[Frontiers in Bioscience 19, 818-824, January 1, 2014]

The role of glioma stem cells in glioma tumorigenesis

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1. ABSTRACT

Malignant gliomas, the most common type of primary brain tumor, account for 80% of malignant tumors in the central nervous system (CNS). There are three principal types of glioma: astrocytomas, oligodendrogliomas, and oligoastrocytomas. Glioma stem cells (GSCs) have been found in all types; however, many fundamental questions about GSCs remain unanswered. This review will examine the current state of knowledge regarding GSC origin and the signaling pathways implicated in GSC tumorigenesis. The outstanding challenges for the study of GSCs in the context of glioma progression will also be discussed.

2. INTRODUCTION

The developmental progression of malignant gliomas follows a sequence of events: transformation from a cell of origin, acquisition of invasive properties, activation of proliferative signals coupled with abrogation of cell cycle control, upregulation of angiogenesis, and clonal evolution (1). According to the 2007 classification by the World Health Organization (WHO), tumors of neuroepithelial tissues can be of several types, including astrocytic, oligodendroglial, oligoastrocytic, ependymal, choroid plexus-derived, or others, according to the cell type with which they share histological features (2). The

WHO also defines tumors according to a malignancy scale (grades I–IV), based on the manifestation of specific characteristics. Grade I applies to lesions with low proliferative potential, which have the possibility of being cured following surgical resection alone. Grade II indicates infiltrative neoplasms with a low level of proliferation but high recurrence. Grade III lesions are malignant by histological criteria. Cytologically malignant, mitotically active, necrosis-prone neoplasms typically associated with rapid pre- and postoperative disease evolution and fatal outcome are assigned to Grade IV. In this grading system, only astrocytic tumors cover all four grades; other types of glioma are more restricted, such as oligodendroglial (grades II and III), oligoastrocytic (grades II and III), ependymal (grades I–III), choroid plexus-derived (grades I–III), and other neuroepithelial tumors (grades I and II).

Cancer stem cells (CSCs), first identified in 1997 (3), are defined by the American Association for Cancer Research as a subpopulation of cells in the tumor which have the capacity to self-renew and differentiate into heterogeneous cancer cells (4). Glioma stem cells (GSCs) are a type of CSC isolated from solid tumors (5). Relatively little is known about GSCs in terms of a cell of origin and their role in tumorigenesis. Moreover, suitable markers for isolating and characterizing GSCs-which could potentially serve as diagnostic markers or drug targets—are conspicuously lacking. Finally, the genetic and epigenetic mechanisms and signaling pathways regulating GSCs still require detailed characterization. Each of these points is considered in greater detail below.

3. GSC CELL OF ORIGIN AND MARKERS

Human prominin-1 (CD133), a five transmembrane domain glycoprotein, was originally identified as a CD34+ hematopoietic stem cell (HSC) surface antigen, and has been widely used to purify CSCs including GSCs. In CD133+ GSCs, CD133 expression level was highly correlated with clonal expansion *in vitro*; these cells were able to generate nonadherent neurospheres, in contrast to non-proliferative CD133− cells. Moreover, the neurospheres had immunophenotypes similar to those of the primary tumor from which they were isolated (6). Intracranial transplantation of as few as 100 CD133+ cells in NOD-SCID mice gave rise to a tumor phenotypically similar to the original tumor, while injection of as many as 10^5 CD133− cells did not result in tumor formation (5). However, in a separate study, CD133− GCSs were isolated that were tumorigenic in immunocompromised mice. Primary tumor cells were cultured from 15 glioblastomas; 11 contained CD133+ cells capable of neurosphere-like, nonadherent growth, while the remaining four contained CD133− cells that were adherent *in vitro*, but were also able to form tumors upon transplantation (7).

The use of CD133 as a definitive marker of GSCs is problematic for a few reasons. First, the commonly used antibodies against CD133, AC133 and AC141, recognize glycosylated but not unmodified extracellular epitopes, which could lead to the false identification of cells as CD133− (8). In theory, this could be circumvented by measuring CD133 transcript levels. However, when designing primers for amplification, the various isoforms of CD133 must be considered. For instance, the predominant CD133 isoform discovered in HSCs, which is presumably recognized by AC133 and AC141, is one that lacks exon 4 (9). In addition, CD133 expression is modulated by oxygen levels. Hypoxic culture conditions (2–3% O2) increase CD133 transcription and the expression of AC133 and AC141 epitopes in glioblastoma cells (10). Conversely, culturing GSCs under high (20%) oxygen tension could lead to a reduction in CD133 expression, thereby altering their potential for tumor initiation. As in most experiments, GSCs are cultured *in vitro* prior to being injected into live animals; the exposure of the cells to two different environments may induce antigenic alterations that could compound the difficulty of GSC detection. Therefore, it is imperative to find additional markers, and recent studies have revealed a few promising candidates. A population of glioma-initiating cells in the perivascular niche was found to express high levels of CD44 and Id1 (11). Knockdown of integrin alpha 6 in GSCs was shown to inhibit self-renewal, proliferation, and tumor-forming capacity (12), while stage-specific embryonic antigen 1 (SSEA-1) was present in all but one (n = 24) of the primary glioblastoma samples examined in another study, in contrast to CD133, which was detected in only 60% of freshly isolated glioblastoma specimens (13).

The failure to detect CD133 in some GSCs may also be because tumor-initiating cells are a naturally heterogeneous population; this diversity could be established at an early stage, further obfuscating the identification of a cell of origin. A recent study showed that Pten-deficient glioblastoma tumors produce both CD133+ and CD133− self-renewing, tumor-initiating cells (14); however, it is not clear at which stage loss of Pten occurs in a natural, pathological process. Various models have been proposed for the origin of GSCs (Figure 1). In the first model, a simple GSC clone derived from neural stem cells (NSCs) undergoes genetic alterations under selective pressure from the environment to become different types of glioma. Alternatively, multiple GSC clones with distinct genetic alterations may differentiate into specific types of glioma. Finally, glioma cells may possess inherent plasticity that allows them to revert to GSCs under appropriate conditions. Evidence in support of the first and second models comes from the observation that targeted deletion of the tumor suppressor genes *p53*, *Nf1*, or *Pten* in NSCs generates gliomas with 100% penetrance, whereas deficiency of these genes in non-neurogenic brain regions does not lead to tumor formation (15). Support for the third

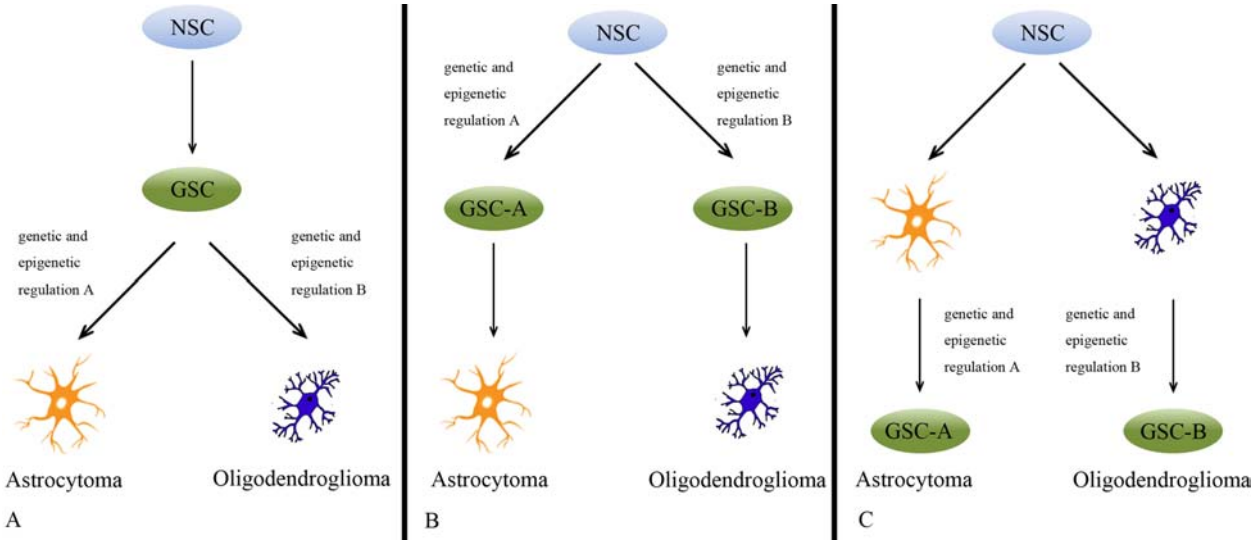

Figure 1. Models of GSC generation from NSCs. (A) NSCs transform into a homogenous pool of GSCs, which subsequently undergo different genetic and epigenetic modifications to become distinct types of gliomas (*e.g.*, astrocytomas or oligodendrogliomas). (B) NSCs undergo various genetic and epigenetic modifications to generate different types of GSC, which then differentiate further into astrocytomas or oligodendrogliomas depending on the conditions of their microenvironment. (C) NSCs first differentiate into normal astrocytes and oligodendrocytes; these cells then de-differentiate into specific types of GSC by unknown mechanisms.

model comes from experiments demonstrating that the reversion of neonatal cortical astrocytes to NSCs in culture can be induced by the combined loss of *p16Ink4a* and *p19Arf* (16). However, *in vivo* data for these models is still lacking. Moreover, there is currently no evidence to distinguish between the first and second models (*e.g.*, the discovery of two GSCs within the same specimen that harbor different mutations would favor the second model).

4. GSCS IN GLIOMA TUMORIGENESIS

Glioma tumorigenesis proceeds by a sequence of steps: genetic ablation, proliferation, invasion, angiogenesis, and clonal expansion. The involvement of GSCs in each of these steps has been demonstrated by various studies. CD133+ GSCs were shown to express high levels of disintegrin and metalloproteinase 17 (ADAM17), which are associated with the invasive and proliferative activities of GSCs; for example, ADAM17 can mediate the proteolytic cleavage of the ectodomain of several EGFR ligands, leading to the activation of the PI3K/Akt and MEK/ERK pathways (17). GSCs from the glioblastoma cell line U251 expressed high levels of matrix metalloproteinase (MMP) 13, and had enhanced migratory and invasive capacity both on Matrigel and in organotypic brain slices (18). Expression of the chemokine receptor CXCR4 was upregulated in CD133+ GSCs, indicating that they had the potential to migrate more efficiently in the presence of the cognate ligand CXCL12 (19).

GSCs are also directly implicated in angiogenesis. High levels of vascular endothelial growth factor (VEGF) secreted by GSCs recruit endothelial cells to the tumor region to support proliferation (20). GSCs also produce stromal-derived factor 1, which induces angiogenesis by local endothelial cells (21). In addition to secreting angiogenic cytokines, GSCs can themselves be induced to differentiate into endothelial cells *in vitro*, and some local subpopulations of endothelial cells have genomic profiles identical to GSCs as detected by *in vivo* quantitative FISH (22, 23). GSCs can also differentiate into other cell types to support angiogenesis and vascular function. In a recent study, GSCs were found to contribute to the microvasculature of glioblastomas by transdifferentiating into mural-like cells (24). Another report demonstrated that GSCs can differentiate into vascular pericytes to support vessel function and tumor growth (25).

5. SIGNALING PATHWAYS IN GSC TUMORIGENESIS

Various signaling pathways are involved in different stages of glioma tumorigenesis, regulated by complex genetic and epigenetic mechanisms. Transforming growth factor beta (TGF-β) signaling controls many normal cellular processes including growth, differentiation, and morphogenesis. TGF-β has an oncogenic function in gliomas, and increases GSC self-renewal through Smad-dependent induction of leukemia inhibitory factor and subsequent activation of JAK-STAT signaling (26). Autocrine TGF-β activates

the stemness factor Sox2 by a Sox4-dependent mechanism to enhance GSC self-renewal. Treatment with SB431542, a selective inhibitor of TGF-β superfamily type I activin receptor-like kinase receptors 4, 5, and 7, can deprive GSCs of tumorigenicity by promoting their differentiation (27). However, the mechanisms underlying the activation of TGF-β signaling in GSCs are unclear.

The tumor-suppressor function of p53 was first discovered in colorectal carcinomas (28), and has since been demonstrated in a variety of cancers to promote tumor cell proliferation. The p53 signaling pathway is also important for GSC self-renewal. Mutated p53 in Olig2+ transit-amplifying progenitor-like cells induced glioma formation (29). Likewise, the PI3K/Pten pathway has also been implicated in both tumor cell and GSC proliferation. CNS-specific knockout of Pten and p53 leads to myc-dependent hyperproliferation of GSCs (30). Furthermore, overexpression of c-myc or activated myr-Akt, or both, in mature p53−/− astrocytes induces the expression of stem cell markers and promotes gliomagenesis in these cells (31).

The Wnt signaling pathway was shown to inhibit glioma tumorigenesis through its neurogenic function and crosstalk with HIF-1α and Notch signaling, inducing differentiation of CD133+ GSCs into neurons with non-malignant phenotypes (32). A similar anti-tumorigenic effect has been attributed to cannabinoids, which induce apoptosis and mediate GSC differentiation through the cannabinoid type 1 and type 2 receptors (33). Although the downstream components have not yet been identified, this nonetheless represents a promising new pathway for future studies to investigate.

Invasion by gliomas has been attributed to epidermal growth factor (EGF) and fibroblast growth factor (FGF) signaling, both of which stimulate tumor cell migration. The EGF receptor activates the Akt-Smad-Id3 axis to promote tumor initiation, progression, and angiogenesis (34). Extracellular signal-regulated kinase (ERK), a downstream effector of FGF signaling, regulates tumor invasion in many types of cancer and has recently been shown to serve as a nexus between N-cadherin and integrin α6 in mediating GSC invasion (35). Integrin α3 can also regulate CD133+ GSC migration and invasion through ERK1/2 pathway activation (36). GSC invasion is also regulated epigenetically by microRNAs. Overexpressing miR-107 can inhibit invasion in the GSC cell line U87 through downregulation of MMP-12 expression (37). Glioma cell proliferation is also suppressed by miR-107-mediated suppression of CDK6 and Notch 2. Interestingly, miR-107 is also a transcriptional target of p53 (38), providing a potential molecular link between glioma cell proliferation and migration.

Vascular endothelial growth factor (VEGF) is a principal mediator of angiogenesis. CD133+ GSCs secrete higher levels of VEGF than CD133− cells, and expression is further stimulated under hypoxic conditions (20). Hypoxia also promotes the expansion of the CD133+ GSCs through the PI3K/Akt, ERK1/2, or Notch signaling pathways (39, 40). Cannabinoids have also been shown to inhibit the production of VEGF and activation of VEGFR in gliomas (41), although it is not known whether this occurs in GSCs. Angiogenesis may also be enhanced by chemokines: CXCL12 activates CXCR4 on CD133+ GSCs to induce the expression of VEGF through PI3K/Akt signaling (19).

6. SUMMARY AND PROSPECTS

Although there have been many new insights into the role of GSCs in recent years, many controversies and questions remain. The discovery of GSCs has provided a plausible explanation for the high recurrence and resistance to treatment of glioblastomas. However, the unambiguous detection of GSCs requires the discovery of more specific and stable markers. In addition, most of what is known about GSCs has come from in vitro experiments using primary glioma cell cultures or GSC lines; while these findings are useful, they require in vivo validation by tissue- or cell type-specific knockout or overexpression in mice to have translational utility. Finally, how interactions between GSCs and their niche regulate their proliferative and invasive potentials is a topic that requires more thorough investigation. Resolving these issues will provide a basis for developing better strategies to treat human malignant gliomas, which could improve the prognosis of patients.

7. ACKNOWLEDGEMENTS

This work was supported by the grant from the Natural Science Foundation of Chongqing, China (No. CSTC, 2009BA5058).

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Abbreviations: CNS, central nervous system; GSCs, Glioma stem cells; WHO, World Health Organization; HSC, hematopoietic stem cell; NSCs, neural stem cells; MMP, matrix metalloproteinase; VEGF, vascular endothelial growth factor; TGF-β, Transforming growth factor beta; EGF, epidermal growth factor; FGF, fibroblast growth factor; VEGF, Vascular endothelial growth factor

Key Words: Central Nervous System; Glioma stem cells; neural stem cells, Review

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