in activating the expression of many genes through several mechanisms, such as recruitment of histone acetylase, chromatin remodeling factors, and interaction with basal transcription factors [85]. Guney et al showed that c-Myc inactivation induces telomere independent senescence [85]. C-Myc is highly expressed in GSC, and c-Myc knockdow reduces cell proliferation, induction of cell apoptosis...
and loss of oncogenic potential [45]. In addition, inactivation of p53 and PTEN — tumor suppressor genes — leads to increased expression of c-Myc and increase in oncogenicity of GSC [86]. Inhibitors of c-Myc were found to induce cells cycle arrest and apoptosis in acute myeloid leukemia cells [87]. To date, there are no c-Myc inhibitors used in the treatment of malignant gliomas.

**Bmi1 Contributes to the GSC Maintenance and Transformation**

Bmi1 is an epigenetic silencer gene [88]. Under normal circumstances it is involved in determination of the differential of stem cell in several tissues [88] and it is a positive regulator of neural stem cells [88]. Bruggeman et al demonstrated that Bmi1 was involved in malignant transformation of both neural and differentiated astrocytes [89]. Bmi1 is over-expressed malignant gliomas [89]. To date efforts are on the way to identify small molecule inhibitors to Bmi1.

**Over-Expression of Chemokine Receptors Leads to GSC Migration**

Over-expression of chemokine receptors such as CXCR4, is a common mechanism related to GSC migration [22]. For glioma cells to migrate, a complex combination of multiple molecular mechanisms is needed, including alteration of tumor cell adhesion molecules, secretion of proteases, modification of actin cytoskeleton, and acquisition of resistance to apoptosis (by affecting PI3K, Akt, mTOR, NF-kappaB regulated pathways), and autophagy (programmed cell death type II) [90]. In *vitro* CXCR4 inhibition synergizes with cytotoxic chemotherapy in gliomas [91]. Inhibiting the migration of GCS contains the tumor and prevents invasion. There are several CXCR4 inhibitors, developed for use specifically in HIV patients, but they have not yet been tested malignant gliomas.

**Adhesion Molecules Play an Important Role in Migration and thus Are Important in Maintaining the Oncogenic Potential of Glioma Stem Cells**

Adhesion molecules play an important role in nervous system development, and in neural migration and differentiation [92]. One such molecule is L1CAM [92], which regulates neuronal cell growth, survival and migration and axonal outgrowth and neurite extension during development. It is expressed in gliomas and other cancers and makes it a good potential cell surface target [19]. The L1CAM knockdown expression in GSC disrupted the sphere formation ability of GSC, suppressed tumor growth while inducing apoptosis [19]. L1CAM is found in association with CD133+ cells, while CD133− cells are L1CAM negative [19]. Studies involving lentiviral shRNA targeting of L1CAM disrupted neurosphere formation induced apoptosis and inhibited growth of CD133+ GSC [19]. Currently there are no drugs targeting specifically the L1CAM molecule. However, based on *in vitro* results, this is seems to be an attractive target for glioma growth inhibition.

Integrins, another class of adhesion molecules, are cell surface receptors that are expressed during development and mediate development specific events by binding matrix ligands [93]. Integrin α6 was shown to be important in the neural migration during olfactory development [93]. Recently it was shown that integrin α6 is highly expressed in GSC [94] and that by directly interacting with lammin on endothelial cells increases the oncogenicity of GSC. Disruption of the interaction and targeting of integrin α6 inhibits self-renewal, and proliferation and tumor formation potential [94]. Cilengitide is a specific inhibitor of αvβ3 and αvβ5 integrins and it was tested in phase II clinical trials with promising activity against glioma [95].

**Bone Morphogenetic Protein (BMP) Increased Expression Leads to Increased Stem Cell Phenotype in Malignant Gliomas**

BMP plays an important role in determining the cell fate during development of the nervous system. Piccirillo et al. showed that BMP can activate receptors in GSCs to induce their differentiation in xenograft models [96]. By direct implantation of BMP beads into malignant gliomas, the researchers obtained slower tumor growth in xenograft models suggesting that this could be a new therapy in the treatment of malignant gliomas [96]. Another group of researchers showed that BMP are capable of regulating and alter the BMP receptors in order to induce a more malignant phenotype for GSCs [97]. Based on this evidence, BMP could be used as a very promising treatment for malignant gliomas.

**CD133 is used for the Identification and Enrichment of GSC Populations and is also a Potential Target for Therapy**

CD133 is a cell surface protein found on the surface of a population of GSC. CD133+ GSC are tumorigenic and have proliferative activity [98], and that the presence of CD133+ cells is an independent risk factor for tumor recurrence and inversely correlates with patient survival in patients with malignant gliomas [99]. Liu et al found that purified CD133+ GSC express a series of genes which are associated with undifferentiated, slow-growing, migrating and an anti-inflammatory and anti-angiogenic phenotype [57]. Multiple studies have shown that CD133+ gliomas cells are resistant to chemotherapy [50,100] potentially because CD133+ GSC express higher levels of proteins associated with chemotherapy resistance, such as the DNA repair protein MGMT [14] and the drug transporter gene ABCG2/BCRP (breast cancer resistance protein) [27]. CD133+ cells also have high mRNA levels of other apoptosis inhibitors, including FLIP, Bcl-2, Bcl-X and some IAP family genes [22].

The role of CD133 marker in targeting GSC holds great potential. However, the task of developing a drug against malignant gliomas targeting this marker remains difficult as there are GSC populations which do not express the marker on their surface [101,102]. Beier et al describe CD133- GSCs, and postulate that this molecular heterogeneity of GSC may contribute to the molecular heterogeneity of GBM [102]. In addition, Beier et al, demonstrated that CD133+ GSC from high grade astrocytomas can give rise to CD133+ progenitor cells [101]. These cells however, had significant telomere shortening when compared to the CD133+ compartment [101]. Beier et al also isolated CD133+ GSC and CD133- GSC populations from different GBM [3]. Both cell subtypes showed similar tumor inducing abilities in nude mice [103]. However, clinically the CD133- GBM subtypes showed lower proliferative index [103]. The researchers also showed by GeneArray analysis of these samples that the 133+ and 133- GSC had as much as 117 genes that were differentially expressed [102].

**Overcoming GSC Immune Surveillance Escape-Immunomodulation Therapy in the Treatment of Glioblastoma Multiforme**

Earlier studies in gliomas showed that CD133+ cells did not express detectable Major Histocompatibility Complex (MHC) class I or natural killer (NK) cell activating ligands [98], thus these cells were resistant to adaptive and innate immune surveillance. Incubating GSC with interferon gamma significantly increased the percentage of CD133+ cells that expressed MHC class I and natural killer ligands [98]. In addition, when the CD133+ cells were pretreated with interferon gamma, they became sensitive to NK cell-mediated lysis in *vitro* [98]. GSC can be attacked using active immunotherapy by designing vaccines that stimulate the host’s intrinsic immune response to the tumor. The initial immunotherapies against gliomas included irradiated whole tumor cell inoculation engineered to secrete cytokines [104], or combined with cytokine secreting cells [105] or cytokines alone [106]. TR2-01849 is a specific antibody to CD133 protein, specifically designed to target glioblastoma stem cells. Various immune strategies, such as adjuvants, heat shock proteins, γδ T cell treatments have been used as immunomodulators of the immune system in the treatment of GBM [107].

A newer method of triggering the active immunity is the development of a dendritic cell (DC) vaccine, which used patient-derived professional antigen presenting cells (APC), such as...
dendritic cells, to initiate the tumor-specific T-cell response when re-injected in the patients [108-112]. Using this procedure, the lysates of GSC produced a more robust immune system response than using lysates of GBM cells. The DC conditioned with GBM GCS lysates present a wide variety of antigens to T cells to stimulate effective anti-tumor immunity and it is believed that DC vaccination strategies using GSC lysates generate more effective stronger immune responses against a series of more specific epitopes. It has been known that in monoclonal gammapathy patients can develop an immune response against specific epitopes such as SOX2 [113]. In the case of gliomas, Pellegratta et al showed that the use of GSC lysates elicited a strong T cell immune response [114]. In addition, DC vaccination using the antigens/lysates derived from the “mesenchymal subtype” of GBM (which has a very poor prognosis) when compared to the pro-neural GBM [97,115]) produced a better survival than those other types of GBM treated with their respective lysate-pulsed DC; i.e., classical or pro-neural types [116].

miRNA can Modulate Tumor Cell Proliferation, Invasion, Apoptosis and Senescence

The miRNA are small, non -coding RNAs, which down-regulate gene expression post transcriptionally during different cell processes such as apoptosis, differentiation and development [117]. miRNA were originally identified as potential tumor suppressor molecules which induces apoptosis in neuroblastoma cells [118]. The miRNA expression in gliomas is associated with GSC mainanince and growth [117,119]. For gliomas, miR-34a was down-regulated when compared to normal brain [117,119]. Mutant p53 expressing glioma tumors had lower levels of miR-34a than wildtype p53 tumors. The presence of miR-34a within those gliomas inhibits their proliferation pathways, cell survival, migration and invasion [117,119]. Transfection of cells with miR-34a inhibited oncogene expression such as c-Met, Notch-1 and Notch-2 [117,119]. miR-34a expression induces gliomas stem cells differentiation and thus has the potential to be used in targeting the oncogenic pathways. Another group identified that miR-204 suppressed self – renewal, stem cell phenotype and migration ability of glioma cells [120]. The mechanism was consistent with targeting the transcriptional factor SOX4 and the migration-promoting receptor EphB2 [120]. miR-204 expression decreased invasiveness and increased host survival [120]. It appeared that the miR-204 is an important regulator of the development of glioma stem cell phenotype in regular malignant gliomas [120]. Currently there are no clinical trials which use the miRNA concept. However, as technology advances, it is very likely that miRNA could be used as a treatment strategy.

LOW GRADE GLIOMAS AND GLIOMA STEM CELLS

Some publications attempted to investigate the relationship between low grade gliomas and glioma stem cells, as GBM frequently develop from low grade gliomas. Thon et al demonstrated that CD133+ cells isolate from gliomas of different grades had the same properties concerning self-renewal and multi-lineage differentiation [121]. Rebetz et al. analyzed the surface marker expression of stem cells (CD133), glial progenitors (PDGFRalpha, A2B5, O4, and CD133); and late oligodendrocyte progenitors (O1) [122]. The data suggested that low grade gliomas cells expressed glial progenitor markers [122]. The oligodendrocyte progenitor was weakly expressed on all low grade gliomas and the majority of high-grade gliomas [122]. This study indicated that low grade gliomas have the potential for glial lineage progenitor cell expansion, and therefore have the potential of becoming malignant.

DISCUSSION AND CONCLUSIONS

A significant challenge in designing treatment against GSC is to avoid targeting the neural stem cells and progenitor cells, which share many genetic similarity and antigen expression profiles with GCS [7]. The adult human stem cells serve important functions in tissue repair after injury, especially after traumatic brain injury, ischemic and tumor associated or treatment induced destruction [9]. These normal neural stem cells (NSC) also possess some intrinsic activity against tumor cells, they are attracted by glioma cells in vivo and they induce apoptosis and inhibit glioma stem cell growth [123]. The neural stem cells and the progenitor cells are, however, more sensitive than GSC to temozolomide and carboplatin chemotherapy [3]. Hypoxic conditions and EGFR expression seem to be most characteristic to GSC and not to NSC. We previously showed that bortezomib, a proteasome inhibitor, and erlotinib, an EGFR tyrosine kinase inhibitor, decrease the viability of GSC, but not to affect NSC [3].

New research also shows the need to target both the GSC and the bulk of the glioma cells. In their new paper, Chen et al indicated that if one targets only the GSC, the mice still die due to their tumor burden [8]. Therefore, there is a need for more effective drugs or immunotherapeutic strategies that target both GSC and the more differentiated glioma cell mass. More clinical trials focused on combination therapies against multiple oncogenic pathways involved in GSC maintenance, division and differentiation are still needed.

CONFLICT OF INTEREST

The author(s) confirm that this article content has no conflict of interest.

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