Published in final edited form as:

Curr Opin Neurol. 2013 December ; 26(6): 701–707. doi:10.1097/WCO.0000000000000032.

The Evolving Landscape of Brain Tumor Cancer Stem Cells

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Abstract

Purpose of review—Recent advances in the role of cancer stem cells (CSC) in glioblastoma (GBM) will be reviewed.

Recent findings—In the decade since the description of brain tumor CSCs, the potential significance of these cells in tumor growth, therapeutic resistance and spread has become evident. Most recently, the interplay between CSCs, tumor genetics and the microenvironment has offered potential nodes of fragility under therapeutic development. The CSC phenotype is informed by specific receptor signaling, and the regulation of stem cell genes by transcription factors and miRNAs has identified a number of new targets amenable to treatment. Like normal stem cells, CSC display specific epigenetic landscapes and metabolic profiles.

Summary—Brain cancers activate core stem cell regulatory pathways to empower self-renewal, maintenance of an organ system (albeit an aberrant one), and survival under stress that collectively permits tumor growth, therapeutic resistance, invasion and angiogenesis. These properties have implicated CSC as contributors in GBM progression and recurrence, spurring a search for anti-CSC therapies that do not disrupt normal stem cell maintenance. The past year has witnessed a rapid evolution in the understanding of CSC biology to inform preclinical targeting.

Keywords

Glioblastoma; glioma stem cells; cancer stem cells

Introduction

Glioblastoma (GBM), World Health Organization grade IV astrocytoma, is the most common primary intrinsic brain tumor in adults and remains uniformly fatal despite

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Conflicts of Interest

We have no conflicts of interest to report.

maximal therapy. Presentation depends on tumor location but symptoms often include headache, paresis, seizures, personality changes, and aphasia. Pathologic grading of astrocytomas is based on a combination of cellular morphology and frequency, mitoses, necrosis and vascular proliferation. Standard-of-care GBM management involves maximal surgical resection followed by chemoradiotherapy and adjuvant chemotherapy with the oral methylating agent, temozolomide. Unfortunately, tumor recurrence is the rule with median survival only 12–15 months for patients under the age of 70 [1].

Neuro-oncology has witnessed an explosion in the molecular modeling of GBM through tumor genetics and mouse modeling. The Cancer Genome Atlas (TCGA) is a large effort with an initial large-scale analysis of primarily new diagnosed GBM designed to quantify common genetic tumor abnormalities that confirmed frequent mutational involvement in the p53, RB, and receptor tyrosine kinase pathways [2]. A competing unbiased whole exome sequencing effort of a smaller number of tumors identified the most exciting novel genetic lesion, isocitrate dehydrogenase 1 (IDH1) gain-of-function mutations, associated with secondary GBM [3]. Gene expression studies divided GBM patients into distinct tumor subtypes -- Classical, Mesenchymal and Proneural -- characterized by mutations in epidermal growth factor receptor (EGFR), neurofibromin 1 (NF1), and platelet-derived growth factor receptor A (PDGFRA)/IDH1, respectively [4]. Few of these genetic findings have entered into clinical practice to date.

A link between brain tumors and fetal tissues has long been recognized but it was only a decade ago that several groups simultaneously demonstrated that brain cancers contain hierarchies with highly tumorigenic cells that display stem cell features able to create a complex tumor upon transplantation [5, 6]. This discovery followed earlier reports of tumor initiating populations in leukemia and breast. Similar to CSCs in other cancers, GBM stem cells (GSCs) are defined by their ability to self-renew and propagate heterogeneous tumors in vivo.

Soon after the initial description of GSCs, GSC resistance to conventional therapy -- radiation and chemotherapy -- was described [7, 8]. In addition, GSCs have an increased capacity for angiogenesis and invasion, hallmarks of GBM [9]. While GBMs, like all cancers, are genetic diseases that evolve during treatment, the CSC hypothesis suggests the importance of non-genetic changes in tumor recurrence in patients -- GSCs that remain following therapy contribute to regeneration of the patient's initial tumor. Chen and colleagues modeled this phenomenon in a mouse model of glioma with a Nestin- TK-GFP transgene. The GFP+ cells were suggested to represent a CSC population that remained following temozolomide chemotherapy with additional therapeutic benefit of targeting tumors with the combination of temozolomide and gancyclovir to target the putative CSCs [10]. Although based on a cell line model limiting its relevance to CSCs, a mathematical model of glioma radiotherapy and recurrence predicted that surviving GSCs increased their rate of self-renewal to maintain a tumor following radiotherapy [11]. The collective evidence that CSCs may contribute to our current failure to achieve cures in patients supports the development of molecular targeting strategies.

Initial models of GSC regulation have been based on neural stem cell biology, the probable normal cellular correlate. Indeed, GSCs are governed by pathways active in brain development, including Notch [12], Wnt [13], bone morphogenetic protein (BMP) [14], transforming growth factor- β (TGF β) [15, 16], and receptor tyrosine kinase (RTK) pathways [17, 18]. More recently, micro-RNAs have been shown play a large role in GSC gene regulation [19, 20] and GSCs display epigenetic DNA changes. Early data suggest that GSCs are metabolically distinct from the bulk tumor population. Furthermore, CSCs are not passive recipients of microenvironmental cues as CSCs stimulate angiogenesis through elaboration of pro-angiogenic growth factors and possible lineage plasticity towards vascular lineages, suggesting both cell autonomous and extrinsically defined CSCs. The CSC state is a plastic phenotype and no single cellular marker is universally informative for CSCs. As such, the study of CSCs requires functional validation and use of patient derived tumors with no or limited time in culture to prevent clonal drift. Thus, CSCs add additional complexity to cancer models with CSCs as potential products of their microenvironment [21–23]. Researchers are attempting to exploit many of these elements of GSC biology to target this population and enhance patient survival.

The Search for GSC-specific Molecular Targets

Interrogating GBM models for GSC targets has posed challenges as the field has experienced growing pains with highly variable rigor in the definition of GSCs. Standard culture conditions with serum induce irreversible changes in gene expression profiles divergent from the original tumor, yet many reports persist in the use of cell lines to derive putative GSCs. The CSC hypothesis requires demonstration of a cellular hierarchy of tumorigenic and non-tumorigenic cells. The derivation of sphere-forming cells in serum-free culture enriches for cells with self-renewing features but the corresponding loss of the non-tumorigenic population prevents interpretation of the cellular hierarchy. GSCs should not be considered solely as a replacement for flawed established cell lines, particularly as GSCs receive essential maintenance cues from the microenvironment. Despite these limitations, potentially important therapeutic targets in GSCs have been published on a frequent basis [Fig. 1]. Space limitation prevents discussion of every potential new target, but several recent reports bear attention. One target of particular interest to the field is CD133, or Prominin-1, which has been used for the past decade as a cell surface marker to isolate GSCs for study [5]. The utility of CD133 has been controversial and dependent on context, but a functional role for CD133 has been elucidated in GSCs, as a regulator of the PI3K-Akt pathway via its interactions with the p85 subunit of PI3K [24]. As a cell surface protein, CD133 has been targeted with antibodies in preclinical studies and a vaccine against CD133 (ICT-121) is entering clinical trials.

Numerous receptors have been implicated in GSC biology. The Eph receptor tyrosine kinases have been investigated in cancer and stem cell biology for years [25], but in the last year, two groups independently recognized the roles of the Eph receptors, particularly EphA2 [26] and EphA3 [27], in GSC maintenance. EphA3 was found to be specifically expressed in mesenchymal GSCs and appeared to modulate downstream MAPK signaling. Importantly, both groups were able to target Ephrins *in vivo* to decrease tumorigenicity in mouse models.

Another receptor that has generated interest in GSCs is platelet-derived growth factor receptor B (PDGFRB). PDGFRA is amplified or mutated commonly in Proneural GBM, suggesting a role in intertumoral heterogeneity. In contrast, PDGFRB is preferentially expressed by GSCs, independent of tumor subgroup, and regulates GSCs through downstream STAT3 signaling to increase invasion and tumorigenicity [28]. Several groups have described MET, the receptor for hepatocyte growth factor (HGF), as a receptor implicated in GSC biology [29, 30]. MET activates wnt/ β -catenin signaling in GSCs [31]. In MET high tumors, treatment with HGF induces proliferation, migration and invasion [32]. c-MET inhibitors are in development and c-MET expression may be targeted to sensitize GBMs to anti-angiogenic therapies, supporting potentially direct translation into clinical trials with dual MET/VEGFR2 inhibitors [33].

Transcriptional regulation in GSCs

A tour de force global epigenomic analysis of GSCs performed by Rheinbay et al. revealed selective activation of transcription factors (TFs) compared to normal human astrocytes (NHAs) and ESC-derived neural stem cells (NSCs), including Olig1, Olig2, En2, Hey2, Lhx2, and Ascl1 [34]. Ascl1 proved essential for the maintenance and *in vivo* tumorigenicity of GSCs through activating Wnt signaling [35]. Targeting TFs is challenging but indirect inhibition of the TF Id1 using the natural compound, cannabidiol, proved useful as an anti-GSC therapy [35]. Examining GSC-specific TFs might also clarify the roles of other proteins involved in GSC biology to further treatment of GBM. For example, the stem cell TF Sox2 is regulated by Polo-like kinase [36] in addition to the TF Myeloid Elf1-like factor (MEF) [37]. Maternal Embryonic Leucine Zipper Kinase (MELK) is a serine/threonine-protein kinase involved in various processes such as cell cycle regulation, self-renewal of stem cells, apoptosis and splicing regulation. As a GSC effector, MELK regulates JNK signaling and its activation of pro-tumorigenic TF FOXM1 [38, 39]. Collectively, the genetic and epigenetic landscape now strongly supports core regulation of a stem-like state in GBM that may inform targeted therapy development.

miRNA and Epigenetic Regulation of GSCs

MiRNAs were first implicated in GSC biology in 2008 [19, 20]. Since then, the field has progressively recognized an essential role of miRNA regulation in many aspects of GSC biology. Last year marked a boon in miRNA research in the field, with a number of novel miRNAs that regulate multiple aspects of GSC signaling. A list of several miRNA targets that were described last year is listed in Table 1. Notably, miR-204 is downregulated in GSCs and targets the transcription factor Sox4 as well as the Eph receptor EphB2 to decrease GSC self-renewal and invasion [49]. Also, miR-18A* promotes the tumorigenic potential of GSCs by targeting DLL3, an inhibitor of Notch signaling [40]. Furthermore, a screen for miRNAs that are altered with serum-induced differentiation identified miR-1275 as a key mediator in oligodendrocytic differentiation of GSCs. miR-1275 is epigenetically silenced by histone H3 lysine 27 trimethylation (H3K27me3) during differentiation, allowing for expression of its target, Claudin11, and a subsequent decrease in proliferation [52].

In addition to the epigenetic regulation of miRNAs and TFs, many other key epigenetic regulators were characterized in GSCs during the past year. BMI1 (B lymphoma Mo-MLV insertion region 1 homolog) is a polycomb ring finger oncogene that serves in the PRC1-like complex to repress gene transcription, including cyclin-dependent kinase inhibitors in cell cycle control. A combination of chromatin immunoprecipitation-sequencing and RNA interference of BMI1 identified Atf3 as a gene upregulated by BMP-mediated differentiation and ER-stress pathways [53]. Atf3 is downregulated in GSCs and its expression promotes the transcription of a number of anti-tumorigenic genes. Another polycomb member, EZH2, has been repeatedly linked to GSCs, with increased significance recently derived from evidence that EZH2 also regulates another GSC signaling node, STAT3, through acetylation [54]. The polycomb family is likely directly involved in GBM tumor initiation as gain-of-function mutations in histone H3 that inhibit polycomb repressive complex 2 (PRC2) activity have been described in pediatric GBM [55]. PRC2 may regulate GSC plasticity through gain or loss of H3K27me3 on pluripotency or development associated genes (e.g. Nanog, Wnt1, BMP5) [56]. Furthermore, mixed lineage leukemia (MLL) maintains GSC characteristics by directly activating the Homeobox gene HOXA10 through its activity of histone methyltransferase at histone H3 lysine 4 [57]. Finally, the expression of CD133, the canonical GSC marker which was functionally characterized this year, was also shown to be regulated by epigenetic mechanisms, as promoter methylation and methyl-DNA-binding proteins cause repression of CD133 by the exclusion of transcription-factor binding [58].

Cancer Metabolism in GSCs

Like all cancers, GBMs display the Warburg effect, a preferential utilization of aerobic glycolysis for energy supplies. This metabolic shift reduces the cells' oxygen dependence and provides a steady supply of anabolic material yet requires a steady supply of glucose [59]. Whether and how the metabolic pattern differs between GSCs and non-GSCs within the tumor remains elusive, but GSCs may primarily employ oxidative phosphorylation with plasticity as required by metabolic demands [60]. The critical importance of glycolysis has been demonstrated in mesenchymal GSCs, featured by the upregulation of ALDH1A3 and glycolytic activity [61]. Inhibition of ALDH1A3 significantly attenuated the growth of mesenchymal GSCs. Interestingly, such metabolic pattern was not observed in proneural GSCs, indicating that distinct metabolic signaling pathways exist in GSCs from different GBM subtypes. The role of oxidative phosphorylation (OXPHOS) has also been studied in GSCs during the past year. As shown by Janiszewska et al. [62], the oncofetal insulin-like growth factor 2 mRNA-binding protein 2 (IMP2, IGF2BP2) is a key regulator in proneural GSCs *in vitro*. IMP2 binds and delivers several mRNAs that encode mitochondrial respiratory chain complex subunits to the mitochondria. It also contributes to the assembly of Complex I and Complex IV. More importantly, inhibition of OXPHOS but not of glycolysis abolishes GBM cell clonogenicity in the proneural GSCs. A unifying model would suggest that that glycolysis and OXPHOS are preferentially utilized in mesenchymal and proneural GSCs, respectively, suggesting a potential strategy for a personalized treatment plan based on the metabolic features of GBM subtypes.

GSCs and the Tumor Microenvironment

No stem cell exists in isolation. Similar to normal stem cells, GSCs exist within a tumor niche surrounded by supporting cells such as endothelial cells, pericytes, reactive astrocytes, and tumor-associated macrophages. In addition, GSCs are intimately regulated by microenvironmental factors, and cluster in perivascular [21], hypoxic [22, 63] and likely other niches.

Two years ago, a pair of manuscripts in *Nature* generated much interest in the field, describing the potential transdifferentiation of glioma stem cells into endothelial cells to contribute to the tumor vasculature [64, 65]. In contradiction, Cheng et al. found such lineage plasticity rare but more common and functionally important transdifferentiation of GSCs into pericytes instead using lineage tracing in vivo [66]. Patient tumor pericytes possess the same genetic abnormalities as tumor cells suggesting a neoplastic origin. The chemokine pathway, CXCL12-CXCR4, mediates an attraction of GSCs to the perivascular niche where TGF β induces GSCs to assume a pericyte lineage [66]. Two additional publications this year highlight the role of TGF β in the tumor microenvironment as well, with clear differences in its role based on context. In one paper, TGF β was implicated as a factor secreted by tumor-associated macrophages to promote GSC-mediated invasion at the tumor edge [67]. In another paper, within tumorspheres – that is, in the environment of other GSCs – GSCs secrete four times more TGF β per cell than isolated cells. In this context, TGF β secretion promoted radioresistance of GSCs [68]. Thus, a single microenvironmental factor may be targeted to alter multiple tumor outcomes.

Conclusion

As the cell population in GBM that may contribute to tumor invasion and therapeutic resistance, GSCs have generated much research interest in the past several years. GSCs may be regulated in distinction from neural stem cells, providing potential nodes of fragility. GSC signaling pathways, miRNAs, and epigenetic regulation permit adaptation with the tumor microenvironment to promote tumor invasion, angiogenesis and therapeutic resistance. Last year, mechanisms that endow GSCs with their unique tumorigenic properties have been reported. In addition, we have improved our knowledge on GSC-specific metabolic processes and the interaction between GSCs and the tumor microenvironment. All of these new discoveries have unearthed new opportunities for potential GSC-specific therapies. However, because of the complexity of GSCs, there is unlikely to be an end-all approach to GSC therapy. In order to fight GSCs we must continue to vigorously explore and target all aspects of their biology. Ultimately, we hope to combine the therapies we find with current drugs to prolong the lives of patients with this terrible cancer.

Acknowledgments

We thank our funding sources: The National Institutes of Health grants CA154130 and CA1129958 (J.N.R), grants TR000441 and CA165892 (K. Yan), American Brain Tumor Association (K. Yang), Research Programs Committees of Cleveland Clinic (K. Yang and J.N.R), and James S. McDonnell Foundation (J.N.R).

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Key bullet points

- Dysregulated cell-surface receptor and TF signaling in GSCs provide potential targets for therapy.
- miRNA regulation and epigenetic mechanisms impact multiple aspects of GSC biology.
- Proneural GSCs use oxidative phosphorylation while mesenchymal GSCs prefer glycolytic mechanisms, highlighting the importance of tailoring treatments based on GBM subtypes.
- GSCs contribute to and interact with their microenvironment, with TGF β playing a context-dependent role.

Figure 1.

Summary of key advances in GSC biology in the past year. (1) New receptors and transcription factor (TF) discoveries in GSCs provide potential new therapeutic targets. (2) Insights into TGF-beta signaling provide clues into the interaction of GSCs and the microenvironment. (3) GSCs activate differential metabolic pathways in a subtype-specific manner. (4) New clues into the epigenetic regulation of the CD133 marker and key stem cell effectors in GSCs.

Table 1

miRNAs implicated in GSC biology in the past year.

miRNA	Authors	Relative Expression	Role
miR-18a*	Turchi et al. [40]	Upregulated	Inhibits DLL3, enhancing Notch signaling
miR-21	Polajeva et al. [41]	Upregulated	Co-expressed with Sox2 and promotes growth and survival
miR-23b	Geng et al. [42]	Downregulated	Induces cell cycle arrest and inhibits proliferation
miR-124	Wei et al. [43]	Downregulated	Enhances T-Cell mediated clearance of GSCs by inhibiting STAT3
miR-125b	Wu et al. [44]	Downregulated	Inhibits proliferation by targeting E2F2
miR-125b	Shi et al [45]	Downregulated	Inhibits invasion by targeting MMP levels via TIMP3 and RECK
miR-137	Bier et al. [46]	Downregulated	Promotes neural differentiation by targeting RTVP-1
miR-143	Zhao et al. [47]	Downregulated	Inhibits glycolysis via hexokinase 2, leading to differentiation
miR-145	Lee et al. [48]	Downregulated	Inhibits migration and invasion by targeting CTGF
miR-204	Ying et al. [49]	Downregulated	Targets Sox4 and Ephrin B2 to inhibit self-renewal and invasion
miR-211	Asuthkar et al. [50]	Downregulated	Inhibits migration and invasion by targeting MMP9
miR-452	Liu et al. [51]	Downregulated	Impacts self-renewal by targeting stem cell factors BMI1, LEF1 and TCF4
miR-1275	Katsushima et al. [52]	Upregulated	Promotes growth and prevents oligodendrocytic differentiation by targeting Claudin11