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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 survival¹. 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

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survival over the last twenty years remains minimal¹. 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 blood³, breast⁴, brain⁵⁻⁹, and colon^{10, 11}. These cancer stem cells appear to be resistant to many current therapies including radiation¹² and chemotherapy¹³ 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 signature¹⁵. 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 tumors⁸ including medulloblastomas⁶. Interestingly, GBM stem cells have been proposed to be highly localized to blood vessels¹⁶ which has been shown to be the case in neural stem/progenitor cells^{17, 18} as well as in regions of hypoxia¹⁹. Additionally, GBM stem cells have been shown to have unique properties which define them from the bulk tumors cells including radiation resistance¹², chemoresistance²⁰, and elevated expression of developmental pathways such as Sonic Hedgehog²¹, Notch²², 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/tumorsphere 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 cells²⁵. 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 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.

Tool 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-Buylla²⁶). The ability to culture mammalian NSPCs *in vitro* by the neurosphere assay²⁷ 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 assay²⁸. 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 NPSCs 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 phenotype³⁰. CD133 has previously been shown to be a marker of normal embryonic neural³¹ and hematopoietic³² stem cells and also been shown to be a useful marker of GBM stem cells⁹ 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 assays³³. Confounding the use of CD133 for fluorescence activated cell sorting (FACS) are recent reports that its expression is cell cycle dependent³⁴ 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 glycoside³⁵ 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 A2B5-positive/CD133-positive and A2B5-positive/CD133-negative cell populations were capable of generating tumors in transplantation models³⁶ 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 cells³⁷. 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 its 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 embryonic³⁸ and adult³⁹ 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 cells⁴⁰. However, like CD133, little is known about the biology of CD15; it is thought that it may be cell cycle dependent³⁴. 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 Hoepfner, unpublished observation) and a recent study showed that CD15 in NSPCs was not an informative marker for *in vitro* cultures²⁴. 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 cells⁴¹. 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 brain⁴⁴, 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 marker⁴⁶. 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 usage⁴⁷. 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 lines⁴⁸. 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 development⁴⁹. 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 embryos⁵⁰. 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 testing⁵¹. 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 NSPCs⁵⁶. 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 NSPCs⁵⁷. 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/SVZ⁶¹.

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 gliomas⁶⁷. 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 formation⁷⁰. 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 transplanted 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 generated⁷¹. 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 mesenchymal⁷⁵. 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 prognosis⁷⁵. Additional interpretation of these tumors 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 groups⁷⁶. 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 on 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 NSPCs⁷⁷. 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 CD133⁹, NG2⁷⁸, Sox2⁷⁹, and Olig2⁸⁰. Additional profiling and phenotypic assessment of the GBM stem cells from each tumor type may yield insight into difference 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 and previous classified solid tumors may represent several 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 ependymoma⁸¹, will be a powerful tool to understand how different classes of cancer stem cell 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.

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References

1. Deorah S, Lynch CF, Sibenaller ZA, Ryken TC. Trends in brain cancer incidence and survival in the United States: Surveillance, Epidemiology, and End Results Program, 1973 to 2001. *Neurosurg Focus*. 2006; 20:E1. [PubMed: 16709014]
2. Stupp R, Hegi ME, Mason WP, et al. Effects of radiotherapy with concomitant and adjuvant temozolomide versus radiotherapy alone on survival in glioblastoma in a randomised phase III study: 5-year analysis of the EORTC-NCIC trial. *Lancet Oncol*. 2009; 10:459–66. [PubMed: 19269895]
3. Bhatia M, Wang JC, Kapp U, Bonnet D, Dick JE. Purification of primitive human hematopoietic cells capable of repopulating immune-deficient mice. *Proc Natl Acad Sci U S A*. 1997; 94:5320–5. [PubMed: 9144235]
4. Al-Hajj M, Wicha MS, Benito-Hernandez A, Morrison SJ, Clarke MF. Prospective identification of tumorigenic breast cancer cells. *P Natl Acad Sci USA*. 2003; 100:3983–8.
5. Ignatova TN, Kukekov VG, Laywell ED, Suslov ON, Vrionis FD, Steindler DA. Human cortical glial tumors contain neural stem-like cells expressing astroglial and neuronal markers in vitro. *Glia*. 2002; 39:193–206. [PubMed: 12203386]
6. Singh SK, Clarke ID, Terasaki M, et al. Identification of a cancer stem cell in human brain tumors. *Cancer Res*. 2003; 63:5821–8. [PubMed: 14522905]
7. Galli R, Binda E, Orfanelli U, et al. Isolation and characterization of tumorigenic, stem-like neural precursors from human glioblastoma. *Cancer Res*. 2004; 64:7011–21. [PubMed: 15466194]
8. Hemmati HD, Nakano I, Lazareff JA, et al. Cancerous stem cells can arise from pediatric brain tumors. *Proc Natl Acad Sci U S A*. 2003; 100:15178–83. [PubMed: 14645703]
9. Singh SK, Hawkins C, Clarke ID, et al. Identification of human brain tumour initiating cells. *Nature*. 2004; 432:396–401. [PubMed: 15549107]
10. O'Brien CA, Pollett A, Gallinger S, Dick JE. A human colon cancer cell capable of initiating tumour growth in immunodeficient mice. *Nature*. 2007; 445:106–10. [PubMed: 17122772]
11. Ricci-Vitiani L, Lombardi DG, Pilozzi E, et al. Identification and expansion of human colon-cancer-initiating cells. *Nature*. 2007; 445:111–5. [PubMed: 17122771]
12. Bao S, Wu Q, McLendon RE, et al. Glioma stem cells promote radioresistance by preferential activation of the DNA damage response. *Nature*. 2006:444.
13. Liu G, Yuan X, Zeng Z, et al. Analysis of gene expression and chemoresistance of CD133+ cancer stem cells in glioblastoma. *Mol Cancer*. 2006; 5:67. [PubMed: 17140455]
14. Li Z, Wang H, Eyler CE, Hjelmeland AB, Rich JN. Turning cancer stem cells inside out: an exploration of glioma stem cell signaling pathways. *J Biol Chem*. 2009; 284:16705–9. [PubMed: 19286664]
15. Ben-Porath I, Thomson MW, Carey VJ, et al. An embryonic stem cell-like gene expression signature in poorly differentiated aggressive human tumors. *Nat Genet*. 2008; 40:499–507. [PubMed: 18443585]
16. Calabrese C, Poppleton H, Kocak M, et al. A perivascular niche for brain tumor stem cells. *Cancer cell*. 2007; 11:69–82. [PubMed: 17222791]
17. Palmer TD, Willhoite AR, Gage FH. Vascular niche for adult hippocampal neurogenesis. *J Comp Neurol*. 2000; 425:479–94. [PubMed: 10975875]
18. Kokovay E, Goderie S, Wang Y, et al. Adult SVZ lineage cells home to and leave the vascular niche via differential responses to SDF1/CXCR4 signaling. *Cell stem cell*. 7:163–73. [PubMed: 20682445]
19. Li Z, Bao S, Wu Q, et al. Hypoxia-inducible factors regulate tumorigenic capacity of glioma stem cells. *Cancer cell*. 2009; 15:501–13. [PubMed: 19477429]

20. Liu G, Yuan X, Zeng Z, et al. Analysis of gene expression and chemoresistance of CD133+ cancer stem cells in glioblastoma. *Mol Cancer*. 2006;5. [PubMed: 16457726]
21. Bar EE, Chaudhry A, Lin A, et al. Cyclopamine-mediated hedgehog pathway inhibition depletes stem-like cancer cells in glioblastoma. *Stem cells (Dayton, Ohio)*. 2007; 25:2524–33.
22. Fan X, Matsui W, Khaki L, et al. Notch pathway inhibition depletes stem-like cells and blocks engraftment in embryonal brain tumors. *Cancer Res*. 2006; 66:7445–52. [PubMed: 16885340]
23. Sherry MM, Reeves A, Wu JK, Cochran BH. STAT3 is required for proliferation and maintenance of multipotency in glioblastoma stem cells. *Stem cells (Dayton, Ohio)*. 2009; 27:2383–92.
24. Ravin R, Hoepfner DJ, Munno DM, et al. Potency and fate specification in CNS stem cell populations in vitro. *Cell stem cell*. 2008; 3:670–80. [PubMed: 19041783]
25. Quintana E, Shackleton M, Sabel MS, Fullen DR, Johnson TM, Morrison SJ. Efficient tumour formation by single human melanoma cells. *Nature*. 2008; 456:593–8. [PubMed: 19052619]
26. Kriegstein A, Alvarez-Buylla A. The glial nature of embryonic and adult neural stem cells. *Annu Rev Neurosci*. 2009; 32:149–84. [PubMed: 19555289]
27. Reynolds BA, Weiss S. Generation of neurons and astrocytes from isolated cells of the adult mammalian central nervous system. *Science*. 1992; 255:1707–10. [PubMed: 1553558]
28. Singec I, Knoth R, Meyer RP, et al. Defining the actual sensitivity and specificity of the neurosphere assay in stem cell biology. *Nature methods*. 2006; 3:801–6. [PubMed: 16990812]
29. Doetsch F, Caille I, Lim DA, Garcia-Verdugo JM, Alvarez-Buylla A. Subventricular zone astrocytes are neural stem cells in the adult mammalian brain. *Cell*. 1999; 97:703–16. [PubMed: 10380923]
30. Zacchigna S, Oh H, Wilsch-Brauninger M, et al. Loss of the cholesterol-binding protein prominin-1/CD133 causes disk dysmorphogenesis and photoreceptor degeneration. *J Neurosci*. 2009; 29:2297–308. [PubMed: 19228982]
31. Uchida N, Buck DW, He D, et al. Direct isolation of human central nervous system stem cells. *Proc Natl Acad Sci U S A*. 2000; 97:14720–5. [PubMed: 11121071]
32. Yin AH, Miraglia S, Zanjani ED, et al. AC133, a novel marker for human hematopoietic stem and progenitor cells. *Blood*. 1997; 90:5002–12. [PubMed: 9389720]
33. Beier D, Hau P, Proescholdt M, et al. CD133(+) and CD133(–) glioblastoma-derived cancer stem cells show differential growth characteristics and molecular profiles. *Cancer Res*. 2007; 67:4010–5. [PubMed: 17483311]
34. Sun Y, Kong W, Falk A, et al. CD133 (Prominin) negative human neural stem cells are clonogenic and tripotent. *PloS one*. 2009; 4:e5498. [PubMed: 19430532]
35. Eisenbarth GS, Walsh FS, Nirenberg M. Monoclonal antibody to a plasma membrane antigen of neurons. *Proc Natl Acad Sci U S A*. 1979; 76:4913–7. [PubMed: 388422]
36. Ogden AT, Waziri AE, Lochhead RA, et al. Identification of A2B5+CD133– tumor-initiating cells in adult human gliomas. *Neurosurgery*. 2008; 62:505–14. discussion 14–5. [PubMed: 18382330]
37. Bao S, Wu Q, Li Z, et al. Targeting cancer stem cells through L1CAM suppresses glioma growth. *Cancer Res*. 2008; 68:6043–8. [PubMed: 18676824]
38. Capela A, Temple S. LeX is expressed by principle progenitor cells in the embryonic nervous system, is secreted into their environment and binds Wnt-1. *Dev Biol*. 2006; 291:300–13. [PubMed: 16458284]
39. Capela A, Temple S. LeX/ssea-1 is expressed by adult mouse CNS stem cells, identifying them as nonependymal. *Neuron*. 2002; 35:865–75. [PubMed: 12372282]
40. Son MJ, Woolard K, Nam DH, Lee J, Fine HA. SSEA-1 is an enrichment marker for tumor-initiating cells in human glioblastoma. *Cell stem cell*. 2009; 4:440–52. [PubMed: 19427293]
41. Ramalho-Santos M, Yoon S, Matsuzaki Y, Mulligan RC, Melton DA. “Stemness”: transcriptional profiling of embryonic and adult stem cells. *Science*. 2002; 298:597–600. [PubMed: 12228720]
42. Georges-Labouesse E, Mark M, Messaddeq N, Gansmuller A. Essential role of alpha 6 integrins in cortical and retinal lamination. *Curr Biol*. 1998; 8:983–6. [PubMed: 9742403]
43. Loulier K, Lathia JD, Marthiens V, et al. beta1 integrin maintains integrity of the embryonic neocortical stem cell niche. *PLoS Biol*. 2009; 7:e1000176. [PubMed: 19688041]

44. Kazanis I, Lathia JD, Vadakkan TJ, et al. Quiescence and activation of stem and precursor cell populations in the subependymal zone of the mammalian brain are associated with distinct cellular and extracellular matrix signals. *J Neurosci.* 30:9771–81. [PubMed: 20660259]
45. Shen Q, Wang Y, Kokovay E, et al. Adult SVZ stem cells lie in a vascular niche: a quantitative analysis of niche cell-cell interactions. *Cell stem cell.* 2008; 3:289–300. [PubMed: 18786416]
46. Hall PE, Lathia JD, Miller NG, Caldwell MA, French-Constant C. Integrins are markers of human neural stem cells. *Stem cells (Dayton, Ohio).* 2006; 24:2078–84.
47. Lathia JD, Gallagher J, Heddleston JM, et al. Integrin alpha 6 regulates glioblastoma stem cells. *Cell stem cell.* 6:421–32. [PubMed: 20452317]
48. Lee J, Kotliarova S, Kotliarov Y, et al. Tumor stem cells derived from glioblastomas cultured in bFGF and EGF more closely mirror the phenotype and genotype of primary tumors than do serum-cultured cell lines. *Cancer cell.* 2006; 9:391–403. [PubMed: 16697959]
49. Rosen JM, Jordan CT. The increasing complexity of the cancer stem cell paradigm. *Science.* 2009; 324:1670–3. [PubMed: 19556499]
50. Grossi-Paoletti E, Paoletti P, Pezzotta S, Schiffer D, Fabiani A. Tumors of the nervous system induced by ethylnitrosourea administered either intracerebrally or subcutaneously to newborn rats. Morphological and biochemical characteristics. *J Neurosurg.* 1972; 37:580–90. [PubMed: 5076376]
51. Barth RF, Kaur B. Rat brain tumor models in experimental neuro-oncology: the C6, 9L, T9, RG2, F98, BT4C, RT-2 and CNS-1 gliomas. *J Neurooncol.* 2009; 94:299–312. [PubMed: 19381449]
52. Zhu Y, Romero MI, Ghosh P, et al. Ablation of NF1 function in neurons induces abnormal development of cerebral cortex and reactive gliosis in the brain. *Genes Dev.* 2001; 15:859–76. [PubMed: 11297510]
53. Gil-Perotin S, Marin-Husstege M, Li J, et al. Loss of p53 induces changes in the behavior of subventricular zone cells: implication for the genesis of glial tumors. *J Neurosci.* 2006; 26:1107–16. [PubMed: 16436596]
54. Kwon CH, Zhao D, Chen J, et al. Pten haploinsufficiency accelerates formation of high-grade astrocytomas. *Cancer Res.* 2008; 68:3286–94. [PubMed: 18451155]
55. Zhu Y, Guignard F, Zhao D, et al. Early inactivation of p53 tumor suppressor gene cooperating with NF1 loss induces malignant astrocytoma. *Cancer cell.* 2005; 8:119–30. [PubMed: 16098465]
56. Doetsch F, Garcia-Verdugo JM, Alvarez-Buylla A. Cellular composition and three-dimensional organization of the subventricular germinal zone in the adult mammalian brain. *J Neurosci.* 1997; 17:5046–61. [PubMed: 9185542]
57. Zheng H, Ying H, Yan H, et al. p53 and Pten control neural and glioma stem/progenitor cell renewal and differentiation. *Nature.* 2008; 455:1129–33. [PubMed: 18948956]
58. Chow LM, Zhang J, Baker SJ. Inducible Cre recombinase activity in mouse mature astrocytes and adult neural precursor cells. *Transgenic Res.* 2008; 17:919–28. [PubMed: 18483774]
59. Burns KA, Ayoub AE, Breunig JJ, et al. Nestin-CreER mice reveal DNA synthesis by nonapoptotic neurons following cerebral ischemia hypoxia. *Cereb Cortex.* 2007; 17:2585–92. [PubMed: 17259645]
60. Burns KA, Murphy B, Danzer SC, Kuan CY. Developmental and post-injury cortical gliogenesis: a genetic fate-mapping study with Nestin-CreER mice. *Glia.* 2009; 57:1115–29. [PubMed: 19115384]
61. Alcantara Llaguno S, Chen J, Kwon CH, et al. Malignant astrocytomas originate from neural stem/progenitor cells in a somatic tumor suppressor mouse model. *Cancer cell.* 2009; 15:45–56. [PubMed: 19111880]
62. Holland EC, Varmus HE. Basic fibroblast growth factor induces cell migration and proliferation after glia-specific gene transfer in mice. *Proc Natl Acad Sci U S A.* 1998; 95:1218–23. [PubMed: 9448312]
63. Holland EC, Hively WP, DePinho RA, Varmus HE. A constitutively active epidermal growth factor receptor cooperates with disruption of G1 cell-cycle arrest pathways to induce glioma-like lesions in mice. *Genes Dev.* 1998; 12:3675–85. [PubMed: 9851974]

64. Holland EC, Celestino J, Dai C, Schaefer L, Sawaya RE, Fuller GN. Combined activation of Ras and Akt in neural progenitors induces glioblastoma formation in mice. *Nat Genet.* 2000; 25:55–7. [PubMed: 10802656]
65. Holmen SL, Williams BO. Essential role for Ras signaling in glioblastoma maintenance. *Cancer Res.* 2005; 65:8250–5. [PubMed: 16166301]
66. Robinson JP, VanBrocklin MW, Guilbeault AR, Signorelli DL, Brandner S, Holmen SL. Activated BRAF induces gliomas in mice when combined with Ink4a/Arf loss or Akt activation. *Oncogene.* 29:335–44. [PubMed: 19855433]
67. Marumoto T, Tashiro A, Friedmann-Morvinski D, et al. Development of a novel mouse glioma model using lentiviral vectors. *Nat Med.* 2009; 15:110–6. [PubMed: 19122659]
68. Liu HK, Belz T, Bock D, et al. The nuclear receptor tailless is required for neurogenesis in the adult subventricular zone. *Genes Dev.* 2008; 22:2473–8. [PubMed: 18794344]
69. Liu HK, Wang Y, Belz T, et al. The nuclear receptor tailless induces long-term neural stem cell expansion and brain tumor initiation. *Genes Dev.* 24:683–95. [PubMed: 20360385]
70. Jacques TS, Swales A, Brzozowski MJ, et al. Combinations of genetic mutations in the adult neural stem cell compartment determine brain tumour phenotypes. *EMBO J.* 29:222–35. [PubMed: 19927122]
71. Read RD, Cavenee WK, Furnari FB, Thomas JB. A drosophila model for EGFR-Ras and PI3K-dependent human glioma. *PLoS Genet.* 2009; 5:e1000374. [PubMed: 19214224]
72. Lindberg N, Kastemar M, Olofsson T, Smits A, Uhrbom L. Oligodendrocyte progenitor cells can act as cell of origin for experimental glioma. *Oncogene.* 2009; 28:2266–75. [PubMed: 19421151]
73. Hambardzumyan D, Amankulor NM, Helmy KY, Becher OJ, Holland EC. Modeling Adult Gliomas Using RCAS/t-va Technology. *Transl Oncol.* 2009; 2:89–95. [PubMed: 19412424]
74. Zhu H, Acquaviva J, Ramachandran P, et al. Oncogenic EGFR signaling cooperates with loss of tumor suppressor gene functions in gliomagenesis. *Proc Natl Acad Sci U S A.* 2009; 106:2712–6. [PubMed: 19196966]
75. Phillips HS, Kharbanda S, Chen R, et al. Molecular subclasses of high-grade glioma predict prognosis, delineate a pattern of disease progression, and resemble stages in neurogenesis. *Cancer cell.* 2006; 9:157–73. [PubMed: 16530701]
76. Verhaak RG, Hoadley KA, Purdom E, et al. Integrated genomic analysis identifies clinically relevant subtypes of glioblastoma characterized by abnormalities in PDGFRA, IDH1, EGFR, and NF1. *Cancer cell.* 17:98–110. [PubMed: 20129251]
77. Lottaz C, Beier D, Meyer K, et al. Transcriptional profiles of CD133+ and CD133- glioblastoma-derived cancer stem cell lines suggest different cells of origin. *Cancer Res.* 70:2030–40. [PubMed: 20145155]
78. He J, Liu Y, Xie X, et al. Identification of cell surface glycoprotein markers for glioblastoma-derived stem-like cells using a lectin microarray and LC-MS/MS approach. *J Proteome Res.* 9:2565–72. [PubMed: 20235609]
79. Gangemi RM, Griffero F, Marubbi D, et al. SOX2 silencing in glioblastoma tumor-initiating cells causes stop of proliferation and loss of tumorigenicity. *Stem cells (Dayton, Ohio).* 2009; 27:40–8.
80. Ligon KL, Huillard E, Mehta S, et al. Olig2-regulated lineage-restricted pathway controls replication competence in neural stem cells and malignant glioma. *Neuron.* 2007; 53:503–17. [PubMed: 17296553]
81. Johnson RA, Wright KD, Poppleton H, et al. Cross-species genomics matches driver mutations and cell compartments to model ependymoma. *Nature.* 466:632–6. [PubMed: 20639864]
82. Huang EH, Hynes MJ, Zhang T, et al. Aldehyde dehydrogenase 1 is a marker for normal and malignant human colonic stem cells (SC) and tracks SC overpopulation during colon tumorigenesis. *Cancer Res.* 2009; 69:3382–9. [PubMed: 19336570]
83. Carpentino JE, Hynes MJ, Appelman HD, et al. Aldehyde dehydrogenase-expressing colon stem cells contribute to tumorigenesis in the transition from colitis to cancer. *Cancer Res.* 2009; 69:8208–15. [PubMed: 19808966]
84. Cheung AM, Wan TS, Leung JC, et al. Aldehyde dehydrogenase activity in leukemic blasts defines a subgroup of acute myeloid leukemia with adverse prognosis and superior NOD/SCID engrafting potential. *Leukemia.* 2007; 21:1423–30. [PubMed: 17476279]

85. Ran D, Schubert M, Pietsch L, et al. Aldehyde dehydrogenase activity among primary leukemia cells is associated with stem cell features and correlates with adverse clinical outcomes. *Exp Hematol.* 2009; 37:1423–34. [PubMed: 19819294]
86. Pearce DJ, Taussig D, Simpson C, et al. Characterization of cells with a high aldehyde dehydrogenase activity from cord blood and acute myeloid leukemia samples. *Stem cells (Dayton, Ohio).* 2005; 23:752–60.
87. Ma S, Chan KW, Lee TK, et al. Aldehyde dehydrogenase discriminates the CD133 liver cancer stem cell populations. *Mol Cancer Res.* 2008; 6:1146–53. [PubMed: 18644979]
88. Ucar D, Cogle CR, Zucali JR, et al. Aldehyde dehydrogenase activity as a functional marker for lung cancer. *Chem Biol Interact.* 2009; 178:48–55. [PubMed: 18952074]
89. Ginestier C, Hur MH, Charafe-Jauffret E, et al. ALDH1 is a marker of normal and malignant human mammary stem cells and a predictor of poor clinical outcome. *Cell stem cell.* 2007; 1:555–67. [PubMed: 18371393]
90. Kadowaki M, Nakamura S, Machon O, Krauss S, Radice GL, Takeichi M. N-cadherin mediates cortical organization in the mouse brain. *Dev Biol.* 2007; 304:22–33. [PubMed: 17222817]
91. Walker MM, Ellis SM, Auza MJ, Patel A, Clark P. The intercellular adhesion molecule, cadherin-10, is a marker for human prostate luminal epithelial cells that is not expressed in prostate cancer. *Mod Pathol.* 2008; 21:85–95. [PubMed: 18084254]
92. Hendrix MJ, Seftor EA, Meltzer PS, et al. Expression and functional significance of VE-cadherin in aggressive human melanoma cells: role in vasculogenic mimicry. *Proc Natl Acad Sci U S A.* 2001; 98:8018–23. [PubMed: 11416160]
93. Wang L, O'Leary H, Fortney J, Gibson LF. Ph⁺/VE-cadherin⁺ identifies a stem cell like population of acute lymphoblastic leukemia sustained by bone marrow niche cells. *Blood.* 2007; 110:3334–44. [PubMed: 17638851]
94. Read TA, Fogarty MP, Markant SL, et al. Identification of CD15 as a marker for tumor-propagating cells in a mouse model of medulloblastoma. *Cancer cell.* 2009; 15:135–47. [PubMed: 19185848]
95. Ward RJ, Lee L, Graham K, et al. Multipotent CD15⁺ cancer stem cells in patched-1-deficient mouse medulloblastoma. *Cancer Res.* 2009; 69:4682–90. [PubMed: 19487286]
96. Li C, Heidt DG, Dalerba P, et al. Identification of pancreatic cancer stem cells. *Cancer Res.* 2007; 67:1030–7. [PubMed: 17283135]
97. Vermeulen L, Todaro M, de Sousa Mello F, et al. Single-cell cloning of colon cancer stem cells reveals a multi-lineage differentiation capacity. *Proc Natl Acad Sci U S A.* 2008; 105:13427–32. [PubMed: 18765800]
98. Chan KS, Espinosa I, Chao M, et al. Identification, molecular characterization, clinical prognosis, and therapeutic targeting of human bladder tumor-initiating cells. *Proc Natl Acad Sci U S A.* 2009; 106:14016–21. [PubMed: 19666525]
99. Chu P, Clanton DJ, Snipas TS, et al. Characterization of a subpopulation of colon cancer cells with stem cell-like properties. *International journal of cancer.* 2009; 124:1312–21.
100. Takaishi S, Okumura T, Tu S, et al. Identification of gastric cancer stem cells using the cell surface marker CD44. *Stem cells (Dayton, Ohio).* 2009; 27:1006–20.
101. Prince ME, Sivanandan R, Kaczorowski A, et al. Identification of a subpopulation of cells with cancer stem cell properties in head and neck squamous cell carcinoma. *Proc Natl Acad Sci U S A.* 2007; 104:973–8. [PubMed: 17210912]
102. Zhang S, Balch C, Chan MW, et al. Identification and characterization of ovarian cancer-initiating cells from primary human tumors. *Cancer Res.* 2008; 68:4311–20. [PubMed: 18519691]
103. Patrawala L, Calhoun T, Schneider-Broussard R, et al. Highly purified CD44⁺ prostate cancer cells from xenograft human tumors are enriched in tumorigenic and metastatic progenitor cells. *Oncogene.* 2006; 25:1696–708. [PubMed: 16449977]
104. Lee A, Kessler JD, Read TA, et al. Isolation of neural stem cells from the postnatal cerebellum. *Nat Neurosci.* 2005; 8:723–9. [PubMed: 15908947]

105. Campos LS, Leone DP, Relvas JB, et al. Beta1 integrins activate a MAPK signalling pathway in neural stem cells that contributes to their maintenance. *Development*. 2004; 131:3433–44. [PubMed: 15226259]
106. Nagato M, Heike T, Kato T, et al. Prospective characterization of neural stem cells by flow cytometry analysis using a combination of surface markers. *Journal of neuroscience research*. 2005; 80:456–66. [PubMed: 15795928]
107. Vaillant F, Asselin-Labat ML, Shackleton M, Forrest NC, Lindeman GJ, Visvader JE. The mammary progenitor marker CD61/beta3 integrin identifies cancer stem cells in mouse models of mammary tumorigenesis. *Cancer Res*. 2008; 68:7711–7. [PubMed: 18829523]
108. Zhang M, Behbod F, Atkinson RL, et al. Identification of tumor-initiating cells in a p53-null mouse model of breast cancer. *Cancer Res*. 2008; 68:4674–82. [PubMed: 18559513]
109. Patrawala L, Calhoun-Davis T, Schneider-Broussard R, Tang DG. Hierarchical organization of prostate cancer cells in xenograft tumors: the CD44+alpha2beta1+ cell population is enriched in tumor-initiating cells. *Cancer Res*. 2007; 67:6796–805. [PubMed: 17638891]
110. Mulholland DJ, Xin L, Morim A, Lawson D, Witte O, Wu H. Lin-Sca-1+CD49fhigh stem/progenitors are tumor-initiating cells in the Pten-null prostate cancer model. *Cancer Res*. 2009; 69:8555–62. [PubMed: 19887604]
111. Matsui W, Huff CA, Wang Q, et al. Characterization of clonogenic multiple myeloma cells. *Blood*. 2004; 103:2332–6. [PubMed: 14630803]
112. Franklin, BJ.; Paxinos, G. The mouse brain atlas in stereotaxic coordinates. New York, NY: Elsevier; 2007.
113. Kazanis, I.; Lathia, J.; Moss, L.; French-Constant, C. StemBook. The Stem Cell Research Community. StemBook; 2008. The neural stem cell microenvironment. <http://www.stembook.org>

Figure 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 Atlas¹¹³ and Kazanis *et al.*¹¹⁴.

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 $\alpha 6\beta 1$ in human fetal neural stem cells ⁴⁶ , integrin $\beta 1$ in rodent NSPCs ^{105, 106}	Human patient specimens (integrin $\alpha 6\beta 1$) 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