

# Seeing is Believing: Are Cancer Stem Cells the Loch Ness Monster of Tumor Biology?

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**Abstract** Tumors are complex systems with a diversity of cell phenotypes essential to tumor initiation and maintenance. With the heterogeneity present within the neoplastic compartment as its foundation, the cancer stem cell hypothesis posits that a fraction of tumor cells has the capacity to recapitulate the parental tumor upon transplantation. Over the last decade, the cancer stem cell hypothesis has gained support and shown to be relevant in many highly lethal solid tumors. However, the cancer stem cell hypothesis is not without its controversies and critics question the validity of this hypothesis based upon comparisons to normal somatic stem cells. Cancer stem cells may have direct therapeutic relevance due to resistance to current treatment paradigms, suggesting novel multimodal therapies targeting the cancer stem cells may improve patient outcomes. In this review, we will use the most common primary brain tumor, glioblastoma multiforme, as an example to illustrate why studying cancer stem cells holds great promise for more effective therapies to highly lethal tumors. In addition, we will discuss why the abilities of self-renewal

and tumor propagation are the critical defining properties of cancer stem cells. Furthermore, we will examine recent progress in defining appropriate cell surface selection markers and mouse models which explore the potential cell(s) or origin for GBMs. What remains clear is that a population of cells is present in many tumors which are resistant to conventional therapies and must be considered in the design of the next generation of cancer treatments.

**Keywords** Brain tumor stem cell · Review · Cell of origin · Cancer stem cell hypothesis

## Once Upon a Time—Introduction

In general, as our understanding of a disease improves, so does our ability to design effective therapies. For some highly lethal tumors, this has not been the case. Since President Nixon declared the war on cancer in 1971, tumors such as the most common primary brain tumor, Glioblastoma Multiforme (GBM), have not seen a major change in survival [1]. For GBMs in particular, the 5 year survival rate for patients treated with radiation alone remains at 2% [2] and current therapies offer mere palliation. Overall, cancer deaths account for the second most common disease related cause in the US and the change in survival over the last 20 years remains minimal [1]. The recent characterization of a fraction of tumor cells in many solid cancers responsible for their maintenance and propagation has brought much excitement and promise to cancer research. This fraction of cells, termed cancer stem cells, stem cell-like cancer cells, tumor initiating cells, or tumor propagating cells have been identified in a variety of tumors including those in the blood [3], breast [4], brain [5–9], and colon [10, 11]. These cancer stem cells appear to be resistant to many current therapies

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including radiation [12] and chemotherapy [13] as a result of potentially primed DNA damage responses. In addition, cancer stem cells appear to have unique regulatory pathways to promote their maintenance (reviewed recently by Li et al. [14]), some of which are shared by stem cells during development and adult homeostasis. As we begin to fully appreciate the role of cancer stem cells in tumor maintenance, we're likely to find cancer stem cell specific targets that can be incorporated into the development of multimodal therapeutic strategies.

### **The Legend of the Loch Ness—Cancer Stem Cells in the Brain**

Despite recent attention being focused on cancer stem cells, the general concept can first be attributed to Virchow's embryonal rest theory which suggested that cancers arose from embryo-like cells which remained remnant in a tissue. Interestingly, this has been shown to be the case at the transcription level as poorly differentiated cancers have an embryonic stem cell-like signature [15]. This link between normal and cancer stem cells is a common theme and has provided much insight into the mechanism of regulation and contribution of cancer stem cells to the biology of the tumor. In the nervous system, cancer stem cells have been identified in GBMs [5, 7, 9], and pediatric brain tumors [8] including medulloblastomas [6]. Interestingly, GBM stem cells have been proposed to be highly localized to blood vessels [16] which has been shown to be the case in neural stem/progenitor cells [17, 18] as well as in regions of hypoxia [19]. Additionally, GBM stem cells have been shown to have unique properties which define them from the bulk tumor cells including radiation resistance [12], chemoresistance [20], and elevated expression of developmental pathways such as Sonic Hedgehog [21], Notch [22], and signal transducer and activator of transcription-3 (stat3) [23]. Despite the identification of this cell population, there appears to be controversy over the existence of cancer stem cells in the brain and throughout the body. There are several reasons for the controversy, many of which originate from inappropriate definitions and expectations of what a cancer stem cell should be or must be able to do. In the following sections, we will use GBM stem cells to examine some of these controversies and suggest functional requirements for cancer stem cells.

### **Lurking in the Fog—Functional Definitions**

While there are many caveats to the work described for GBM stem cells, and for that matter other cancer stem cell types, what is clear is that it is possible to distinguish

fractions of a tumor which have different capabilities with respect to tumor initiation. This ability to recapitulate a phenotypically similar tumor in itself is the single most important feature of a cancer stem cell. As discussed previously, many somatic stem cells are solely defined by functional aspects and with regards to GBM and other cancer stem cells, the most logical functional definition would be the ability to generate tumors upon secondary transplantation. Another important feature of cancer stem cells is the ability to self-renew. However, conclusively showing self-renewal using current methodologies is not a trivial task. The first hurdle is to establish what is self? Currently, it is possible to measure non-invasive aspects of an individual cell (presence/absence of a marker, growth) and use subsequent analysis to determine if these properties are maintained. A recent demonstration of this has been shown nicely at the single cell level of neural stem/progenitor cells (NSPCs) [24]. However, establishing self-renewal in the most literal sense *in vivo* is not currently possible at the single cell level. The neurosphere/tumor-sphere assay has been used as a measure of self-renewal *in vitro* but it must be carefully interpreted. First, it measures more than one aspect of cell behavior taking into account survival, proliferation, and self-renewal. Second, careful interpretations are required to link sphere formation to the *in vivo* setting as these *in vitro* studies are often done in growth permissive conditions and many microenvironmental cues present in tissue are lacking.

The controversy over cancer stem cells has resulted, in part, from assigning additional features to cancer stem cells that are present in somatic stem cell such as low frequency and marker expression. A recent report has called into question both the transplantation model as well as frequency of cancer stem cells [25]. However, in the developing embryo, many organs have a large fraction of stem/progenitor cells during histogenesis. In addition, the growth requirements of a tumor are far different from that of a homeostatically stable organ. For many highly aggressive tumors (such as GBMs), it is not unreasonable to consider that there may be many cancer stem cells driving tumor growth. Hence, while there is a low frequency of stem cells present in many tissues, this may not be the case in all tumors and should not be a consideration in defining a cancer stem cell. The additional requirement for cancer stem cells posed by some investigators is marker expression. While informative, marker expression alone cannot be a singular determining factor of a cancer stem cell just as it should not for a somatic stem cell. For tumors which display a great heterogeneity, such as GBMs, marker expression may serve as a good starting point but functional analysis is a critical determining factor for the presence of a cancer stem cell. The single most critical factor in defining a cancer stem cell is the ability to recapitulate the parental tumor

upon translation into a secondary site. With such a definition in place, much of the controversy and confusion with regards to cancer stem cells can be avoided.

### Tools for Sighting the Monster—How to Identify a GBM Stem Cell

One of the major controversies surrounding the existence of cancer stem cells is their identification and enrichment from bulk tumor tissue. For the brain, examining stem/progenitor cells present in the normal embryonic and adult brains is likely to provide insight into what will be successful in defining GBM stem cells. Despite being located in distinct anatomical regions and possessing somewhat unique marker expression, NSPCs are defined strictly by function which appears to be the case with stem/progenitor cells in various systems. In the nervous system, the functional definition(s) a NSPC must fulfill remains relatively unclear. Ideally, NSPCs would be able to self-renew and generate all lineages within the brain but the assays used to examine these cells are unable to provide appropriate insight. To date, there are several neural stem cell (embryonic ventricular zone, adult subependymal zone, adult dentate gyrus) and progenitor cell (embryonic subventricular zone, embryonic glial progenitors, adult white matter/glial progenitors) populations which have been described for the developing and adult brain (see review by Kriegstein and Alvarez-Buylla [26]). The ability to culture mammalian NSPCs *in vitro* by the neurosphere assay [27] provided a substantial advance in our ability to understand their biology and allowed for the examination of which populations of cells are putative NSPCs. However, it has become clear that the neurosphere assay measures more than self-renewal (also adhesion independence, proliferation, and survival) and has forced us to be careful with our evaluation of results generated from the assay [28]. While culturing NSPCs as neurospheres has been useful in expanding these populations, the anatomical complexity (cell to cell, cell to extracellular matrix interactions) is lost as is the appropriate mitogenic balance. The *in vivo* examination of regeneration first shown by Doetsch et al. [29] is another way to examine NSPCs. While this assay is complex, it allows for the examination of quiescent NSPCs repopulation *in vivo*, which is not possible *in vitro*. While both these assays have provided a greater understanding of NSPCs, they both do not adequately measure self-renewal, which given our current technology, appears to remain a difficult endeavor.

It is logical to draw parallels between NSPCs in the brain to GBM stem cells and in principle, both appear to have similar functions (maintain a normal or aberrant organ). As such, our understanding of GBM stem cells has greatly

benefited from assays used for NSPCs. Using identical culture media, it is possible to expand GBM stem cells as spheres similar to neurospheres, also called tumorspheres. In addition, the majority of cancer stem cell models, including those for GBM stem cells, have relied on mouse transplantation models. With *in vitro* and *in vivo* evaluation assays, it would theoretically be possible to evaluate which marker(s) are best for enrichment of GBM stem cells. While this has been an area of major effort for those working in GBM and other cancer stem cell systems (Table 1), there are several caveats which must be considered in marker evaluation. First off, in the normal brain (both in the embryo and adult), unlike in other systems, there is no single defining NSPC marker. Second, given the heterogeneity of GBMs, it is unlikely that a single defining marker will be capable of enriching for GBM stem cells in all cases. As such, approaches integrating several markers, either by positive or negative selection are likely to prove successful. Against this backdrop, several cell surface markers have been proposed for GBM stem cell enrichment:

#### CD133

CD133 (also known as Prominin-1) is a pentaspan membrane protein with relatively unknown function where the mutant mice have a photoreceptor defect phenotype [30]. CD133 has previously been shown to be a marker of normal embryonic neural [31] and hematopoietic [32] stem cells and also been shown to be a useful marker of GBM stem cells [9] and other cancer stem cells [10, 11]. However, CD133 is not consistently expressed in all GBMs and recent reports have demonstrated that CD133-negative cells are capable of giving rise to tumors in transplant assays [33]. Confounding the use of CD133 for fluorescence activated cell sorting (FACS) are recent reports that its expression is cell cycle dependent [34] so it is possible to miss a population of CD133-positive cells during sorting. When present, CD133 expression can be a useful enrichment method for GBM stem cells, however the low expression in some tumors suggests additional markers need to be explored. In addition, as we begin to understand the biological function of CD133 in greater detail, we are likely to fully appreciate its contribution to GBM stem cell and cancer stem cell biology.

#### A2B5

A2B5, an antibody against a surface glycoside [35] which is a commonly used glial/white matter progenitor cells marker has also been explored as a potential GBM stem cell marker. While expressed in a large percentage of brain tumor and GBM cells, transplantation studies showed both

**Table 1** Summary of markers used for NSPC, GBM, and other cancer stem cell enrichment

Cell surface marker (s)	NSPC	GBM stem cell	Other cancer stem cell systems
ALDH	Activity unknown	Activity unknown	Colon [82, 83], Leukemia [84, 85], AML [86], Liver [87], Lung [88], Mammary [89]
Cadherins	N-cad is critical in NSPC adhesion in embryonic brain but utility for sorting unknown [90]	Present but utility for sorting unknown	Prostate—absence of Cadherin-10 [91], Leukemia—VE Cadherin [92, 93]
CD15	Embryonic [38] and adult [39] mouse NSPCs	Human patient specimens [40]	Medulloblastoma [94, 95]
CD24	Present but utility for sorting unknown	Present but utility for sorting unknown	Pancreas—in combination with CD44 and ESA [96, 97], Colon—in combination with CD133 [97]
CD44	Present but utility for sorting unknown	Present but utility for sorting unknown	Bladder—in combination with CK5+/CK20- [98], Breast [4], Colon [99], Gastric [100], Head and Neck [101], Ovarian—in combination with CD117 [102], Prostate [103]
CD133	Fetal human NSPCs [31], mouse postnatal cerebellum [104]	Human patient specimens [6, 9]	Colon [10, 11], Other CNS tumors (Medulloblastomas, Pilocytic astrocytoma, Anaplastic ependymoma) [6]
Chemokine receptors	Present but utility for sorting unknown	Present but utility for sorting unknown	Present but utility for sorting unknown
Integrins	Integrin $\alpha\beta 1$ in human fetal neural stem cells [46], integrin $\beta 1$ in rodent NSPCs [105, 106]	Human patient specimens (integrin $\alpha\beta 1$ ) [47]	Breast—integrin $\beta 3$ [107] and integrin $\beta 1$ [108] in mouse models, Colon—integrin $\beta 1$ [97], Prostate—integrin $\alpha 2\beta 1$ [109], and integrin $\alpha 6$ in mouse model in combination with Lin-Sac1+ [110]
Syndecans	Syndecan-1 in mouse NSPCs [106]	Present but utility for sorting unknown	Syndecan-1 overexpressed in multiple myeloma [111]

A2B5-positive/CD133-positive and A2B5-positive/CD133-negative cell populations were capable of generating tumors in transplantation models [36] suggesting that A2B5 may be used to enrich for CD133-negative GBM stem cells. In addition, this suggests that there may be a link between glial/white matter progenitor cells and GBM stem cells. Further work will be required to fully understand the role of A2B5 as a GBM stem cell marker and suggests that progenitor cell markers should be evaluated in other cancer stem cell system.

### L1CAM

L1CAM, a neural cell adhesion molecule found to be overexpressed in GBMs has been shown to also enrich for GBM stem cells [37]. Interestingly, while the biology in the normal brain is yet to be fully elucidated, targeting L1CAM in GBM stem cells showed therapeutic benefits in vitro (tumorsphere assays) and in vivo (increased tumor latency

in transplantation models). Furthermore, it is worth noting the L1CAM is also overexpressed in other solid cancers so it use as a cancer stem cell marker would be worth exploring.

### CD15

A recently proposed cell surface marker which has been explored for GBM stem cell enrichment is CD15 (also known as SSEA-1 or LewisX (LeX)). This cell surface carbohydrate is expressed in embryonic [38] and adult [39] NSPCs and has been linked to bFGF responses. Recently, it has been shown to be useful to enrich for GBM stem cells in scenarios where CD133 is either not present or present in a small fraction of cells [40]. However, like CD133, little is known about the biology of CD15 and it is thought that it may be cell cycle dependent [34]. In addition, it may be the case that CD15 is marking proliferating tumor cells rather than actual GBM stem cells (DM Park, R Ravin, DJ

Hoeppner, unpublished observation) and a recent study showed that CD15 in NSPCs was not an informative marker for in vitro cultures [24]. Clearly, further studies to define the role of CD15 in GBMs will be required but the preliminary data which exists suggest that it can be an informative GBM stem cell enrichment marker.

### Integrin $\alpha 6$

Integrin  $\alpha 6$  is a member of the heterodimeric integrin family of extracellular matrix (ECM) receptors and interacts with the laminin family of ECM proteins. Integrin  $\alpha 6$  was identified as a core stemness marker by meta-analysis of microarray studies from multiple labs profiling embryonic, hematopoietic, and neural stem cells [41]. In addition, integrin  $\alpha 6$  has been shown to be critical in embryonic NSPCs during development [42, 43], expressed by several progenitor cell types in the adult brain [44], and used by adult NSPCs to interact with blood vessels in the niche [18, 45]. Integrin  $\alpha 6$  can also be used as a NSPC enrichment marker [46]. Recently, an examination of the perivascular microenvironment, a region enriched in GBM stem cells identified integrin  $\alpha 6$  as an additional GBM stem cell marker with potential therapeutic usage [47]. Integrin  $\alpha 6$  targeting, either by RNA interference or blocking antibodies resulted in decreased growth in vitro (tumorsphere formation assays) and in vivo (increased tumor latency in transplantation models). The use of integrin  $\alpha 6$  in the context of GBM therapy will need to be carefully assessed in the context of normal tissue. The use of integrin  $\alpha 6$  as a GBM stem cell enrichment marker highlights the importance of evaluating the microenvironment in order to identify additional GBM enrichment markers and potential therapeutic pathways.

While all of these enrichment markers have shown success in selecting for GBM stem cells from bulk tumors, several issues have become apparent. First, selection using fluorescence activated cell sorting (FACS) has been a critical tool but it should be understood that the phenotype of the selected cells are only known at the time of selection. Enzymatic treatment, additional culture, or transplantation has the potential to influence the phenotype in addition to the possibility that certain markers are cell cycle dependent and could be missed at the time of sorting. Second, no single marker has been demonstrated to enrich for all GBM stem cells, which is likely due in part to the heterogeneity of the disease. Third, the time in culture and culture conditions must be considered as if not controlled properly, will result in phenotypes similar to highly passages GBM cells lines [48]. Finally, and possibly most interesting, all tumor initiating populations do not appear to share the same marker profile suggesting that there could be more than one cell of origin or original clone driving the tumor maintenance.

As progress moves forward, it will be critical to develop additional markers for GBM stem cell enrichment, such as those which have been shown to be successful for neural stem cell as well as other cancer stem cell system, and understand how they relate to other markers (Table 1). A systematic screen of cell surface markers with combined functional assessments will likely yield additional markers for GBM stem cell enrichment. Future multi-marker sorting schemes may allow for the selection of multiple tumor initiating cell populations and assist in the development of putative GBM and cancer stem cell hierarchies.

### Birth of the Legend—Cell(s) of Origin for GBMs

Another point of contention for the cancer stem cell hypothesis is that the cell of origin for the tumor must be a stem cell. For GBMs, like other solid tumors, there could be a variety of cells that could be responsible for generating the tumor and thus the term “cancer stem cell” implies the phenotype of the tumor cells, not the cell of origin. With our ability to genetically engineer mouse disease models, attempts have been made to create an accurate model which fully recapitulates GBMs and could be used, at least in part, to infer the cell type(s) of origin. However, GBMs are a complex heterogeneous disease and to date, no single mouse model has been able to accurately recapitulate the disease. In addition, genetic models rely on the principle that tumor initiation is a cell intrinsic process and excludes cell extrinsic influences which are likely to also be critical in the initial steps of tumor development [49]. With that said, several models exist which have been shown to be useful in the understanding of the disease as well as potential cell type(s) of origin. The earliest animal model of brain tumors was based on mutagenesis using DNA alkylating agents such as *N*-ethyl-*N*-nitrosourea (ENU). The ENU model induces sporadic brain tumors of various grades by injection of ENU into gestating rat embryos [50]. This experimental approach has resulted in a cohort of rat brain tumor models that have yielded insight into the biology of the disease as well as allowed for therapeutic testing [51]. However, this model fails to allow for interrogation of the cell of origin or the tumor-initiating mutations.

Genetically engineered mice (GEMs), on the other hand, provide an experimental system that allows researchers to manipulate the genetics of a specific cell type within the adult animal and evaluate for tumor formation. This is accomplished by a germline modification that is conditionally manipulated in the adult or by somatic cell gene transfer in a targeted cell population. Conditional transgenic models take advantage of drug administration (e.g.; 4-hydroxytamoxifen or tetracycline) to temporally activate

a gene (such as an oncogene) or recombination by cell type specific expression of *Cre/Flp* to delete a gene (such as a tumor suppressor). Somatic cell gene transfer, on the other hand, involves the stereotactic injection of a virus engineered to express a gene of interest to a defined location within the brain and at a specific time. Furthermore, these systems can be used together to provide for the multiple genetic alterations required for cancer progression. Utilizing the aforementioned murine systems, numerous groups have attempted to evaluate whether NSPCs are the source of malignant gliomas. For the purpose of the following discussion, we will focus on those studies aimed at modeling GBM. The link between glioma formation and NSPCs was highlighted by reports from knockout studies that described alterations in pathways commonly mutated in GBM (namely p53, Neurofibromatosis type 1 (NF1), and PTEN) led to deregulation of NSPCs within the subependymal/subventricular zone (SEZ/SVZ), a germinal region known to harbor adult stem cells [52–55]. To more specifically target this population, the glial fibrillary acidic protein (*GFAP*) or *nestin* promoters have been used as they have been shown to be active in adult NSPCs [56]. Of note, both of these genes are selective rather than specific for this population, underscored by the expression of GFAP in differentiated astrocytes and nestin in additional non-NSPC populations. Nonetheless, deletion of both the *p53* and *PTEN* tumor suppressor genes in GFAP positive cells (using a *hGFAP-Cre* transgenic line) led to GBM-like tumors and revealed a role for these proteins in regulating differentiation and self-renewal of NSPCs [57]. However, this system failed to allow for temporal regulation and therefore carries the caveat of loss of gene expression at an early developmental stage. Use of the recently generated *Nestin-creER* or *GFAP-creER* mice overcomes this limitation by allowing for cre activation upon 4-hydroxytamoxifen injection in the adult animal [58–60]. Using such an approach, it was demonstrated that tumor suppressor inactivation (p53, NF1, and PTEN) under the control of the *Nestin-creER* promoter led to tumors in adult mice and results were confirmed using viral-mediated cre recombinase injections into the adult SEZ/SVZ [61].

Even with these improved GEMs, validating the cell of origin using models based on conditional induction via the *GFAP* or *nestin* promoter is problematic due their being selective rather than specific for NSPCs, therefore allowing for potential transformation of more differentiated cell types. Somatic cell gene transfer using systems such as the RCAS/tv-a retroviral model circumvents this issue by allowing for both spatial and temporal control, targeting the NSPC niche in adult animals only. RCAS vectors stereotactically injected into a defined brain region are engineered to express a transforming element (oncogene or tumor suppressor) only upon infection in cells expressing the TVA receptor. Mouse

lines driving tv-a by the *GFAP* or *nestin* promoters have been generated and have indeed generated gliomas from NSPCs in adult mice in the context of various genetic disruptions [62–66]. In a similar vein, Cre-*loxP*-controlled lentiviral vectors have been used to transform GFAP cells in the neurogenic zones via activated H-Ras and AKT overexpression in a p53 heterozygous background, resulting in malignant gliomas [67]. Moving beyond Nestin and GFAP, the orphan nuclear receptor tailless (Tlx) has recently been reported to be neural stem cell specific and tumorigenic when overexpressed, yielding invasive GBMs when combined with the loss of p53 [68, 69].

Lending support to the idea that NSPCs may be a potential cell of origin is a recent study in which mutations were introduced into NSPCs and astrocytes ex-vivo and then transplanted to assess tumor formation [70]. The study demonstrated that mature astrocytes were not capable of forming tumors upon the introduction of mutations whereas deletion of PTEN and p53 in NSPCs resulted in transformed cells that gave rise to tumors, suggesting in part that mature astrocytes are not likely to be the cell of origin in this mouse transplant model. In addition to traditional GEMs, several other model systems have been developed which are likely to be useful in understanding cellular contributions to GBMs. A recent report has demonstrated that by mutating epidermal growth factor receptor (EGFR) and phosphoinositide 3-kinase (PI3K) signaling in drosophila glia and glial precursors, GBM-like tumors can be generated [71]. This model may be a good candidate to screen drugs or identify additional pathways contributing to the formation and maintenance of GBMs. Although powerful, many of the systems discussed above rely on the mutation of a defined cell type to produce tumors. These model systems have not ruled out other potential cells or origin and assuming the multiple-hit hypothesis holds true, it is likely that any cycling cell population in the brain subject to the appropriate set of mutations could generate a tumor. In support of this, recent studies have reported the generation of gliomas by cells outside of the neurogenic niches [72–74].

Another potential way of evaluating the cell(s) or origin for GBMs is by using microarray analysis. Attempts to generate distinct gene signatures have not produced a unified profile but rather three main classes of tumors: proneural, proliferative, and mesenchymal [75]. The proneural gene signature is associated with neuronal marker expression and longer survival while the proliferative and mesenchymal signatures have higher neural stem cell marker expression and poorer prognosis [75]. Additional interpretation of these tumor signatures suggests that the proneural tumors resembled neurogenic processed and specifically neuroblast cells, the proliferative tumors resembled proliferation processed and neural stem and/or

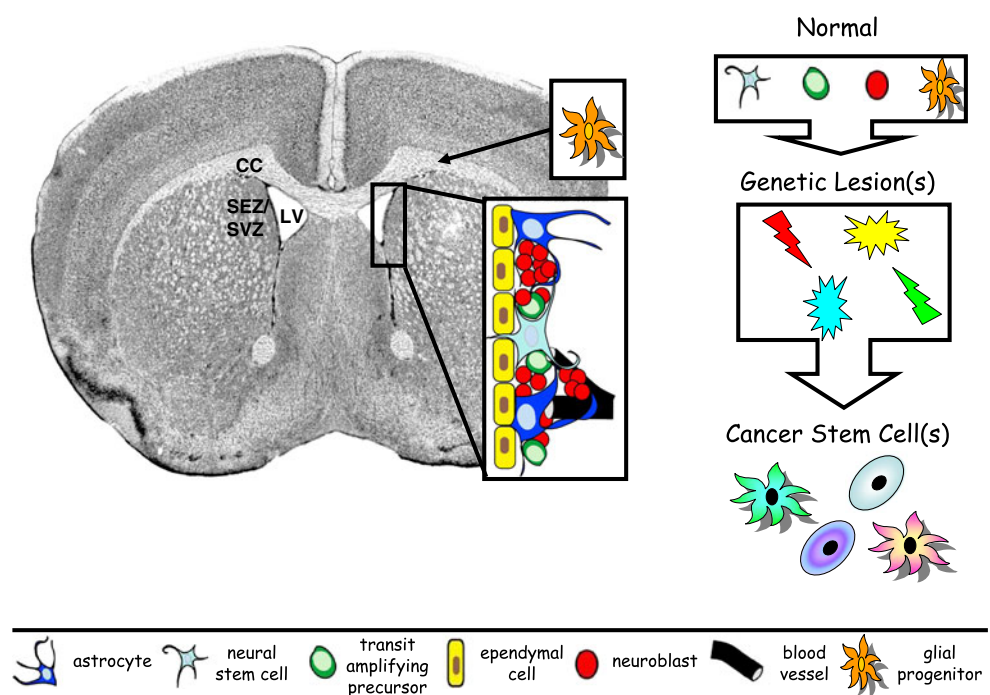
transit amplifying progenitors, and mesenchymal tumors resembles angiogenic processes and NSPCs. No uniform mutation pattern exists for each tumor type but there are some associations: loss of PTEN, gain of EGFR, and Akt activation in mesenchymal and proliferative groups, elevated presence of notch pathway members in the proneural group. A recent follow up study by the Cancer Genome Atlas Network (TCGA) delineated four GBM molecular subclasses (proneural, neural, classical, and mesenchymal), expanding on the previous three groups [76]. In their analysis, the TCGA found that the following mutation patterns: the proneural group has frequent mutations in p53 and isocitrate dehydrogenase 1 (IDH1), the classical group had increased mutations in EGFR and the presence of EGFRvIII, and the mesenchymal group had increased mutations in NF1. In addition, an evaluation of survival after therapy (which included radiation and chemotherapy) demonstrated that the proneural group was the worst to respond as assessed by patient survival. Additional classification of GBMs based on CD133 status has suggested that CD133-negative tumors have a proneural gene signature and resemble fetal NSPCs while CD133-positive tumors have a mesenchymal gene signature and are associated with adult NSPCs [77]. The studies discussed above demonstrate the heterogeneity present with GBMs and a simplistic interpretation would be that these data suggest that more than one cell type is capable of generating a GBM. Lending support to this idea would be data which demonstrates that GBMs contain populations of cells that share markers present in embryonic and adult stem/progenitor cell populations such as CD133 [9], NG2 [78], Sox2 [79], and

Olig2 [80]. Additional profiling and phenotypic assessment of the GBM stem cells from each tumor type may yield insight into differences between tumor classes and potential molecular subclass-specific therapies. Furthermore, a systematic comparison of GBM stem cells to that of normal stem and progenitor cells present from the embryo and adult are likely to yield core pathways that are conserved between populations. Such efforts will also aid in the identification of additional GBM stem cell pathways and provide conserved pathways which will need to be considered when designing targeted therapies. Overall, these microarray studies have provided a good starting point for additional genomic analysis linking tumors classes to survival and will be critical as will attempting to assess which therapies are most effective to which tumor classes.

### Rising from the Water—Final Thoughts

In this review, we have used GBM as an example to explore the cancer stem cell hypothesis. GBM, like many other solid tumors, is a heterogeneous disease and as such, requires the appreciation of the diversity (both cellular and genetic) present within the tumor. To improve our understanding of cancer stem cells and their contribution to cancer, additional enrichment paradigms must be developed that are likely to include multiple markers. In addition, current models will need to be improved to address the possibility that multiple cells of origin may exist (Fig. 1) and previous classified solid tumors may represent several

**Fig. 1** Schematic depicting potential cell(s) of origin for GBMs. Proliferating cell populations are present in several regions of the CNS, including the subventricular/subependymal zone (SEZ/SVZ) adjacent to the lateral ventricle (LV), the corpus callosum (CC), and the subgranular zone of the hippocampus (not shown). Based on heterogeneity present in cell surface marker expression, molecular subclasses, and GBM models, it is plausible that multiple cells of origin exist for GBM, consisting of various proliferating cell populations that acquire genetic lesions. Adapted from Mouse Atlas [112] and Kazanis et al. [113]



distinct tumor types with variant genetic abnormalities, such as is the case with GBM. Finally, additional genetic profiling will be critical to understand the diversity present within a tumor and if combined with animal models, such as demonstrated recently with ependymoma [81], will be a powerful tool to understand how different classes of cancer stem cells contribute to different subclasses of tumors. Amidst the controversy surrounding the cancer stem cell hypothesis, what is clear is that the existence of cancer stem cells has helped explain certain aspects of tumor behavior and demonstrates the complexity of cancer. Moving forward, efforts to detect the monster at a distance (i.e. diagnostic studies) and kill the monster (multi-modal tumor therapies) will likely result in improved patient outcomes.

**Acknowledgements** We sincerely apologize to those whose work we were unable to discuss due to space limitations. We would like to thank members of the Rich lab for stimulating discussion and critical review of this manuscript. Work in the Rich laboratory is supported by the Childhood Brain Tumor Foundation, the Pediatric Brain Tumor Foundation of the United States, Accelerate Brain Cancer Cure, Alexander and Margaret Stewart Trust, Brain Tumor Society, Goldhirsh Foundation, Duke Comprehensive Cancer Center Stem Cell Initiative Grant, and NIH grants NS047409, NS054276, CA112958, and CA116659. J.N.R. is a Damon Runyon-Lilly Clinical Investigator supported by the Damon Runyon Cancer Research Foundation. M.V. is supported by an American Brain Tumor Association Basic Research Fellowship and a National Service Research Award (NINDS F32 NS058042). J.D.L. is supported by an American Brain Tumor Association Basic Research Fellowship (sponsored by the Joelle Syverson Fund) and a National Service Research Award (NCI F32 CA142159).

**Conflict of Interest** Dr. Mahendra S. Rao is an employee of Invitrogen Corporation, co-founder of Q Therapeutics, and currently serves at its Chief Scientific Consultant.

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