Neuron
Review

Glioma Stem Cells: A Midterm Exam

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DOI 10.1016/j.neuron.2008.05.031

Several years ago, the discovery of a highly tumorigenic subpopulation of stem-like cells embedded within fresh surgical isolates of malignant gliomas lent support to a new paradigm in cancer biology—the cancer stem cell hypothesis. At the same time, these "glioma stem cells" seemed to resolve a long-standing conundrum on the cell of origin for primary cancers of the brain. However, central tenets of the cancer stem cell hypothesis have recently been challenged, and the cellular origins of stem-like cells within malignant glioma are still contended. Here, we summarize the issues that are still in play with respect to the cancer stem cell hypothesis, and we revisit the developmental origins of malignant glioma. Do glioma stem cells arise from developmentally stalled neural progenitors or from dedifferentiated astrocytes? Five separate predictions of a neural progenitor cell of origin are put to the test.

Introduction

Primary tumors of the central nervous system account for less than 1.5% of all new cancer cases reported in the United States each year (Central Brain Tumor Registry of the United States, http://www.CBTRUS.org). However, the majority of these cancers are either anaplastic astrocytomas or malignant gliomas. The more aggressive manifestations of these cancers (WHO grades III and IV, see Figure 1) are for all intents and purposes incurable. Accordingly, astrocytic tumors are the third leading cause of cancer-related death among middle aged men and the fourth leading cause of death for women between 15 and 34 years of age (Prados and Wilson, 1993). Pilocytic astrocytomas (WHO grade I) are the most common form of brain cancer in children (Kleihues and Cavenee, 2007). Some of these pediatric tumors are surgically curable, and many of the others are responsive to radiation (Kortmann et al., 2003) or conventional chemotherapy (Packer et al., 1997; Prados et al., 1997); however, the clinical impact of surgery, radiation, and cytotoxic drugs on growing children can be significant.

Against this backdrop, malignant gliomas and pediatric brain cancers have become one of the favored scientific vehicles for "the cancer stem cell hypothesis." The central tenet of this hypothesis is that solid and liquid tumors alike are composed of (1) a relatively small subset of slowly cycling cells that undergo self renewal for an indefinite/unlimited period of time and (2) a larger population of cells that have committed to a particular fate and have finite division capacity (Reya et al., 2001). A practical corollary of the cancer stem cell hypothesis is that cancer therapies frequently fail because they are directed toward the wrong cellular targets (Tan et al., 2006).

An important and testable notion with direct relevance to the cancer stem cell hypothesis is that tumors arise from mutated, developmentally arrested progenitor cells that normally drive organogenesis and/or tissue repair. For several reasons, cancers of the brain lend themselves especially well to critical assessment from this developmental perspective. Adult neural progenitors have been identified and mapped to spatially restricted regions of the postnatal brain. Marker proteins and antibodies are available for most of the specific cell types that comprise the adult brain. Protocols for the culture of normal, multipotent neural progenitors are well established. Above all, the "wall charts" for development of the normal brain, though far from complete, are surpassed in detail only by those for development of blood.

Cancer stem cells have now been isolated from a wide range of CNS neoplasms, including gliomas (both adult and pediatric) anaplastic oligodendrogliomas, and malignant medulloblastomas (Table 1). For neuro-oncologists, the notion that brain tumors arise from developmentally arrested neural progenitors seems to resolve some long standing puzzles: Why are these tumors (especially high-grade gliomas) so notoriously resistant to radiation and chemotherapy? What accounts for the bizarre mixture of cell types found in the most aggressive gliomas (WHO grade IV, a.k.a. glioblastoma mulitiforme; see Table 1)? Above all, how do any cancers arise in an organ that is well isolated from environmental toxins and—to a first approximation—mitotically inert?

Despite its considerable teleological charm, the cancer stem cell hypothesis remains a work in progress. Many aspects of cancer biology do not lend themselves well to a stem cell perspective. Recent developments in the area of stem cell research refresh a decades-long polemic on the cell of origin for malignant gliomas. We will start this review by framing the issues that are currently in dispute with respect to the cancer stem cell hypothesis in general and glioma stem cells in the particular. We will then critically evaluate five testable predictions of the cancer stem cell hypothesis.

The Cancer Stem Cell Hypothesis and Astrocytic Tumors: The Issues at Hand

One practical component of the cancer stem cell hypothesis addresses the issue of intrinsic resistance to radiation and chemotherapy. Cancer stem cells are predicted to be difficult targets for cancer therapeutics because (1) they cycle slowly, (2)

	Pilocytic Astrocytoma (WHO grade I)	Diffuse Astrocytoma (WHO grade II)	Anaplastic Astrocytoma (WHO grade III)	Glioblastoma a.k.a. Glioblastoma Multiforme (WHO grade IV)
Age of Onset	First two decades of life	30 to 40 yrs	Early 40s	Mid 50–60s
Typical Location	Throughout the neuraxis. Optic pathway tumors are frequent	Cerebral hemispheres. Pons/brainstem, esp. in children	Cerebral hemispheres	Cerebral hemispheres
Average Survival	Years to decades	Five years	Two to five years	Fourteen months

Figure 1. An Overview of the Astrocytic Tumors

The World Health Organization (WHO) recognizes ten major classes of neuroepithelial tumors and more than 40 subclasses. Of these, 60% belong to one major class—the astrocytic tumors, which are parsed into four grades in accord with histopathological appearance and clinical prognosis. As indicated, there is a direct relationship between tumor grade and age of onset and an inverse relationship between tumor grade and time to death. A subset of grade IV gliomas are thought to arise via progression of diffuse astrocytomas (WHO grade II) or anaplastic astrocytomas (WHO grade III). Grade IV gliomas that arise via progression are referred to as "secondary gliomas." However, most high-grade gliomas are diagnosed in patients with no evidence of a pre-existing, less malignant astrocytoma and are termed "primary gliomas." See Kleihues and Cavenee (2007) for further reading.

express high levels of drug export proteins, and (3) they may not express or may not depend upon the oncoproteins that are targeted by the new generation of "smart drugs," such as Gleevec and Iressa. Collectively, these observations and considerations (and speculations) provide a fresh rationale for tumor resistance to therapy and suggest that a new class of agents should be designed to specifically target cancer stem cells (Al-Hajj et al., 2004).

However, some cancers respond dramatically to chemotherapy, including cancers that arise from germ cells (i.e., testicular cancer) and cancers that clearly have a stem cell component, such as chronic myelogenous leukemia (Polyak and Hahn, 2006). Acquired resistance to radiation or chemotherapy is even more difficult to reconcile with the cancer stem cell hypothesis. Selective pressure and clonal expansion of tumor cells with preexisting mutations for drug resistance provides a plausible (albeit more prosaic) explanation for these typical failures of cancer medicine (Weinberg, 2007). In accord with the clonal expansion view, high-throughput DNA sequence analysis of breast cancer stem cells (marked by the cell surface antigen CD44) shows that the CD44-positive tumor stem cells are genetically more dissimilar to their CD44-negative counterparts than would be predicted by the cancer stem cell hypothesis (Shipitsin et al., 2007).

For brain cancers in particular, skeptics complain that glioma stem cells are defined in operational ways that have little or no bearing on brain development (see below). Moreover, even if their existence is conceded, the cellular and developmental origins of "glioma stem cells" can be contended. Do these cells actually arise from postnatal neural progenitors? Alternatively, are glioma stem cells the progeny of mature glia or committed glial progenitors that have dedifferentiated to a more stem-like state? The latter point of view, though somewhat counterintuitive, has gained credibility with recent reports on genetic reprogramming of adult skin cells to pluripotent embryo stem cells by transfection with small sets of transcription factors (Nakagawa et al., 2008; Okita et al., 2007; Takahashi et al., 2007; Wernig et al., 2007). These extreme examples of genetic reprogramming are low-frequency events in culture. Moreover, insertional mutagenic events may contribute to the process because retroviral expression vectors have thus far been required to transduce the transcription factors. Nevertheless the skin-to-stem cell transformations do illustrate the fundamental genetic plasticity

Table 1. Milestones in the History of the Glioma Stem Cell

• Mid-to-late 19th century: Lobstein, Cohnheim, and others comment on similarities between embryogenesis and the biology of cancer cells (1).
• 1926: Bailey and Cushing develop the brain tumor classification system from which modern taxonomies derive. The system emphasizes the histological resemblance of brain tumor cells to cells of the developing CNS (2).
• Mid 60s: Metcalf, Sachs, and others develop in vitro clonogenic assays to display the cellular progenitors of blood (3 and 4).
• 1966: Altman and Das describe postnatal neurogenesis in rats (5).
• 1988: Weissman and coworkers isolate the multipotent hematopoietic stem cell (6).
• 1992: Reynolds and Weiss identify postnatal neural progenitors (neurosphere cultures) (7).
• 1994: Dick and coworkers isolate malignant stem cells from human acute myeloid leukemia (8).
• 2000: Prospective isolation of human CNS stem cells (9).
• 2002–2004: Cancer stem cells isolated from adult and pediatric astrocytomas (10–13).

(1) Rather (1978); (2) Bailey and Cushing (1926); (3) Bradley and Metcalf (1966); (4) Ichikawa et al. (1966); (5) Altman and Das (1966); (6) Spangrude et al. (1988); (7) Reynolds and Weiss (1992); (8) Lapidot et al. (1994); (9) Uchida et al. (2000); (10) Ignatova et al. (2002); (11) Galli et al. (2004); (12) Hemmati et al. (2003); (13) Singh et al. (2003).

of replication-competent cells. Within this context, the conversion of committed astrocytes or astrocyte progenitors into multipotent neural progenitors seems to be a small and easily surmountable challenge. Indeed, one cannot rule out that reversion to a less-differentiated state might be a normal, albeit rare, event in healthy CNS tissue.

These unresolved issues in cancer cell biology and malignant glioma are the focus of this review. We will begin with a quick synopsis of glioma stem cell biology and a brief overview of the candidates for "glioma cell of origin." We will then address the cell of origin issue by considering five testable predictions of the cancer stem cell hypothesis.

Glioma Stem Cells: Definitions

Neural stem cells are classically defined as cells active in development, cell turnover, or repair that are (1) self-renewing and (2) multipotent. Although the definition of "glioma stem cell" was initially coined to reflect these two cardinal properties, there is growing recognition that cancer stem cells, while self-renewing, cannot be considered multipotent because the differentiated progeny derived from a transformed precursor are genetically abnormal. The operational definition of glioma stem cell is a tumor subpopulation that can self-renew in culture, perpetuate a tumor in orthotopic transplant in vivo, and generate diversified neuron-like and glia-like postmitotic progeny in vivo or in vitro.

Glioma stem cells meeting these criteria grow as neurospheres in culture and can express CD133 (a.k.a. Prominin-1)—a cell surface antigen that is also a known marker of multipotent stem cells in blood and other tissues, including the brain (Galli et al., 2004; Singh et al., 2003; Yuan et al., 2004). The CD133-positive cells isolated from human brain tumors can initiate formation of "neurospheres" when cultured under nonadherent conditions in medium supplemented with EGF and FGF. When these growth factors are removed and the tumor neurospheres are cultured under adherent conditions in serum-supplemented medium (or, in some studies, LIF-supplemented medium), individual cells began to express marker proteins associated with neurons and glia (Galli et al., 2004; Hemmati et al., 2003; Ignatova et al., 2002; Singh et al., 2003). Notably, the repertoire of neuronal and glial marker proteins that are expressed under these conditions recapitulates, the repertoire of multipotent neural progenitors (Galli et al., 2004; Hemmati et al., 2003; Singh et al., 2003).

Such CD133-positive, neurosphere-forming cancer stem cells are typically a minor subset of the total cells within glioma. However, they are by far the most tumorigenic component of the tumor. For example, disaggregated cells from fresh surgical isolates of malignant glioma will form tumors when inoculated into the cranium of immune suppressed mice; typically though, an inoculum of at least 10^5 cells is required to initiate tumor growth. By contrast, as few as 100 CD133-positive cells will suffice to initiate tumor formation (Singh et al., 2004). Interestingly, in accord with the cancer stem cell hypothesis, the CD133-positive glioma stem cells are relatively resistant to radiation (Bao et al., 2006a).

Glioma Stem Cells: Caveats

There are exceptions to the stem cell properties noted above and experimental pitfalls for the unwary. For example: (1) Not all of the cells that fit the operational definition of a glioma stem cell are marked by CD133. Some gliomas contain CD133-negative cells that, in every other respect, fit the operational definition of a cancer stem cell (Beier et al., 2007). (2) CD133 is expressed on cells that are not glioma stem cells—for example, endothelial cells. Slowly cycling endothelial cells are expected to be intrinsically resistant to radiation and could (in principle at least) complicate the analysis of radiosensitivity studies such as those conducted by Bao et al. (2006a). (3) Experiments with tumor xenograft models may lead to underestimation of the percentage of tumor-initiating or "tumor-sustaining" cells (Kelly et al., 2007). (4) Some of the cellular heterogeneity seen in high-grade gliomas may reflect the recruitment of nonmalignant neural or glial precursors into the tumor milieu (Assanah et al., 2006). These nonmalignant progenitors could contribute to the neurosphere population in vitro and obfuscate the interpretation of cell culture experiments. (5) Single neurospheres are not usually derived from single cells. Even in the absence of agitation or manipulation, free-floating neurospheres are highly motile entities. Time-lapse video microscopy shows that individual neurospheres are rapidly drawn toward each other and merge to form larger neurospheres, irrespective of whether cultures are passaged at conventional density or very low density (Singec et al., 2006). Neurosphere merger events are clearly germane to the property of multiple fate choice—one of the defining characteristics of a cancer stem cell.

Overshadowing all of these tactical concerns is a strategic problem: Stem-like properties in a neoplastic cell cannot be taken as evidence prima facie of a developmental origin from a "stalled" neural progenitor. The cancer stem cell hypothesis and the cellular origins of cancer are separate and distinct issues. Graphic evidence of the disconnect between a stem-like phenotype and a stem cell origin comes from studies on leukemias where it has been shown that committed progenitor cells can be transformed into leukemia stem cells by misexpression of the oncogenic fusion protein MLL-AF9 (Krivtsov et al., 2006). In the case of malignant glioma, there are at least three neural cell types that could, in principle, serve as cell of origin for glioma stem cells. These are (1) mature "dedifferentiated" glia, (2) "restricted" neural progenitors that are normally unipotent, and (3) multipotent neural progenitors (see Figure 5).

Cellular Progenitors of Glioma Stem Cells I: The Case for Mature Glia

Prior to the discovery of replication-competent neural progenitors in the postnatal brain (Reynolds and Weiss, 1992), mature astrocytes or committed astrocyte progenitors were thought to be the only replication-competent cells in the postnatal brain and thus the only cells capable of malignant transformation. The conceptual problem with mature or committed glia as tumor progenitors is that the transformed glia would have to dedifferentiate to produce the malignant, multipotent cells that are embedded within human gliomas. This "retrograde differentiation" might seem counterintuitive; however, it has recently been shown that genetic cocktails of just a few transcription factors can convert normal skin cells into totipotent embryonic stem cells (Nakagawa et al., 2008; Okita et al., 2007; Takahashi et al., 2007; Wernig et al., 2007). Within this context, the

conversion of a terminally differentiated astrocyte into a multipotent neural progenitor seems to be a small and easily surmountable challenge.

Multiple studies have shown that early cortical astrocytes can be targeted in vitro or in vivo with oncogenes or activated signal-generating proteins to produce tumors in animal models with convincing glioma histology (Bachoo et al., 2002; Blouw et al., 2003; Ding et al., 2001; Holland et al., 1998a; Rich et al., 2001; Sonoda et al., 2001; Uhrbom et al., 2005; Weiss et al., 2003; Xiao et al., 2005). For example, Bachoo et al. (2002) isolated neonatal astrocyte cultures from $p16^{Ink4a}/p19^{Arf}$ null mice and infected these cells with a retrovirus encoding a mutated, constitutively active version of the EGF receptor (EGFRvIII). Neurospheres generated from these genetically modified astrocytes could form gliomas when implanted into the brains of SCID mice (Maher et al., 2001). However, there are important caveats to most of these studies.

Several lines of evidence suggest that in vitro and in vivo cellular targeting paradigms select for a less mature cell in the astroglial lineage. First, retroviral expression vectors can only infect proliferating cells and are thus biased toward immature progenitors relative to their terminally differentiated progeny. Second, such transformation-competent astrocyte cultures can be generated from the neonatal cortex but not the adult cortex, arguing for selection of a less mature astrocytic cell type (Laywell et al., 2000). Indeed, culture of adult cortical astrocytes has proved extremely difficult. Third, cultured "astrocytes" are generally characterized by only one marker, GFAP, which is found on multipotent precursors as well as reactive astrocytes (see below). Finally, in vivo targeting studies suffer from a lack of reliable and specific regulatory sequences to drive gene expression specifically in mature cortical astrocytes. In this regard, the commonly used marker GFAP is quite problematic because it is expressed in neural stem cells as well as some committed astrocyte progenitors and cortical astrocytes (Doetsch et al., 1999). Thus, a major remaining challenge for the dedifferentiation hypotheses of glioma is the development of genetic systems capable of selective transmission of oncogenic events solely to mature astroglial cells. The critical need to discover novel markers of mature astrocytes was articulated in the summary statement of the NINDS-sponsored "Workshop on Astrocyte Function in Health and Disease (http://www.ninds.nih.gov/news_and_events/astrocyte_function_health_disease.htm).

Cellular Progenitors of Glioma Stem Cells II: The Case for 'Restricted' Neural Progenitors

The discovery of replication-competent multipotent neural progenitor cells in the postnatal brain (Reynolds and Weiss, 1992) created an attractive alternative candidate for the glioma cell of origin. Because such stem cells have the machinery for self-renewal already activated, maintaining this activation may be simpler then turning it on de novo in a more differentiated cell; that is, fewer mutations might be required to maintain self-renewal then to activate it ectopically (Reya et al., 2001).

"Restricted progenitors" of the brain are able to proliferate but are generally defined as unipotent. Examples include granule cell neuron precursors of the cerebellum and oligodendrocyte precursors in vivo. These restricted progenitors might first need to acquire the self-renewal potential of multipotent progenitors to have the opportunity to experience additional mutations that would lead to transformation. Some insight into this process can be taken from the hematopoietic system wherein restricted lymphoid and myeloid progenitors fail to self-renew detectably upon transplantation (Mikkola and Orkin, 2006; Tenen, 2003). Introduction of the MLL-AF9 fusion protein into committed granulocyte-macrophage precursors is sufficient for leukemic transformation including generation of self-renewing stem cells (Krivtsov et al., 2006).

Kondo and Raff have shown that committed oligodendroglial progenitors can reacquire stem-like properties after extensive treatment in vitro (Kondo and Raff, 2000), which results in chromatin remodeling and reactivation of the primitive neural epithelial marker, Sox2 (Kondo and Raff, 2004). Interestingly, Sox2 expression is prevalent in human gliomas (Schmitz et al., 2007), consistent with the possibility that similar mechanisms could be involved in the process of transformation of a restricted neural (glial) precursor to transformed cell type.

In the postnatal brain, cycling neural progenitors encompass the diffuse NG2 progenitor cell population (Diers-Fenger et al., 2001; Levine and Stallcup, 1987). NG2 cells are the most actively cycling cells in the adult brain, and they have been reported to have multipotent qualities (Belachew et al., 2003; Liu et al., 2007). The majority of NG2 cells express Olig2, which is required for oligodendrocyte lineage development (Ligon et al., 2006b), consistent with the proposal that NG2 cells are fundamentally similar to, or give rise to, oligodendrocyte precursors (Baracskay et al., 2007). Others have argued that NG2 cells comprise a distinct (fourth) neuroepithelial lineage (Greenwood and Butt, 2003; Nishiyama, 2007). In any case, NG2 cells can undergo reprogramming in culture to produce neurons and astrocytes via epigenetic mechanisms as described for oligodendrocyte precursors (Liu et al., 2007). Fate mapping in vivo also supports contributions to gray matter astrocytes (Zhu et al., 2008), although it is unclear whether such NG2 cells with increased potential represent a distinct functional subset or whether NG2 marks several classes of cells with distinct capabilities to form astrocytes or oligodendrocytes (Tamura et al., 2007).

Cellular Progenitors of Glioma Stem Cells III: The Case for Multipotent Neural Progenitors

Multipotent neural progenitors, found in specialized niches such as the dentate gyrus and subventricular zone (SVZ), have been extensively scrutinized, and have recently been suggested as cells with potential to form gliomas (for reviews see Sanai et al., 2005; Vescovi et al., 2006). Alvarez-Buylla and colleagues have described several basic subclasses of SVZ progenitor cells on the basis of histology and immunohistochemical and ultrastructural characteristics (Alvarez-Buylla et al., 2001; Doetsch et al., 1999). As indicated in Figure 2, quiescent "type B" stem cells that expresses the astrocytic marker GFAP and exhibits other morphological features of astrocytes are capable of responding to growth factors such as EGF and PDGF. Mitogen-treated type B cells give rise to transit-amplifying type C cells that in most cases will go on to form neuroblasts of the rostral migratory stream. However, type B cells can also give rise to

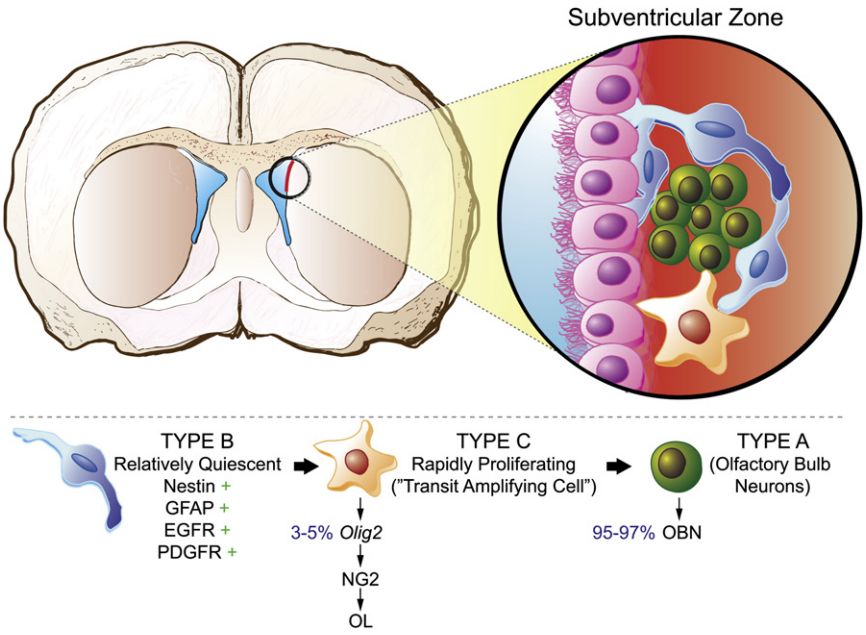

Figure 2. Neural Progenitor Subtypes in the Subventricular Zone

(Top) Coronal section through the postnatal adult mouse forebrain depicts subventricular zone (SVZ) progenitors in situ including type B (blue), C (amber), and A (green) cells, as well as ciliated ependymal cells (pink) that line the lateral ventricle (see Alvarez-Buylla et al. [2001] for further reading). (Bottom) Type B cells divide very slowly and express the markers indicated. They give rise to transient amplifying type C cells that in turn generate type A neuroblasts that contribute to the rostral migratory stream in most cases. However, a fraction of type C cells express Olig2 and NG2 and can form myelinating oligodendrocytes. The potential of type B cells to produce oligodendrocyte precursors might relate in principle to tumor-competence for glioma.

oligodendrocytes during normal development and after white matter injury (Hack et al., 2005; Menn et al., 2006). Interestingly, treatment with antimitotic agents such as cytosine-β-D-arabinofuranoside destroys type C but not type B cells, a finding that could be relevant for understanding the recurrence of brain cancer after chemo- and radiotherapy (Sanai et al., 2005).

As illustrated in Figure 2, these functional subgroups of the normal SVZ provide a conceptual framework for investigating features of gliomagenic stem cells as well as the cell of origin for glioma. For example, the normal type B cell captures essential properties of quiescence, self-renewal, and multipotentcy that one might ascribe to a cancer stem cell. Do glioma stem cells arise from developmentally stalled neural progenitors such as the type B cell? In the sections below, five separate predictions of a neural progenitor cell of origin are put to the test.

Prediction 1: Neural Progenitors and Brain Tumor Stem Cells Are Driven by Common Signaling Pathways

Four examples of signal transduction pathways that modulate growth and differentiation of neural progenitor cells during development and also in the postnatal brain are depicted schematically in Figure 3. As indicated, mutations of key regulatory elements within these pathways are associated with adult gliomas and pediatric medulloblastoma.

During development, Notch signaling promotes formation and suppresses differentiation of radial glia (Gaiano et al., 2000). In the postnatal brain, Notch promotes survival of neural progenitors and thereby expands the population of these cells both in vitro and in vivo (Androutsellis-Theotokis et al., 2006). The Notch signaling pathway is constitutively active in many high-grade gliomas and glioma cell lines—conceivably through an autocrine/juxtacrine loop mechanism involving coordinate expression of Notch and Notch ligands (Purow et al., 2005). The activation state of Notch in these tumors and tumor cell lines has lead to the proposal that Notch pathway inhibitors might be employed in glioma therapy (Kanamori et al., 2007).

Mitogens such as EGF, FGF, PDGF, and LIF promote the growth of adult neural progenitor cells from the subventricular zone or dentate gyrus and are used routinely for neurosphere cell culture (Jackson et al., 2006; Reynolds and Weiss, 1992; Shimazaki et al., 2001; Vescovi et al., 1993). Amplification and/or activating mutations in EGF and PDGF receptors are seen in adult high-grade gliomas (Kesari et al., 2006). PDGF ligand/receptor autocrine loops are also a common feature of malignant gliomas in all grade levels (Guha et al., 1995; Hermanson et al., 1992; Lokker et al., 2002). The receptors for EGF, FGF, PDGF, and LIF all activate the canonical Ras/Raf/MAPK signaling axis (Weinberg, 2007). Loss-of-function mutations in NF1 (encoding a GTPase) promote activation of the Ras/Raf/MAPK signaling axis and are associated with low-grade astrocytomas in patients with hereditary neurofibromatosis (Hochstrasser et al., 1988).

A parallel signaling pathway for growth factors and their receptor tyrosine kinases involves the formation of phosphatidylinositol 3 phosphate via activation of phosphatidylinositol 3 kinase (PI3K). Activating mutations in this PI3K are etiologic in a subset of high-grade gliomas (Samuels et al., 2004). The trophic functions of PI3K on anabolic metabolism and survival are opposed by the tumor-suppressor gene PTEN, which is deleted in a high percentage of malignant gliomas (Duerr et al., 1998; Furnari et al., 2007; Liu et al., 1997; Rasheed et al., 1997).

Although they are less frequent than low-grade astrocytomas, malignant medulloblastomas are actually the most common malignant brain tumor of childhood (Grovas et al., 1997). Medulloblastomas are thought to originate from granule neuron precursor cells in the external granular layer of the developing cerebellum. Sonic hedgehog is a critical mitogen for these precursor cells (Wechsler-Reya and Scott, 1999). Hereditary loss-of-function mutations in the Sonic hedgehog receptor Patched lead to constitutive activation of the Sonic hedgehog pathway and a predisposition to medulloblastoma in Gorlin syndrome (Bale et al., 1998; Goodrich et al., 1997; Gorlin, 1987). Loss-of-function mutations in "Suppressor of Fused" (SF) have been detected in

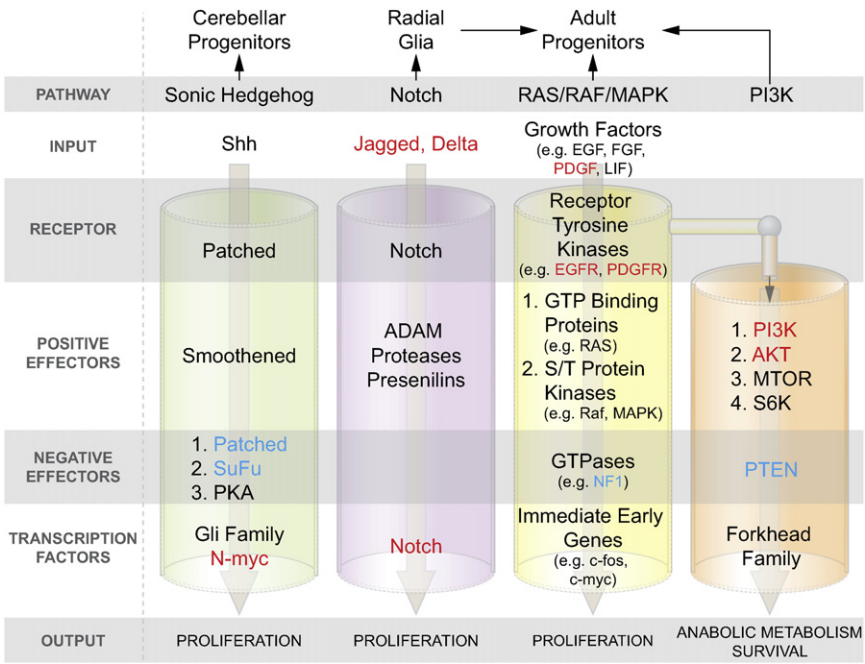

Figure 3. Common Mitogenic Cues for Neural Progenitors and Brain Tumor Stem Cells

Common gene amplifications, activating mutations, or autocrine loops are indicated in red font. Common deletions or loss-of-function mutations are indicated in blue font. See text for details. Note that the Hedgehog pathway is implicated strongly in the cerebellar tumor, medulloblastoma, whereas its roles in glioma are contended.

sporadic medulloblastomas (Taylor et al., 2002), and these mutations would likewise be expected to activate the Sonic hedgehog signaling axis (Figure 3).

What do these data say about the cancer stem cell hypothesis and glioma cell of origin? As predicted by the hypothesis, CNS cancer stem cells do appear to co-opt mitogenic cues that regulate the growth of normal neural progenitors. However, the receptors for EGF, FGF, Shh, and Notch ligands are broadly expressed. In cell culture, EGF and FGF are known to promote the growth of normal astrocytes derived from the neonatal cortex. Thus, the outcome of prediction 1 is ambiguous with respect to the cellular origins of CNS cancer stem cells.

Prediction 2: Signaling Pathways that Constrain the Growth of Normal Progenitor Cells Are Suppressed in Brain Cancers

Cell cycle progression of all mammalian cell types is governed by the "RB signaling axis." The central, cell-intrinsic features of this signaling axis have been extensively reviewed (Adams and Kaelin, 1998; Gartel and Radhakrishnan, 2005; Pei and Xiong, 2005; Sherr and Roberts, 1999). As indicated in Figure 4, there are three key negative regulators in the RB signaling axis. All three of the negative regulator elements in the RB signaling axis modulate the proliferation of neural progenitor cells.

Targeted disruption of $p16^{INK4a}$ has little effect on the anatomy of the SVZ in young mice or on the replicative potential of SVZ progenitor cells derived from young mice; however, deletion of $p16^{INK4a}$ can partially oppose an age-related decline in the abundance and self-renewal potential of SVZ neural progenitors (Molofsky et al., 2006). Targeted deletion of p21 opposes the relative quiescence of neural progenitors in the adult forebrain, leading to a transient surplus of these cells. This initial increase in the absence of p21 function is followed by a depletion and long-term decrement in stem cell replicative potential. In aggregate, the p21 null phenotype suggests that p21 contributes to the relative quiescence of neural progenitors, which is necessary for life-long maintenance of neural stem cell self-renewal (Kippin et al., 2005).

Mice lacking p53 display an elevated rate of cell proliferation in the adult SVZ. This enhanced proliferation in vivo correlates with an increase in the relative number of SVZ cells capable of forming neurospheres in vitro and with an increased number of stem cells within the neurospheres that form (Meletis et al., 2006).

The Polycomb group gene silencer Bmi1 lies genetically upstream of the RB signaling axis and plays key roles in sustaining the replication-competent state of normal neural progenitors. At postnatal stages, knockout of Bmi1 dramatically reduces forebrain SVZ neural progenitors (Molofsky et al., 2003; Zencak et al., 2005). As indicated in Figure 4, the postnatal requirement for Bmi1 function is thought to reflect the repressive functions of Bmi1 on the $p16^{Ink4a}/p19^{Arf}$ gene products (Molofsky et al., 2005). Targeted gene disruption reveals little or no requirement of Bmi1 in the developing CNS. However, recent studies using shRNA technology to achieve an acute knockdown of Bmi1 have shown a critical role for Bmi1 function for neural stem cell self-renewal in the developing CNS. Surprisingly, the cell cycle target for Bmi1 in the developing brain is not $p16^{Ink4a}/p19^{Arf}$ but rather *p21* (Figure 4) (Fasano et al., 2007).

In addition to general regulators of the cell cycle, lineage-restricted control mechanisms have recently been described. The bHLH transcription factor Olig2 is expressed exclusively within the central nervous system, where it contributes to the cell cycle control of neural progenitors. During development, Olig2 is expressed in progenitors that give rise to oligodendrocytes and certain neuronal subtypes, including motor neurons in the developing spinal cord (Lu et al., 2002, 2000; Novitch et al., 2001; Takebayashi et al., 2000; Zhou and Anderson, 2002; Zhou et al., 2000). In the adult CNS, Olig2 is expressed in myelinating oligodendrocytes but is observed also in mitogen-treated "transit-amplifying cells" of the SVZ (Jackson et al., 2006). Olig2 is also expressed in NG2-positive glia and required for the development of these cells (Ligon et al., 2006b). Olig2 sustains the replication-competent state of neural progenitors (Lee et al., 2005b), perhaps in part through suppression of p21 gene expression (Ligon et al., 2007).

Overlaps between the genes and gene products that regulate stem cell proliferation and the genetic lesions that underlie

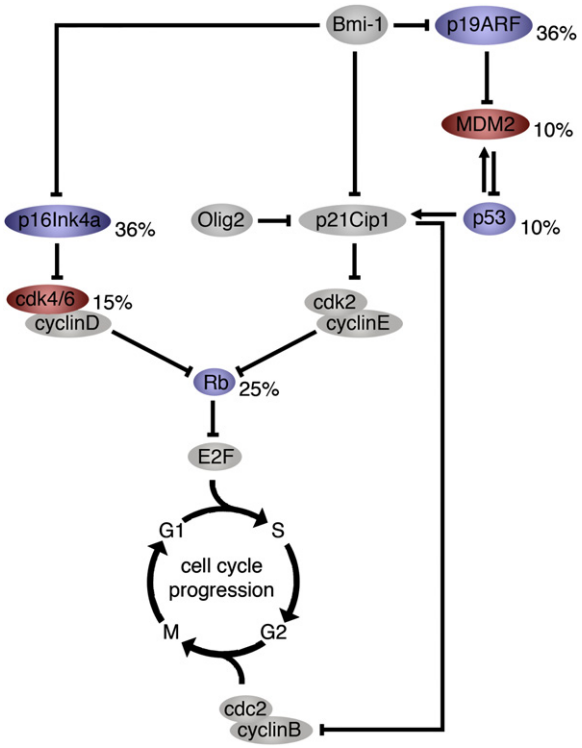

Figure 4. Common Cell-Intrinsic Mechanisms Sustain Replication Competence of Normal and Gliomagenic Neural Precursors

Generic components of the retinoblastoma (RB) cell cycle regulatory apparatus are shown. Negative regulators shown in blue are often deleted in malignant glioma, while positive regulators shown in red are frequently amplified, overexpressed, or mutated to a constitutively active state (reported frequencies indicated to the right of each symbol) (Sauvageot et al., 2007). The Polycomb gene silencer Bmi1 is thought to be a critical regulator of p21 gene expression during development and of $p16^{Ink4a}/p19^{Arf}$ expression in the postnatal brain. The bHLH transcription factor Olig2 is expressed in essentially 100% of glioma stem cells (as defined by coexpression of CD133) and directly suppresses expression of *p21*. See text for details.

malignant gliomas are readily apparent. The most common genetic lesions found in primary high-grade gliomas consist of loss-of-function mutations in the $p16^{Ink4a}$ and $p19^{ARF}$ negative effectors of the RB signaling axis. However, RB itself is sometimes targeted. Gain-of-function mutations in the positive effector CDK4 are also observed (Figure 4) (Sauvageot et al., 2007). The Bmi1 gene product is expressed in almost all human brain tumors irrespective of grade. The expression of Bmi1 has been shown to contribute to the malignancy of $p16^{Ink4a}/p19^{ARF}$ double null murine gliomas (Bruggeman et al., 2007). This observation is superficially at odds with the known role of Bmi1 in repressing the function of $p16^{Ink4a}/p19^{Arf}$ (Molofsky et al., 2005). As indicated in Figure 4, the $p16^{Ink4a}/p19^{ARF}$-independent function of Bmi1 might reflect an epistatic relationship with Olig2 and p21 (Fasano et al., 2007). The Olig2 transcription factor is expressed in 100% of adult astrocytomas, irrespective of grade (Ligon et al., 2004). In adult gliomas, Olig2 expression marks essentially 100% of the CD133-positive putative glioma stem cells. Olig2 is also required for tumor formation in the same $p16^{Ink4a}/p19^{ARF}$ double null murine model of adult gliomas used by Bruggeman et al. (2007). Finally, as noted above, Olig2 suppresses the expression of p21 (Ligon et al., 2007).

What do these data say about the glioma cell of origin? Glioma mutations found in general regulators of the cell cycle are equally compatible with neural progenitors or mature astrocytes as tumor cell of origin. However, Olig2 is not found in mature astrocytes (Lu et al., 2000; Takebayashi et al., 2000; Zhou et al., 2000). The gliomagenic requirement for Olig2 (albeit in a mouse tumor model) provides a small edge in favor of neural progenitors as cell of origin for glioma.

Prediction 3: Neural Progenitors Are Competent for Transformation by Mutations Found in Human Brain Tumors

"Tumor competence" is a necessary feature of glioma progenitor cells. The property of tumor competence in humans is provided by hereditary cancer syndromes such as Li-Fraumeni, retinoblastoma, or familial breast cancer syndromes caused by germline deletions of *p53*, *RB*, or *BRCA*, respectively. Patients with these hereditary cancer syndromes develop a narrow spectrum of spontaneous tumors despite tissue-wide distribution of the affected genes (Weinberg, 2007). Of particular relevance to the CNS, germline mutations of the *PTCH* gene, encoding a repressor of the Hedgehog pathway (see Figure 3), give rise to medulloblastoma in patients with Gorlin syndrome (Hahn et al., 1996; Johnson et al., 1996). The fact that Gorlin syndrome patients—and also $Ptch^{+/-}$ mice (Goodrich et al., 1997)—typically develop cerebellar tumors, but not forebrain glioma, indicates a particular susceptibility of a subset of precursor cells in the anterior posterior axis to respond to this particular oncogenic pathway.

There are several strategies to define progenitor compartments that are "competent" to produce tumors in vivo. Because the many studies employing this strategy in brain cancer research have been comprehensively reviewed (Furnari et al., 2007), we will confine our own discussion to a few illustrative cases. For example, expression of V(12) Ha-ras under control of GFAP regulatory sequences directs activated Ras signaling to this cellular compartment, yielding molecular insight into glioma formation and a highly penetrant model (Ding et al., 2001; Shannon et al., 2005).

Oncogenic rodent ecotropic retroviruses have provided useful insights into gliomagenesis (Assanah et al., 2006), but this approach targets a relatively broad range of proliferating cells including multipotent and restricted progenitors. Other viral systems select for certain classes of precursor cells. For example the adeno-associated virus serotype 5 (AAV5) utilizes PDGF receptors for initial entry into the host cell (Di Pasquale et al., 2003; Lotery et al., 2003). AAV5 expression vectors have been used to fate map the progeny of PDGF receptor-positive type B progenitor cells in the SVZ (see Figure 2) (Jackson et al., 2006); however, a potential complication in this experiment is that PDGFRa is also expressed on NG2 cells and oligodendrocyte precursors that are also present in the SVZ might also be targeted.

Holland and Varmus developed a more flexible means of targeting replication-competent avian sarcoma ("RCAS") virus expression vectors to specific cellular subcompartments in the

brain. The RCAS protocol uses promoter/enhancer regulatory sequences of interest to drive expression of TVA, the cell surface receptor for ALV avian retrovirus (Holland et al., 1998a; Holland and Varmus, 1998). Subsequent infection with avian sarcoma virus expression vectors in such transgenic mice is possible only in TVA-positive cells. This RCAS system has been used to introduce oncogenes (e.g., Ras), cell cycle regulatory factors (e.g., CDK4), growth factors (FGF, PDGF), growth factor receptor mutants (e.g., activated EGFRvIII), and signal-generating proteins (e.g., Akt) into subsets of nestin- or GFAP-expressing cells with additional temporal-spatial control conferred by infection (Dai et al., 2001; Holland et al., 2000, 1998a, 1998b; Holland and Varmus, 1998; Holmen and Williams, 2005; Tchougounova et al., 2007). The RCAS, GFAP-tva system has also been used to target type B adult neural stem cell populations (Figure 2) in fate-mapping studies (Menn et al., 2006) and has provided a means to introduce oncogenic events into this subclass of neural stem cells, which are implicated in gliomagenesis based on other studies. Despite its intrinsic flexibility as a platform for glioma modeling, the RCAS system is ultimately limited by reliability and specificity of the regulatory sequences to drive TVA gene expression in the rodent brain. Based on drawbacks discussed above regarding the available GFAP and Nestin promoters, new TVA-based transgenics based on regulatory elements from lineage- and/or stage-specific markers of stem cells and glial subtype developmental programs could provide important new tools for gliomagenic studies.

Parada and his associates have developed a true in vivo genetic model for malignant glioma that nicely illustrates the phenomenon of tumor competence. In this model, *GFAP-cre* recombinase transgenic mice are used to achieve targeted deletion of a floxed *Nf1* tumor suppressor gene in a p53 null background. The underlying genetic lesions for the tumors that arise in this model system are present from the onset in all *GFAP*-positive cells of the brain, including SVZ progenitors and the more mature astroglial cells of cortical white matter. Nevertheless the majority of the tumors that arise in this system at early times appear to originate from the SVZ (Zhu et al., 2005) wherein reside the GFAP-positive type B neural progenitor cells (Doetsch et al., 1999). As a whole, the phenomenon of tumor competence makes a rather compelling argument for neural progenitor cells as cell of origin for malignant gliomas. Multiple murine models show that neural progenitors are susceptible to malignant transformation by genetic lesions found in human tumors. By contrast, other cell types bearing identical lesions in vivo are not transformed.

It should be noted that none of the animal studies show that malignant gliomas arise directly from multipotent neural progenitors. In the genetic model described by Zhu et al. (2005) for example, all descendants of the GFAP-positive multipotent B cells will be genetically equivalent. The ultimate list of genetic equivalents includes type D transit-amplifying cells, some NG2-positive glia, and some oligodendrocyte progenitors (see Figure 2). Oligodendrocyte progenitors and NG2-positive glia are capable of migrating extensive distances away from the SVZ, and yet the majority of tumors arise initially within the SVZ. The spatial restriction could mean that only B cells and/or transit-amplifying D cells are competent for malignant transformation. However, there is an alternative explanation. One principle weakness in the competence argument is the question of cell autonomy. It could be argued for example that mature astrocytes in the model of Zhu et al. (2005) actually are competent for malignant transformation, but their malignant potential is not displayed because the cells are not positioned within a permissive "mitogenic niche." As has been noted, mature astrocytes or astrocyte progenitors are competent for malignant transformation when oncogenic mutations are introduced in vitro under conditions permissive for growth (Bachoo et al., 2002; Bruggeman et al., 2007). Although it is beyond the scope of this review, considerable attention is now being focused on symbiotic relationships between neural stem cells, glioma stem cells, and the blood vascular system (Bao et al., 2006b; Calabrese et al., 2007; Shen et al., 2004; Yang and Wechsler-Reya, 2007). Disruption of the support system afforded by the mitogenic niche might be a viable therapeutic strategy.

Prediction 4: Brain Tumors Will Initiate and/or Cluster near the Germinal Centers of the Brain

If gliomas are derived from multipotent type B cells of the SVZ in humans, then brain cancers might be expected to cluster within or near this germinal region, at least at the time of inception (Figure 2). If gliomas arise from committed astrocytes or NG2 cells, a more diffuse distribution of tumors might be expected. As indicated in Figure 5, the raw clinical data are actually more consistent with astrocytes or NG2-positive glia as tumor cells of origin. At the time of diagnosis, malignant gliomas are typically imaged within the brain parenchyma at a distance from the SVZ in subcortical white matter, along blood vessels and in subpial collections. However, malignant gliomas proliferate rapidly and also migrate rapidly along white matter tracts and throughout all layers of the brain. Thus, at later stages of glioma development, when patients become symptomatic from the mass effect of a large tumor, the initial anatomical ties to the SVZ might be obscured.

This hypothetical scenario is gaining support from several lines of evidence. In the *GFAP-cre*, floxed *Nf1*, $p53^{-/-}$ astrocytoma model (described above), gliomas arise with high penetrance and at predictable times (Zhu et al., 2005). Accordingly, the tumors can be tracked from their onset by cranial MRI and by immunohistochemical analysis of brains from animals euthanized at sequential stages of development. These data show that the SVZ is the site where gliomas first develop. At early times, focal zones of hyperplasia are noted in the SVZ of affected mice. At later times, thin streams of mitotic cells are observed along tracts of white matter leading to the cortex. Ultimately, these migratory stalks are obscured. The trophic and/or chemotactic factors that regulate migration of glioma progenitors in this model, as well as human glioma, are unclear. In this respect, it is interesting to consider that EGFR overexpression, found commonly in human glioma, is sufficient to confer migratory properties to neural progenitors (Aguirre et al., 2005). Similarly, SVZ c-cells are EGF responsive and respond with mass-like proliferations, high motility, and migration along white matter tracts and blood vessels (Doetsch et al., 2002). Other studies suggest that PTEN and HIF signaling in tumor cells and stroma, respectively, also

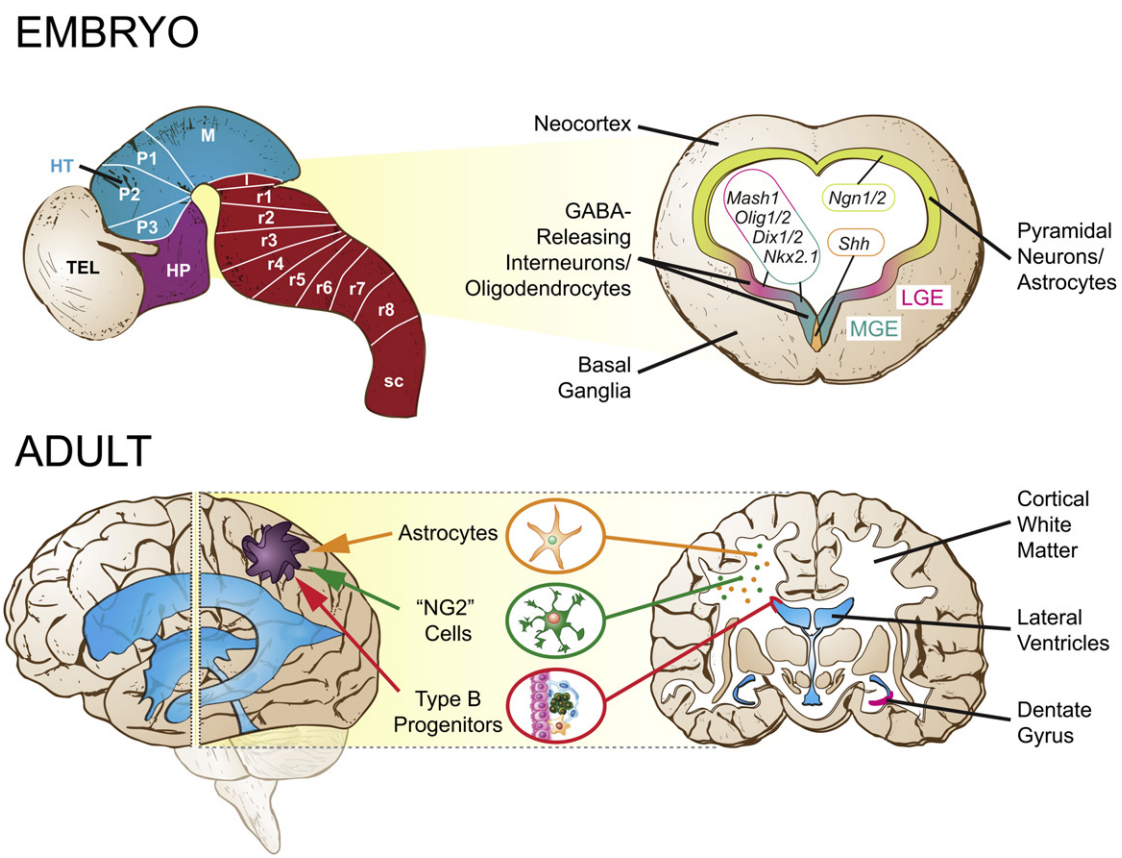

Figure 5. Speculative Relationship of Glioma Cell of Origin to Subsets of Developmentally Distinct Neural Progenitors
(Top) The embryonic CNS is patterned by organizing signals (e.g., Sonic hedgehog [Shh]) and activity of homeodomain and bHLH transcription factors into discrete domains of progenitor cells in the anterior-posterior and dorsal-ventral axes. Such pattering could relate to ultimate heterogeneity and tumor competence of subsets of persistent neural progenitors in the adult brain. Shown at left are prosomere and rhombomere boundaries of the embryonic brain. At right, restricted domains of neuronal and glial progeny subtype production suggested by recent work are indicated. (Bottom) If gliomas are derived from multipotent type B cells of the SVZ in humans, then brain cancers might be expected to cluster within or near this germinal region, at least at the time of inception. Subsequently, they could migrate to the cerebrum. Alternatively, gliomas might arise from committed astrocytes or NG2 cells. See text for details.

dramatically regulate tumor cell migration (Blouw et al., 2003; Xiao et al., 2005).

As previously mentioned, because human gliomas usually present at a late stage, and due to the relatively low resolution of current imaging modalities, it has not been possible to serially track tumor progression from progenitors of the SVZ. In recent work, the spatial relationship of the contrast-enhancing gliomas to the SVZ was examined, revealing a significant subset of tumors that either contacted or were located with proximity to the SVZ (Lim et al., 2007). Thus, the possibility that human glioma could arise from fundamentally similar cells as those resident in the SVZ remains open and is a compelling area for further research (Sanai et al., 2005; Vescovi et al., 2006). In summary, the weight of the imaging data from mouse models and a recent clinical study are modestly supportive of the hypothesis that adult neural progenitors in the SVZ serve as cell of origin for glioma stem cells. However, if brain cancers indeed arise in the SVZ, one must assume that the tumorigenic precursors migrate rather rapidly to more favorable "mitogenic niches" of the brain for tumor expansion before clinical symptoms arise.

Prediction 5: Genes that Govern Replication Competence of Neural Progenitors Will Serve as 'Gatekeepers' for Development of CNS Cancers

A final prediction of the cancer stem cell hypothesis is that similar lineage-restricted genetic requirements for development and survival of stem cells during organogenesis will apply to cognate primary cancers (Garraway and Sellers, 2006). For example, the transcription factor *MITF* is required both for normal melanoblast development and generation of melanoma (McGill et al., 2002). Common genetic requirements for tissue development and tumor formation support the proposition that proliferation and survival of tumor cells may be dependent on the same "gatekeeper" pathways that govern early phases of organogenesis. In principle, these tissue-type-specific gatekeeper functions could be targeted for cancer therapy with the benefit that "off target" collateral damage to other organs would be limited.

In the CNS, OLIG2 might be considered an example of one such gatekeeper for malignant stem cells in glioma because it regulates two cardinal features of stem cells: multipotency and self-renewal. The archetypal hematopoietic stem cell can

produce multiple differentiated lineages depending on environmental conditions. In the CNS, neurospheres capable of generating neurons, astrocytes, and oligodendrocytes can be cultured from certain parts of the brain in the presence of mitogens such as FGF and/or EGF. The mitogen-induced expansion of these multipotent neurospheres seems to select for cells that express Olig2 (Ligon et al., 2007). Olig2 function, moreover, is necessary to regulate the full potential of such cells to produce all three neural lineages: neurons, astrocytes, and oligodendrocytes (Zhou and Anderson, 2002). Pathological overtones of the link between Olig gene function and cell proliferation are found in human astrocytomas, wherein 85% of all proliferating cells and virtually 100% of all CD133-positive cells are positive for Olig2 expression. In "genetically relevant" $p16^{Ink4a}/p19^{Arf}$ null, EGFRVIII-positive tumor neurospheres, a model of WHO grade III astrocytoma (Bachoo et al., 2002), Olig2 function is necessary for tumorigenesis (Ligon et al., 2007).

In summary, there is a common genetic requirement for replication competence in a subset of neural progenitors and for tumor formation in a murine model of primary glioma. This observation supports the cancer stem cell hypothesis and cannot be reconciled with committed astrocyte progenitors or their progeny as cell of origin for glioma. Further work is needed to determine whether these observations are relevant to the biology of other forms of CNS cancer in adults and children and, in particular, whether gliomas with other oncogenic mutations than those tested above also require OLIG2 function.

Cancer Stem Cells and the Cell of Origin for Malignant Glioma: Reprise

Malignant cells with stem-like properties have been found in a wide range of adult and pediatric brain cancers. Do these cancer stem cells arise from developmentally arrested progenitor cells that drive normal brain development and tissue repair? Five testable predictions of this hypothesis are more or less supported by the literature reviewed here. However, in the words of Enrico Fermi, "*Experimental confirmation of a prediction is merely a measurement. An experiment disproving a prediction is a discovery*." We have noted ambiguities, loopholes, gray areas in the data, and additional work to be done for each of the predictions tested here. Committed progenitors for blood or brain can clearly acquire stem-like properties when transformed in vitro (Bachoo et al., 2002; Krivtsov et al., 2006). A retrograde, somatic-to-stem cell path may be an occasional or even a frequent route to the neoplastic state for some types of brain cancer. It is only Ockham's razor, applied to the data in toto, that currently favors a multipotent stem cell origin for malignant glioma.

In accord with the subjective state of the field, terms such as "tumor-initiating cells" or "tumor-propagating cells" are sometimes substituted for "cancer stem cells" because the later term implies unwarranted insight into the tumor cell of origin (Hill and Perris, 2007; Kelly et al., 2007). We agree that the "stem cell" descriptor is somewhat prejudicial. However, for CNS tumors, the cancer stem cell hypothesis has thus far provided (1) a template for experimental design and (2) an incentive for neuro-oncologists to think about fundamental problems in brain development. Going forward, we believe that the benefits of terms such as "cancer stem cell" or "glioma stem cell" as consciousness-raising devices justify some modest concessions to semantic rigor.

The Road Ahead

Prominent milestones in the cancer stem cell hypothesis and glioma stem cells are summarized in Table 1. Note the pivotal role of hematology and the hematologic malignancies in development of the cancer stem cell paradigm. However, analogies between cancers of the blood and cancers of the brain can only be taken so far. Further progress may come at the points where development of blood and brain diverge.

Unlike hematopoiesis, CNS development proceeds in precise relation to position in the dorsal-ventral and anterior-posterior axes (Kiecker and Lumsden, 2005; Tanabe and Jessell, 1996) (Figure 5). For example, bHLH and homeodomain proteins expressed in spatially restricted domains of the ventral neural tube and forebrain have been shown to govern oligodendrocyte and astrocyte subtype specification (Hochstim et al., 2008; Lu et al., 2002; Muroyama et al., 2005; Petryniak et al., 2007; Zhou and Anderson, 2002). Recent studies show that progenitor zones within the adult SVZ are likewise spatially diversified. Stem cells that are separated from each other by distances of far less than a millimeter can have very different fates (De Marchis et al., 2007; Merkle et al., 2007; Young et al., 2007). Differences in progenitor pools within the anterior-posterior axis of the CNS (Klein et al., 2005; Lee et al., 2005a) might also be reflected in the molecular phenotype or behavior of gliomas (Sharma et al., 2007). In future years, a precise characterization of progenitor cell subtypes with specific markers may shed light on new tumor-competent subsets in the postnatal brain.

Neural progenitors also obey temporal restrictions that determine their ability to produce neurons or glia during development (Anderson et al., 2001; Edlund and Jessell, 1999; Ligon et al., 2006a; Liu and Rao, 2004). With respect to human glioma, temporal restriction on progenitor cells is probably best exemplified by dramatic differences observed in the location and underlying mutations observed in pediatric versus adult glioma. Adult gliomas are typically found in the supratentorial region and are characterized by mutation of *PTEN*, *INK4A/ARF*, *P53*, and activation or upregulation of EGFR expression (Figures 3 and 4). In contrast, pediatric astrocytomas are prominently found in infratentorial locations such as the brainstem. Few of the genetic lesions associated with adult gliomas (e.g., PTEN mutation and EGFR amplification) have been found in pediatric gliomas (Biegel and Pollack, 2004; Pollack et al., 1997, 2006; Ullrich and Pomeroy, 2006). The time of onset, location, and different genetic properties of pediatric and adult glial tumors suggest different modes of tumorigenesis.

Overshadowing all of these academic concerns is the issue of targeted therapy. All of the mutations currently associated with malignant glioma lie in genes and signaling pathways that are broadly expressed and generally required for cell growth and survival (Figures 4 and 5). In principle (if not quite in practice), small molecule inhibitors of growth factor receptors such as EGFR and intracellular signal-generating proteins such as PI3 kinase are no more selective for brain cancer cells than radiation or conventional chemotherapeutic drugs. If cancer genetics cannot

deliver targeted therapy, are there opportunities at the interface of development biology and cancer cell biology?

Already, monoclonal antibodies to a drug export protein found in progenitor cell subsets of normal skin have been shown to have therapeutic value against human melanoma stem cells (or melanoma-initiating cells) in mouse xenograft models (Schatton et al., 2008). Other potential drug targets may be embedded in transcription factors that direct the fate choice decisions of uncommitted progenitor cells. As noted above ("Prediction 5"), the MITF and Olig2 transcription factors, required for development of normal melanoblasts and CNS progenitors, respectively, serve also as "gatekeeper" genes for malignant melanoma and malignant glioma. Transcription factors are currently regarded as suboptimal targets for drug development. However, some transcription factor functions can be opposed at posttranslational levels, and new chemical technologies may enable direct inhibition of critical protein-protein interactions (Bernal et al., 2007; Walensky et al., 2004).

These practical overtones of the cancer stem cell hypothesis cannot come soon enough. A new generation of targeted therapeutics would be a boon to growing children with low grade astrocytomas who are currently being treated with radiation and cytotoxic drugs. For adults with malignant glioma, the need is for effective therapies of any sort. The median interval from diagnosis to death for adult patients with glioblastoma multiforme is currently only 14 months.

ACKNOWLEDGMENTS

The authors are grateful to Eric Smith and Claire Sauvageot for graphic artwork and to Ben Barres and Nader Sanai for helpful comments. Work from the authors' laboratories cited here was supported by grants from the NIH (PO1NS047572 and R01NS057727) and by the Pediatric Low Grade Astrocytoma Program. D.H.R. is an HHMI Investigator.

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