The potential origin of glioblastoma initiating cells

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TABLE OF CONTENTS

1. ABSTRACT

Despite intensive clinical and laboratory research and effort, Glioblastoma remains the most common and invariably lethal primary cancer of the central nervous system. The identification of stem cell and lineage-restricted progenitor cell populations within the adult human brain in conjunction with the discovery of stem-like cells derived from gliomas which are themselves tumorigenic and have been shown to have properties of self-renewal and multipotency, has led to the hypothesis that this population of cells may represent glioma initiating cells. Extensive research characterizing the anatomic distribution and phenotype of neural stem cells in the adult brain, and the genetic underpinnings needed for malignant transformation may ultimately lead to the identification of the cellular origin for glioblastoma. Defining the cellular origin of this lethal disease may ultimately provide new therapeutic targets and modalities finally altering an otherwise bleak outcome for patients with glioblastoma.

2. INTRODUCTION

Glioblastoma is the most common and lethal primary malignancy of the central nervous system (CNS) representing approximately 50% of all gliomas (1). With a median survival of 14-16 months, and 2-year survival rate of less than 10-15% using today's best treatment modalities, improvements in survival over the past 100 years can be measured in weeks (2). These tumors represent a relentless disease state with a clear propensity towards migration and invasion within and into the neural parenchyma along with recurrence at both local and distant sites (3).

Traditional views have held that these tumors originate from the malignant transformation of differentiated glial components of the CNS (i.e. astrocytes and oligodendrocytes) (4). The discovery of multipotent stem cell and lineage-restricted progenitor cell populations in the CNS of the postnatal mammalian brain, and

subsequent identification of stem-like cells in several primary brain tumors including glioblastoma has led to the development of the Cancer Stem Cell (CSC) theory of gliomas challenging this hypothesis (4-7). This theory posits that rather than the de-differentiation of mature cell populations being the mechanism behind gliomagenesis, gliomas are instead derived from the malignant transformation of cells derived from this newly defined neural stem cell or lineage-restricted progenitor population. Moreover, accumulating evidence suggests that these glioblastoma-derived stem-like cells are responsible for the invasiveness and resistance to treatment that characterizes glioblastoma (8-15).

Based on the cancer stem cell theory, extensive research defining the phenotypic as well as genetic similarities between adult neural stem cells (NSCs) and glioma initiating cells (GICs) is on going in the hopes of decisively identifying the cellular origin of this tumor, potentially opening the door to new therapeutic targets and modalities. In this article, we discuss evidence supporting the neural stem cells and lineage restricted progenitor cells as the origin of glioblastomas, and specifically as a source of glioma initiating cells (GICs).

3. HYPOTHESES REGARDING THE CELLULAR ONTOLOGY OF GLIOBLASTOMA: CANCER STEM CELL (CSC) MODEL

Traditionally it was thought that mature cells of astroglial or oligodendroglial lineage, through a number of genetic changes, underwent neoplastic transformation to yield gliomas (16). According to this theory, mutations permitting mature, differentiated cells to re-enter a relatively undifferentiated state through the removal of cell-cycle check points, blockade of apoptotic pathways, and the re-activation of developmental pathways were required for the formation of gliomas (16). This theory was in large part based on the widely held belief that the brain was a quiescent organ, incapable of post-natal neurogenesis and the idea that astrocytes and oligodendrocytes were the only cell populations capable of division in the mature brain (17).

Beginning with the findings of Altman and Das demonstrating neurogenesis in the adult rat brain (18, 19) and the subsequent findings in the canary by Goldman and Nottebohm (20), this dogma has been increasingly challenged. In the years following these discoveries, numerous studies have demonstrated that adult mammalian brain is capable of continued neurogenesis; a finding that has been confirmed in the adult human brain (21, 22). This process of neurogenesis in the adult brain has been primarily confined to the forebrain subventricular zone (SVZ) and the subgranular zone (SGZ) of the dentate gyrus (21-32). Within these locations in both rodents and humans, a population of astrocytes has been described which have the properties of self-renewal and multipotentiality consistent with a neural stem cell identity (22, 27, 33, 34). Subsequent to the discovery of stem cell populations residing within the SVZ and SGZ of the central nervous system, several groups reported the presence of

stem cell-like populations isolated from glioblastoma tissue (6, 7, 35). In the appropriate culture conditions, it was found that a subset of tumor cells from surgical explants could generate neurospheres which could give rise to neurons, astrocytes and oligodendrocytes, a previously described property of NSCs (6, 35). Additionally, glioblastoma-derived neurosphere cells could recapitulate histologically and cytologically, a tumor resembling the initial glioblastoma in an immunocompromised murine xenograft model (6, 7).

Growing evidence from a number of cancers has supported the theory that most if not all tumors in humans are comprised of a heterogeneous population of cells which have varying tumorigenic potential (36-38). Initially demonstrated in leukemia and subsequently in several solid tumors including those from breast, head and neck, colon, pancreatic, and prostatic cancer, it was shown that only a subset of cells isolated from patients possessed the ability to proliferate *in vitro*, differentiate into mature forms under the proper conditions, and form tumors in xenograft models having characteristics resembling the parent malignancy (39-44). These findings led to the hypothesis that within a tumor, a stem cell-like population is primarily responsible for its tumorgenicity; hence they are referred to as Cancer Stem Cells. The discovery of cell populations derived from glioblastoma which demonstrated these properties has led to the hypothesis being extended to include glioblastoma. Taken together the hypothesis has been made that GICs are derived from NSCs and by extension, arise from the SVZ (4, 7, 21, 45-50).

4. SOURCES OF NSCs AND LINEAGE-RESTRICTED PROGENITOR CELLS IN THE POST-NATAL MAMMALIAN BRAIN

As mentioned earlier, NSCs and lineage-restricted progenitor cells, which are defined as cell subpopulations capable of self-renewal and differentiation into multiple lineages (51), have been described in several regions of the adult mammalian brain including the SVZ, SGZ of the hippocampal dentate gyrus, striatum, frontal and temporal cortex, as well as the subcortical white matter (24, 51-55). Of these regions, the SVZ has been found to be the largest reservoirs of these cells in both the adult rodent and human brain.

4.1. Organization of the Rodent Subventricular Zone

In the adult rodent brain, the SVZ lines the lateral wall of the lateral ventricles bilaterally underlying the ependymal layer (Figure 1). Within this region, resides a population of slowly dividing astrocyte-like NSCs termed type B1 cells (27, 56). These cells, believed to be derived from a neuroepithelial lineage that has also been shown to be the source of the ependymal lining of the ventricular wall, is thought to be responsible for neurogenesis throughout the adult life (57).

Through asymmetrical divisions, type B1 cells within the SVZ give rise to type C cells, a rapidly proliferating cell population, alternatively referred to as transit-amplifying cells. Immature neuroblasts or type A
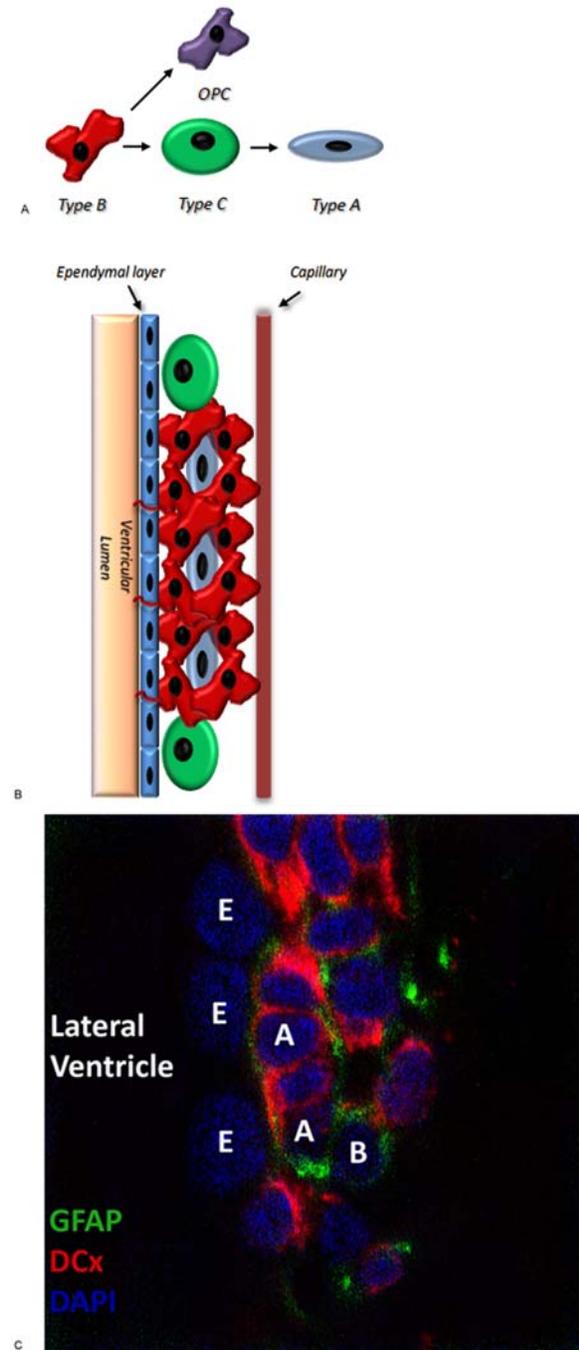
Figure 1. Structure of the Rodent SVZ. (A) Drawing depicting the progression of neural stem cell (type B) to oligodendroglial precursors (OPC) or transit amplifying cells (type C) to immature neuroblasts (type A). (B) Drawing depicting the cytoarchitecture and cellular composition of the rodent SVZ. (C) Immunohistochemistry (40x) of the rodent SVZ. GFAP – glial fibrillary acidic protein; DCx – doublecortin; DAPI - 4',6-diamidino-2-phenylindole; E—ependymal cells; A—type A cells; B—type B cells.

cells, derived from this pool of transit-amplifying cells subsequently migrate from the SVZ in chains surrounded by the glial processes of type B2 cells towards the olfactory bulb comprising the rostral migratory stream (RMS). These migrating neuroblasts ultimately contribute to various populations of interneurons of the granule layers of the olfactory bulb (26, 58-60).

Interestingly, the SVZ has also been shown to be a source of oligodendrocytes both during development and in response to demyelinating disease (61, 62). Menn *et al.* found that the source of these SVZ-derived oligodendrocytes was a subpopulation of Olig2 expressing type B cells which migrate into the subcortical white matter in a manner orthogonal to the RMS where they became local oligodendrocyte progenitor cells (OPCs) (63). Though the majority of research is focused on cells within the SVZ, this population of subcortical progenitor cells cannot be excluded as a possible source of GICs. Consistent with this hypothesis, using a murine model of oligodendroglioma, Persson *et al.* recently found that OPCs, and not NSCs, enriched for a tumor-forming cell population (64).

4.2. Organization of the Human Subventricular Zone

In contrast to the structure of the rodent SVZ where astrocyte-like stem cells directly oppose the ventricular ependyma, a feature also described in the SVZ of canines and non-human primates, the human SVZ possesses a more complex organization comprised of four distinct layers including a hypocellular gap separating the presumed stem cell population from the ependyma (Figure 2; (12, 21, 22, 65-67)). From innermost to outermost layers these are: layer I is a monolayer of ependymal cells lining the ventricular cavity; layer II is an immediately adjacent is the hypocellular gap; layer III is a ribbon of astrocytes in which a population of neural stem cells is felt to reside; and layer IV is referred to as the transitional zone comprised primarily of myelinated fibers.

Unique to the adult human SVZ, the hypocellular gap is rich in glial fibrillary acidic protein (GFAP) expressing processes with ependymal expansions and an abundant network of astrocyte-astrocyte and astrocyte-ependymal interconnections. The function of these interconnections is unclear though it has been hypothesized that they may regulate neuronal function, play a role in metabolic homeostasis, or control NSC proliferation and differentiation (21, 68-70). Layer III, comprised principally of large astrocyte-like cells is the layer in which mitotic bodies consistent with dividing stem cells have been identified, although not to the degree seen in the SVZ of other mammals such as the rodent and non-human primates (21, 22). While neuron-like cells have been identified between layers III and IV which appear to be migratory based on their morphology, the presence of definitive migrating chains of neuroblasts has not been shown (21, 22, 71). Curtis *et al.* however have argued that an RMS-like structure in fact does exist in the adult human brain with chains of neuroblasts arranged around a ventricular remnant extending from the anterior horn of the lateral ventricle to the olfactory bulb. Subsequent studies
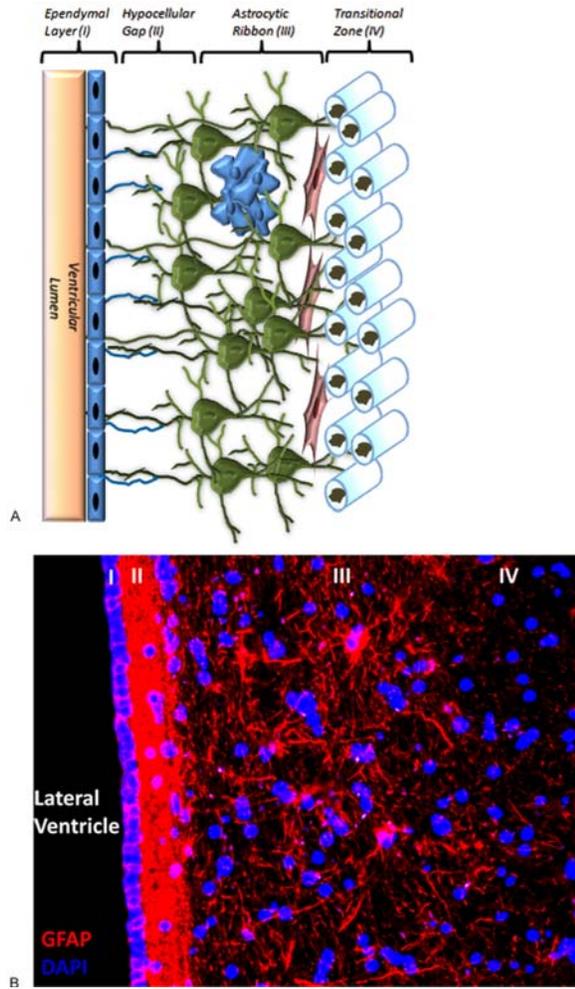
Figure 2. Structure of the Human SVZ. (A) Drawing depicting the cellular composition and cytoarchitecture of the human SVZ. Green cells represent layer III astrocytes, blue cells are a trapped ependymal cell rest, and elongated red cells are migratory neuron-like cells characterized at the layer III-IV interface (see text for more information). (B) Immunohistochemistry (20x) depicting the four layers that make up the human SVZ (Layer I – Ependymal layer, Layer II – Hypocellular gap, Layer III – Astrocytic ribbon, Layer IV – Transitional zone). GFAP – glial fibrillary acidic protein; DAPI – 4',6-diamidino-2-phenylindole.

by Bradford *et al.* recently provided further support of this finding demonstrating expression of Neogenin, a Netrin/RGMa receptor thought to be a marker of neurogenesis descriptive of the rodent RMS, in basal forebrain of humans along what is purported to represent the human equivalent of the RMS (71). Research in our laboratory has confirmed the presence of a structure analogous to the RMS of rodents in human tissues of fetal origin though their applicability to the adult human cortex remains controversial (71-75).

Given the fundamental differences in SVZ anatomy noted between the rodent and human brain, it is not unreasonable to expect that different developmental pathways and structures are at work explaining the failure to definitively demonstrate populations of stem cells and progenitor cells having the same or similar properties in both. In rodents, robust neurogenesis can be seen in the SVZ extending out through the RMS which experiences a decline as the animal ages (76). In contrast with this finding, neurogenesis in the human SVZ, both in the fetal and adult brain is of relatively smaller magnitude though clearly still present (21). This noted disparity should however be interpreted with caution; studies characterizing the anatomy of the rodent SVZ and its associated patterns of neurogenesis have been performed in both embryonic and relatively young mice in comparison to investigations in humans where though fetal tissues have been examined, adult neurogenesis has largely been evaluated in the aged brain which is unlikely to be equivalent to that of the young rodent.

Further illustrating the disparities between rodent and human SVZ architecture, Hansen *et al.* recently described a population of radial glia-like cells populating the outer layers of the SVZ (OSVZ) of the human neocortex, not seen in the rodent brain, which are capable of undergoing both proliferative divisions and self-renewing asymmetric divisions giving rise to neuronal progenitor cells which are themselves capable of further proliferation indicating yet another potential source of stem cells associated with the SVZ (77). These radial glia cells are distinctly different from the traditionally defined population in that while they project apical processes toward the pial surface, the majority fail to show basal processes extending to the ventricular wall (77). Though of unclear significance, this population of radial glia-like cells is thought to represent in part, a mechanism allowing for the neocortical expansion defining of humans and higher primates (77). Furthermore, it is not unreasonable to consider the possibility that these cells may represent yet another potential source of GICs.

Despite the controversies surrounding the functional anatomy of the human SVZ and the striking differences seen between it and other mammalian SVZs, it is clear that neural stem cell-like populations can be isolated from SVZ explants though the exact *in situ* localization of these populations remains elusive for now (22, 77).

5. PUTATIVE MALIGNANT TRANSFORMATION OF NSCs AND LINEAGE-RESTRICTED PROGENITOR CELLS

Unlike mature, differentiated components of the CNS such as astrocytes and neurons, stem cells and lineage-restricted progenitor cells remain highly proliferative resulting in increased susceptibility to malignant transformation of neural stem cells. Exposure to *N*-ethyl-*N*-nitrosourea (ENU) or avian sarcoma virus has been shown to result in tumor formation preferentially within the SVZ of the canine and rodent brain rather than in areas with less proliferative capacity (78-81). Feldkamp *et al.* found in a murine model that exposure of progenitor
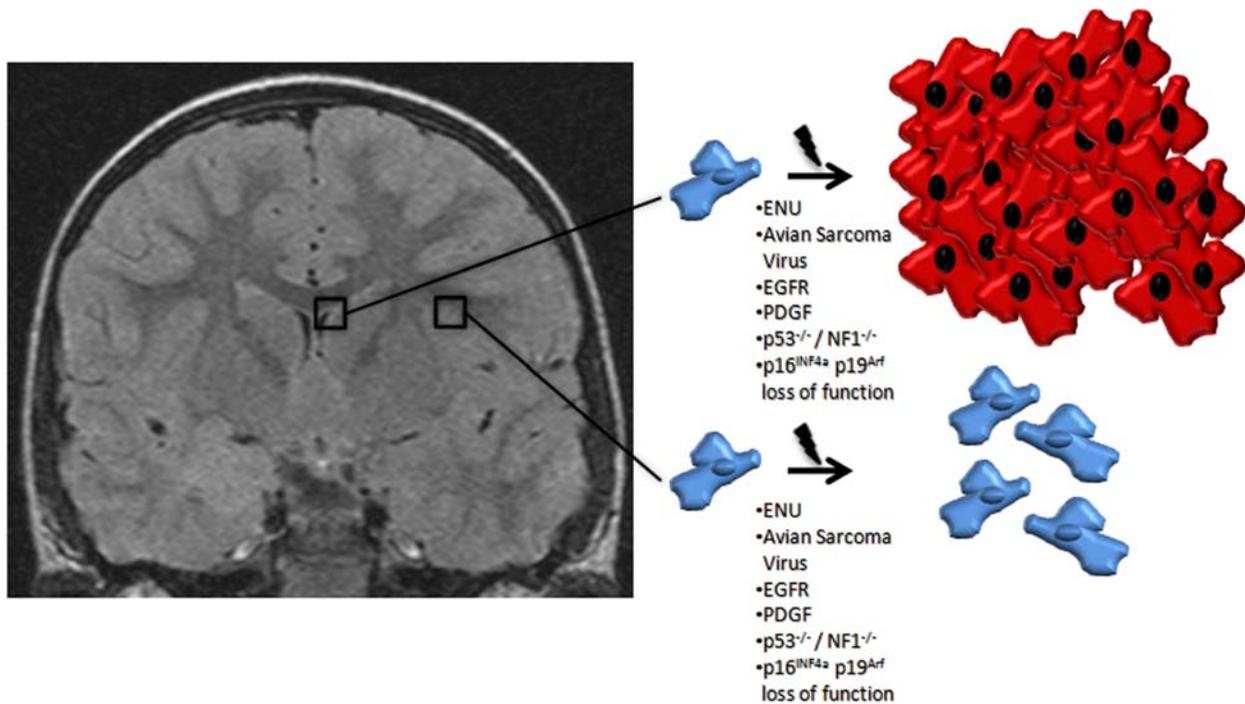
Figure 3. Differential oncologic effects upon cells of the human brain with varying proliferative potential. Left: T1-weighted coronal MRI of the human brain. Top right: Drawing depicting the putative effect of chemical and mutational insults experimentally proposed to be involved in glioblastoma formation upon SVZ derived cells with proliferative capacity. Bottom right: Drawing depicting the absent or reduced effect upon subcortical/cortical cells known to have decreased proliferative capacity compared with the SVZ of chemical and insults experimentally proposed to be involved in glioblastoma formation. ENU - *N*-ethyl-*N*-nitrosourea; EGF - epithelial derived growth factor; PDGF - platelet derived growth factor, NF – neurofibromatosis.

cells to high levels of epithelial derived growth factor (EGF) or platelet derived growth factor (PDGF) resulted in the formation of tumors histologically resembling human gliomas (82). Furthermore, ventricular infusion of EGF or PDGF results in increased proliferation of SVZ progenitor cells and the development of highly invasive glioma-like masses (46, 83). Agreeing with these findings, constitutively activating mutations and/or increased expression of EGF and PDGF receptors as well as autocrine PDGF/PDGFR signaling are known to be present in malignant gliomas (84-87). Both PDGF and EGF signaling can feed through the Ras/Akt pathways, widely known to be significant in oncogenesis, and retroviral delivery of activated Ras and Akt to mouse progenitor cells induces high-grade glioma formation (88). As with other malignancies, disruption of tumor suppressor function has also been found to result in glioma formation; $p53^{-/-}/NF1^{-/-}$ dual knockout mice develop spontaneous lesions associated with the SVZ closely mimicking human glioblastoma in appearance and behavior (Figure 3; (89)).

Interestingly but not necessarily surprising is the decline noted in the size of the stem cell population as the brain ages (90). Central to this decline is the upregulation of $p16^{INK4a}$ and $p19^{Arf}$ expression which are involved in cellular senescence. Deletion of $p16^{INK4a}$ partially rescues the age-related decline in the stem cell population of the mouse SVZ (90). $p16^{INK4a}/p19^{Arf}$ loss of function mutations are one of the most prevalent genetic alterations

in glioblastoma (91); these changes however are not sufficient to initiate gliomagenesis on their own, but can in conjunction with EGFR activation, stimulate glioma formation in both murine NSCs and differentiated astrocytes (92).

The Notch signaling pathway represents another developmentally important transcriptional pathway implicated in glioma propagation. Under normal circumstances, Notch is involved in the maintenance, but not the generation of the neural stem cell population. Mice with homozygous deletion of the Notch1 gene have rapidly depleted neural stem cell populations during embryonic development (93, 94). *In vitro*, blockade of Notch by gamma secretase inhibitors (GSIs) reduced neurosphere formation through reduced proliferation and increased apoptosis where in contrast, expression of active Notch1 increased tumorgenicity (95). *In vivo,* GICs pretreated with GSIs failed to form tumors upon subcutaneous injection into nude mice. Similarly, delivery of GSIs via drug impregnanted polymer beads blocked tumor growth and promoted survival (95).

6. SIMILARTIES BETWEEN NSCs AND GLIOMA INITIATING CELLS

NSCs and lineage-restricted progenitor cells of the SVZ have unique expression patterns of tumor suppressor genes, cell surface markers, cytoskeletal

proteins, transcription factors and growth factors/growth factor receptors which are largely shared by glioma stem cell-like populations. Additionally, glioblastomas have been shown to orchestrate a vascular niche mimicking the normal neural stem cell niche thereby helping to maintain the glioma initiating cell pool (45).

6.1. Transcription factors

One of the arguments for NSCs being a source of GICs is the expression of early developmental genes which persist in GICs contributing to proliferation and self-renewal. The background upon which these genes act in conjunction with possible changes in the regulation of a given pathway likely makes the distinction between the normal, and the tumorigenic state seen in glioblastoma.

For example, Sonic Hedgehog (Shh) is a central regulator of patterning and proliferation in the cerebellum during development (96-98). Dysregulation of the Shh pathway in neural progenitors in the external granular layer of the cerebellum by mutations of Patched, the Shh receptor, constitutive activation of Smoothened, or over expression of N-myc lead to the development of medulloblastoma (99-102). Similar to medulloblastoma, Shh signaling is also implicated in the pathogenesis of glioblastoma. Shh modulates the activity of three zinc-finger transcription factors, Gli1, Gli2, and Gli3, which are involved in proliferation and self-renewal by promoting cell-cycle entry and DNA replication (103, 104). Gli1 is normally expressed by progenitor cells in the SVZ where they are help to maintain the stem cell population (105-107). Gli1 expression has also been found in both low- and high-grade gliomas where the Shh-Gli1 axis is thought to be involved in maintenance of GICs and tumorigenesis (96). Supporting this theory, administration of cyclopamine, a Shh inhibitor inhibits the *in vitro* growth of some glioma stem cell-like lines (108).

6.2. Tumor suppressor genes

Phosphatase and tensin homologue deleted on chromosome 10 (PTEN) is a tumor suppressor gene which lowers phosphatidylinositol phosphate (PIP3) levels enhancing rates of apoptosis and also limits cell motility through a G protein-coupled mechanism (109, 110). PTEN expression in the SVZ is involved in control of stem cell and precursor cell proliferation, precursor cell migration, as well as being required for neuronal differentiation from precursor cells of the SVZ (111-114). Deletion of PTEN leads to persistent neural stem cell self-renewal and expansion of the SVZ while decreasing apoptosis (115, 116). Similarly, deletion of PTEN in gliomas increases the size of the side population and hence ABCG2 transporter expression and activity, which are thought to be mainly associated with stem cell populations (9). In primary or *de novo* glioblastoma, PTEN mutations are frequently described; interestingly epigenetic silencing of PTEN through promoter methylation is described in low-grade tumors and secondary glioblastoma (117).

p53, another tumor suppressor gene, with a key role in regulating DNA repair pathways, is also frequently deleted or mutated in glioblastoma (4, 118). By itself, p53

mutation is insufficient to induce glioma formation requiring additional carcinogenic or genetic insults such as exposure to ENU, constitutive activation of the Ras pathway, or increased PDGF signaling (89, 119, 120). Co-deletion of the neurofibromatosis (NF)-1 tumor suppressor has been shown to cooperate with p53 deletions, inducing glioma formation in mouse models (89). Additionally, NF-1 patients are characterized by an increased frequency of glioblastoma occurrence (121, 122).

6.3. Growth factors / cytokines and their receptors

As was described above, EGF and PDGF have a pronounced effect upon NSCs, capable under the right circumstances of causing hyperplasia and proliferation resulting in the development of periventricular neoplasms akin to early gliomas (46, 83-87). In human gliomas, EGFR amplification or expression of EGFRvIII, a constitutively active mutation, is seen in nearly half of all high-grade tumors (123-125). In the rodent SVZ, EGF prevents C cell differentiation and encourages infiltrative behavior similar to that seen in high-grade gliomas (83). Similarly for PDGF, a role has been defined in the normal stem cells niche of the adult SVZ; a population of type B cells expressing PDGF can give rise *in vivo* to both oligodendrocytes and neurons (46). Experimental over-expression of PDGF induces areas of proliferation and hyperplasia within the SVZ similar to those seen in early gliomagenesis (46).

Transforming growth factor (TGF)-beta has also been shown to have a place in normal SVZ function, having a role in progenitor cell differentiation (126, 127). In glioblastoma, TGF-beta expression has been demonstrated by several groups including our own (unpublished observation). In glioblastoma, TGF-beta expression has been described in both whole tumor samples and specifically in GIC populations *in vitro* and in *in vivo* experimental models. Under these circumstances, TGF-beta likely contributes to the development of the immunosuppressive phenotype described in glioblastoma involving both the Treg subset of T cells and macrophage/microglial populations (128-132).

6.4. Vascular niche

Stem cells of all tissues and organisms reside in unique microenvironments or niches (133, 134). Within the SVZ of the mammalian brain, clusters of capillaries, associated growth factors such as brain-derived neurotrophic factor (BDNF), vascular endothelial growth factor (VEGF), and extracellular matrix components such as tenascin-C and chondroitin sulfate, create an environment which fosters stem cell growth and regulates proliferation and cell fate through cross talk and maintenance of local environmental factors such as pH and oxygen tension (135-142). Similarly, gliomas are typically highly vascularized tumors often with extensive capillary beds that can provide a similar vascular niche for GICs to reside in. Calabrese *et al* found that CD133^{+}/Nestin^{+} glioma-derived stem cells were closely associated with capillary associated endothelial cells; experimentally the number of blood vessels or endothelial cells in an orthotopic brain tumor model increased the fraction of self-

renewing cells within the tumor and accelerated tumor growth (45). Expectedly, depleting blood vessels in the same model arrested tumor growth and depleted the self-renewing cell populations in these tumors. Recently, using a mouse model of PDGF-induced glioma, Charles *et al.* found that nitric oxide (NO) production via endothelial Nitric Oxide Synthase (eNOS) expressed by the endothelium within the perivascular niche activated Notch activity in GICs (143) . Activated Notch increased their capacity to form neurospheres *in vitro*, and enhanced their tumorigencity *in vivo*, tying together the importance of dysregulated developmental pathways and, the association of GICs and the perivascular niche in gliomagenesis (143).

6.5. Cytoskeletal proteins

Nestin, a type IV intermediate filament protein, is expressed during development by neural progenitors throughout the brain but becomes restricted to the SVZ in the adult brain (144). Nestin$^+$ cells are particularly sensitive to ENU exposure persisting throughout tumor progression (145, 146). Nestin$^+$ cells have also been described in human gliomas, which while not an adequate marker of GICs suggests a link between stem cell populations and glioma formation (147). The microtubule-associated protein (MAP), doublecortin, is preferentially expressed in high-grade human gliomas (148). In a non-tumorigenic context, doublecortin is encoded by a locus on the X chromosome associated with lissencephaly, contains two actin-binding domains and is involved in neuronal migration (149). In the adult brain, it is primarily expressed in migrating neuroblasts such as the type A cells of the rodent RMS (149, 150). Additionally, over expression of doublecortin *in vitro* is protective against hypoxia and glucose deprivation in glioma lines (151).

7. CLINICAL EVIDENCE FOR THE SVZ AS A SOURCE OF GLIOMA INITIATING CELLS

Although populations of cells from both glioblastoma and the SVZ can be shown to possess stem cell-like properties *in vitro*, this in and of itself does not provide for the suspected connection between stem or progenitor cells of the SVZ and glioblastoma. Several clinical observations from glioma patients do however provide indirect support for this hypothesis. It has been noted by several groups that a large proportion of gliomas are found to associate with and involve the SVZ at the time of diagnosis; this relationship appears to have a negative impact on outcome (47, 152). In 2007, Lim *et al.* defined an MRI-based classification scheme for glioblastoma correlating in a limited series with predicted tumor recurrence pattern (47). In their schema, glioblastomas were stratified into four groups based on the association of the identified contrast enhancing lesion (CEL) with the SVZ and the cortex. CELs involving both the SVZ and cortex were most likely to present with multifocal disease and have noncontiguous recurrences. Where in contrast, those tumors which spared both the cortex and SVZ were never multifocal and recurrences were always adjacent to the initial site of diagnosis. Consistent with this classification scheme, Chaichana *et al.* in an observational study, found that median survival for patients with lateral

ventricle associated glioblastoma was significantly decreased compared with glioblastoma patients in whom the tumor was not associated with the lateral ventricle (8 months vs 11 months; (152)). Further supporting a central role for the SVZ in glioblastoma, Evers *et al.* retrospectively evaluated a cohort of 55 patients previously diagnosed with WHO Grade III (anaplastic astrocytoma) or Grade IV gliomas (glioblastoma), examining the correlation between the amount of radiation delivered to the periventricular tissues and progression-free survival (PFS) (153). Within their cohort, PFS was significantly increased and relative risk of progression (RR) significantly reduced in those receiving a high dose of radiation to the bilateral periventricular regions (>59Gy) compared to the groups receiving a lower dose (<59Gy). This effect was not seen when comparing high versus low doses of radiation to the SGZ nor was the effect seen when stratifying groups by total dose delivered indicating radiation specifically delivered to the periventricular region and hence the SVZ as important(153).

8. ASSIGNING A SOURCE FOR GLIOMA INITIATING CELLS

A substantial amount of clinical and experimental results suggest that the cell of origin likely derives from the NSCs and/or progenitor cells of the SVZ. Clinically, most though not all glioblastomas are found to be associated with the periventricular regions of the lateral ventricles in humans (21, 47). Additionally, delivery of higher than average doses of radiation to the bilateral periventricular regions correlates with longer PFS compared with those receiving lower doses to the same regions suggesting that cell populations arising from or residing in this region are important in tumor recurrence (153). Experimentally, manipulation of pathways known to be abnormal in glioblastoma such as EGF/EGFR, PDGF/PDGFR, SHH, and Notch signaling in NSCs can cause phenotypic changes closely mimicking the human disease.

Several difficulties are readily apparent when trying to assign a specific origin to GICs. Much of our understanding of neural stem cell biology comes from studies of the rodent brain. Within the brain of postnatal rodents, several distinct populations of neural stem cell and lineage-restricted progenitors have been described. Both EGF and PDGF have been implicated as important growth factors in gliomagenesis with amplification or activating mutations of the EGFR being described in almost half of all malignant gliomas, similarly PDGF and PDGFR are upregulated in glioblastoma (84, 87, 154). EGF responsive type C cells of the SVZ are a sizable population of migratory, rapidly dividing transit-amplifying cells. Stimulation with EGF blocks type C cell differentiation, promotes neurosphere formation *in vitro*, and enhances their migration along white matter tracts and blood vessels similar to gliomas. B cells of the SVZ have been shown to express PDGF which in the rodent SVZ causes progenitor cell proliferation and hyperplasia similar to that seen in early gliomagenesis (46). Moreover, treatment with antimitotic agents (e.g cytosine-b-D-arabino-furanoside) kill type C cells while sparing type B cells. Given that the

GIC population is hypothesized to be responsible for both invasive spread and recurrence characteristic of glioblastomas, this finding can suggest that type B cells are the origin of GICs explaining in part their resistance to chemotherapy agents (4, 155). Limited evidence supports the possibility of OPCs being a source of GICs; Kondo and Raff demonstrated that OPCs manipulated *in vitro* could acquire stem-like properties along with the re-expression of *Sox2,* an immature neuro-epithelial marker associated with glioblastomas (156, 157). Additionally, in a murine model of oligodendroglioma, Persson *et al.* demonstrated that OPC-like rather than NSC-like cells populations enriched for tumor-initiating cells; a finding further supported by the finding that NG2^{+}-enriched populations from human oligodendroglioma explants could recapitulate oligodendrogliomas in a xenograft model (64). In some cases glioblastomas fail to contact the ventricle and hence the SVZ, suggesting that for a subset of glioblastomas, OPCs or perhaps OSVZ associated radial glia may be the origin of GICs (47, 77).

Though evidence from rodent models can be cited in favor of multiple cellular origins for GICs, fundamentally the ability to generalize these results to the human disease is limited. The structure and function of the human SVZ and associated NSCs are disparately different from those of the rodent. The presence of a hypocellular gap, lack of a clearly defined RMS, absence of human equivalents to types A, B, and C cells, and the presence of outer SVZ layer radial glia that fail to contact the ependyma all stand in clear contrast to the rodent SVZ.

Additionally, the initial supposition that CD133^{+} cells represented the GIC subpopulation of gliomas is increasingly being called into question (4, 7, 38, 50). Initial studies suggested that the CD133^{+} population represented GICs due to the observation that neurospheres were more readily established from these sorted populations compared with CD133^{-} cells, and as few as 100 CD133^{+} cells were sufficient to establish tumors in xenograft models (7). Recently however data is accumulating to suggest while more rare, CD133^{-} populations can demonstrate a stem-like phenotype and propagate tumors in xenograft models. It is unclear however if CD133^{+} and CD133^{-} cells represent entities along a developmental spectrum or instead reflect distinct stem-like populations based on transcriptional profiles, both able to act as GICs (158, 159).

9. CLINICAL IMPLICATIONS OF GLIOMA INITIATING CELL IDENTITY

As previously discussed, the CSC population within glioblastomas has a pronounced resistance to both radiation and chemotherapy suggesting in the context of the CSC hypothesis that these populations are a likely source of resistance to treatment and recurrence inherent in this disease (8, 10). Not surprisingly, numerous groups are studying various molecular pathways involved in glioma-associated CSC biology which may ultimately find their way into the clinical armamentarium. Heimberger's group at MD Anderson for example has shown that constitutive activation of the Signal Transducer and Activator of Transcription Protein (STAT)-3 in CSCs is involved in the establishment of an immunosuppressive state; pharmacologic inhibition of STAT3 with WP1066 was found to partially restore glioma-specific immune responses in allogeneic T cells suggesting a possible role in the treatment of glioblastoma by aiding the host anti-tumor response (14). Modulation of the SHH and Notch pathways has been shown *in vitro* and *in vivo* to deplete stem cell-like populations derived from gliomas and inhibit tumor growth in xenograft models; in pediatric populations, a phase I study for medulloblastoma using the small molecule inhibitor of SHH, GDC-0449, is currently underway (95, 108, 160). Driving the differentiation of glioma-associated CSCs through bone morphogenic protein (BMP)-4 treatment similarly inhibited CSC proliferation and tumor growth (161). Furthermore, radiation directed at the bilateral periventricular regions of glioblastoma patients has correlated with improved PFS and reduced RR of progression (153).

These experimental and clinical observations represent an attractive target for future treatment modalities. Though like most oncologic treatments, need be considered carefully. Radiation and chemotherapy most often represent a balancing act between efficacy against tumor and normal tissues, a concept known as the therapeutic index. Targeting STAT3 in stem-cell populations is an attractive avenue for aiding the host anti-tumor immune response; STAT3 however is a key signaling molecule in the response to leukemia inhibitory factor (LIF), important in neural stem cell maintenance (14, 162). Similarly, radiation targeted at the periventricular regions, a proposed source of GICs and known location of NSCs, improves PFS (153). Acharya *et al.* however, have recently demonstrated low dose radiation exposure (1-2 Gy) can have detrimental effects on NSCs of the SGZ promoting apoptosis and increased oxidative and nitrosative stress in surviving NSC populations suggesting a mechanism for radiation-associated cognitive decline (163). Thus while, treatments targeting the GIC population may provide a new avenue for the treatment of glioblastoma, the effects on the normal parenchyma will have to be weighed.

10. CONCLUSIONS

Starting with the initial identification of a CD133^{+}-expressing cell subpopulation derived from gliomas which demonstrated properties of self-renewal, multipotentiality, and recapitulation of glioblastomas in a xenograft model, the role of stem-like cells in the ontology of glioblastoma has been a central topic of interest and investigation among glioma researchers and clinicians (7). The cancer stem cell hypothesis represents an important advancement in our understanding of the origin of glioblastoma, opening a new avenue for therapeutic modalities to target a currently incurable and invariably lethal disease. Despite intensive research by a number of groups, the definitive identity of GICs remains elusive.

Glioblastoma is a highly heterogeneous disease entity with a spectrum of pathologic features which can

vary from patient to patient. Numerous genetic derangements can be demonstrated in tumor samples from both primary and secondary glioblastomas, which can recapitulate glioblastomas in experimental models. Differences in anatomic locations can also be shown to have prognostic significance. Furthermore, experimental evidence can be provided that implicates several different stem cell and lineage-restricted progenitor populations as the source of GICs. While research continues to hunt for the true origin of GICs, the possibility that glioblastomas are in fact several different disease entities which differ only in relatively subtle ways as evidenced by the apparent differences in anatomic origins (periventricular vs subcortical vs cortical), genetic derangements (p53 mutations, PTEN mutations, EGFR amplification and EGFRvIII mutations, etc), as well as pathologic subtypes (glioblastoma, gliosarcoma, giant cell glioblastoma) might be considered. Only continued investigation will shed light on this dilemma.

11. ACKNOWLEGEMENTS

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Abbreviations: CNS: central nervous system, CSC: cancer stem cell, NSC: neural stem cell, GIC: glioma initiating cell, SVZ: subventricular zone, SGZ: subgranular zone, RMS: rostral migratory stream, OPC: oligodendrocyte precursor cell, GFAP: glial fibrillary acidic protein, ENU: *N*-ethyl-*N*-nitrosourea, EGF: epithelial growth factor, PDGF: platelet-derived growth factor, GSI: gamma secretase inhibitor, Shh: sonic hedgehog, PTEN: phosphatase and tensin homologue deleted on chromosome, PIP3: phosphatidylinositol phosphate, NF: neurofibromatosis, TGF: transforming growth factor, BDNF: brain-derived neurotrophic factor, VEGF: vascular endothelial growth factor, NO: nitric oxide, eNOS: endothelial-derived nitric oxide synthase, MAP: microtubule associated protein, CEL: contrast enhancing lesion, PFS: progression-free survival, RR: relative risk, DAPI: 4',6-diamidino-2-phenylindole, STAT: signal transducer and activator of transcription, LIF: leukemia inhibitory factor, BMP: bone morphogenic protein.

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