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REVIEW ARTICLE

Cellular Origin of Glioblastoma: Current Evidence, Challenges, and Implications

Grace R. Qian[1], Yiwei Fu[1], Albert H. Kim[1, 2 *]

1) Taylor Family Department of Neurosurgery, Washington University School of Medicine, St. Louis, MO, 63110, USA
2) The Brain Tumor Center, Siteman Cancer Center, Washington University School of Medicine, St. Louis, MO, 63110, USA; alberthkim@wustl.edu

ABSTRACT: Glioblastoma (GBM) is the most common primary cancer of the central nervous system (CNS) in adults, with a 5-year relative survival rate of 6.9%. For the past 30 years, standard treatment has included a combination of surgical resection with radiation therapy and temozolomide. Progress in treatment for GBM has been hindered due to the brain's limited repair abilities, GBM's diffuse nature into eloquent brain areas that make full resection essentially impossible, and the heterogeneous tumor, which contributes to treatment resistance and inevitable recurrence. As single-cell RNA sequencing allows for identifying subtumoral cellular populations, a potential research area is to study the cell of origin—the cells that accumulate specific mutations in the right conditions to become tumorigenic. Studying the basic science behind transforming the cell of origin into GBM offers insight into how GBM may recur and develop targeted drugs. Here, we provide a comprehensive literature review on possible cells of origin, including neural stem cells (NSCs), oligodendrocyte progenitor cells (OPCs), and astrocytes. We conclude that the cellular origin of GBM in addition to specific mutations and environmental conditions, may better define a specific patient's GBM, which has implications for diagnosis, therapy, and prognosis.

KEYWORDS: Biomedical and Health Sciences, Genetics and Molecular Biology of Disease, Glioblastoma.

Introduction

Glioblastoma (GBM) is a grade IV highly aggressive primary malignant glioma, which accounts for 48.6% of malignant central nervous system tumors, making it the most common primary cancer of the central nervous system (CNS) in adults.[1,2] The median age of diagnosis is 64 years, and the incidence increases with age, peaking at 75–84 years.[3] The median survival is 15 months post-diagnosis with a 5-year survival rate of 6.9%. Among individuals with GBM, prominent figures including Beau Biden and senators Ted Kennedy and John McCain have been afflicted with this disease.[4]

Multiple challenges arise in treating recurrent GBM, including the brain's limited regenerative capacities, the unique and selectively permeable blood-brain barrier (BBB) vascularization that makes drug delivery difficult, GBM's high invasiveness and infiltration into eloquent brain areas that renders full resection essentially impossible, and tumor resistance to radiation and chemotherapy — which all lead to inevitable recurrence.[2,5] GBMs have been classified into three transcriptional subtypes – Proneural, Classical, and Mesenchymal.[6] Currently, standard treatment includes surgical resection to debulk the tumor, followed by fractionated radiation therapy and chemotherapy with temozolomide.[7,8] Despite intensive treatment, GBM has a near 100% recurrence rate, with a 10-year survival rate of only around 1%.[4]

There has been renewed interest in the "cell of origin" concept in recent years. Exact definitions of the cell of origin vary, but consensus agrees that the normal cell is malignantly transformed into the first GBM cell.[9] There are multiple possible cells of origin for GBM. The cell of origin, combined with specific mutations, may determine the specific molecular features of an individual's GBM, thus offering a potentially useful means of stratifying GBM tumors for distinct therapeutic strategies. Identifying the cell of origin of GBM could also help us understand the biological mechanisms of tumor initiation and provide additional and possibly earlier targets in the process of GBM pathogenesis. Here, we discuss evidence for neural stem cells (NSCs), oligodendrocyte progenitor cells (OPCs), and astrocytes for being key cell types of origin in GBM development. The mechanism of transformation is debated, with theories of accumulating somatic genetic mutations, dedifferentiation of progeny cells, and epigenetic changes accounting for recurrence. Importantly, molecularly distinct tumors formed when the same mutations—NF1, Trp53, and PTEN—were mutated in NSCs and OPCs, suggesting that the cell of origin has implications for tumor phenotype.[10] The mutational signature distinctions that arise from cell-of-origin differences may enable the characterization of properties and therapeutic vulnerabilities. Importantly, there is the distinction between cells of origin and cells of mutation; The cell of mutation is the cell in which the DNA mutation occurs, potentially because of DNA damage. On the other hand, the cell of origin is the cell in which the mutation itself is manifested biologically and acquires malignant features. For example, in familial cancer, mutations may be harbored in multiple cell types (cells of mutation), but only certain cells progress into tumors (cells of origin). Distinguishing between cells that transform into malignant tumors and those that aquire initial mutations is important since identifying the cell of origin may provide insight into tumor development, GBM subtypes, and potential future

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therapies. Thus, identifying and considering the cell of origin in combination with the specific mutations and environmental conditions may improve personalized and patient-specific treatment in the future.

Discussion

In understanding the cellular origins of GBM, it is important to introduce normal glial development (Figure 1). Neural stem cells in specialized niches give rise to glial and neural progenitor cells, which differentiate into oligodendrocyte and astrocyte lineages. There is support for these progenitor pools as potential cells of origin. This evidence is summarized in Table 1.

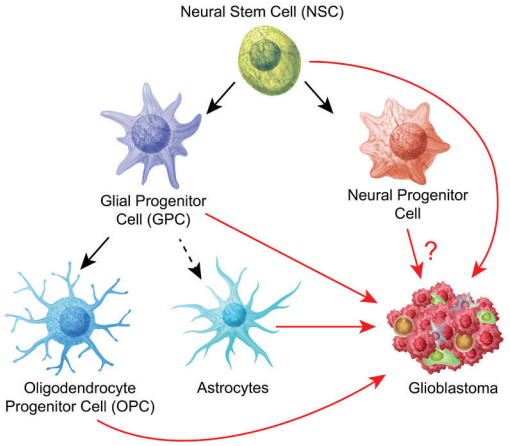

Figure 1: Overview of developmental lineage and GBM cells of origin, highlighting the developmental relationship between possible cells of origin. Neural stem cells (NSCs) give rise to neural progenitor cells and glial progenitor cells (GPCs), which in turn differentiate into oligodendrocyte progenitor cells (OPCs) and astrocytes. These cell types are all able to accumulate mutations and form GBM.

Table 1: Summary and key findings of experimental evidence for specific cell of origin-mutation combinations and environmental conditions. NSCs, OPCs, and astrocytes were all able to successfully produce GBM tumors in various mouse models and cell lines.

Cell of Origin	Driver Mutation	Key findings/ important notes	Reference
NSCs	Nf1/p53 or Nf1/p53/Pten	Tamoxifen-inducible *nestin-creER*^{T2} in vivo mice model	Alcantara Llaguno et al. 2009 [18]
	Trp53/Pten/Egfr	Mouse model, only SVZ injections produced tumors	Lee et al. 2018 [13]
	Rb/p53, Rb/p53/Pten, or p53/Pten	Mouse, mature astrocytes did not produce tumors	Jacques et al. 2010 [21]
	Pten	Mesenchymal stem cells did not produce tumors	Duan et al. 2015 [19]
OPCs	Pdgfa	Retroviral vector, tumors expressed OPC markers (NG2, PDGFRA)	Assanah et al. 2006 [33]
	Pdgfb	PDGF is the mitogen for OPCs	Lindberg et al. 2009 [30]
	S100b promoter Egfr/p53	S100b promoter is not expressed in NSCs	Persson et al. 2010 [67]
	p53/Nf1	Mosaic Analysis with Double Markers (MADM), no tumor production in NSC lines	Liu et al. 2011 [47], Zong et al. 2005 [48]
Astrocytes	P16INK4a/p19ARF	SCID mouse, both NSCs and astrocytes formed GBM	Bachoo et al. 2002 [38]
	p53/Pten/Rb	Fully penetrant mouse model, >20% tumors formed in non-neural progenitor niches	Chow et al. 2011 [39]
	Reinduction of Erbb2	Mouse, formed radial glial progenitors (dedifferentiation)	Ghashghaei et al. 2007 [35]

Abbreviations: Subventricular zone (SVZ), Platelet-derived growth factor (PDGF) – A/B denotes subunits, Neurofibromatosis Type 1 (NF1), Phosphatase and tensin homolog deleted on chromosome 10 (PTEN), Epidermal Growth Factor Receptor (EGFR), Human Epidermal Growth Factor Receptor 2 (HER2/ERBB2), Retinoblastoma (Rb), **Tumor suppressor genes:** PTEN, p53, NF1, Rb, **Tumor oncogenes:** PDGF, EGFR, ERBB2 **Activating mutations:** PTEN, p53, EGFR, ERBB2 **Repressing mutation:** NF1, Rb, PDGF

Neural Stem Cells as a Cell of Origin:

NSCs are a fundamental cell type in the development of the brain, as they give rise to all of the cells of the CNS.[13] NSCs divide both asymmetrically, generating differentiated cell types like neurons, astrocytes, and oligodendrocytes, and progenitor cells like OPCs or GPCs, and symmetrically, generating more NSCs (Figure 1). Their replication and growth contribute to cortical expansion during development. Historically, all neurons were believed to be generated during development before adulthood.[14] However, pioneering work by Nottebohm using labeled DNA precursors found evidence for producing new neurons in adulthood in avian models,[15] suggesting that neurogenesis also occurs later in life through the maintenance and division of a pool of NSCs that persists into adulthood. These postnatal NSCs are localized to the astrocytic ribbon of the brain's subventricular zone (SVZ), adjacent to the lateral ventricles,[16] and the hippocampus's subgranular zone (SGZ). Growing evidence suggests that NSC localization in the SVZ is significant in creating a seed-to-soil relationship for gliomagenesis. The SVZ microenvironment may be a neurogenic niche for NSCs through the release of chemoattractants like pleiotrophin.[17] The SVZ's interactions with cerebrospinal fluid (CSF) and vascularization also provide a rich pro-tumor microenvironment.[18,19] Signals to the SVZ from the CSF and blood are helpful during development in transferring growth factors to proliferate NSCs. Still, disruption of the control system can corrupt tumor formation and growth.[18] Furthermore, in an MRI-based study, 93% of GBMs contacted at least some part of the lateral ventricular wall lined by the SVZ.[20] Thus, the location of GBM in the SVZ offers a unique look into NSCs as a potential cell of origin for GBM. These studies suggest that NPCs are important for glioma invasion by releasing factors like pleiotrophin, which help create an attractive and supportive niche for glioma cells, with further implications that these NSCs themselves may transition into tumor cells.

- NSC Evidence:

Several studies have investigated the tumorigenic potential of neural stem cells harboring oncogenic mutations in mouse models.[21] Deletion of p53, NF1, and PTEN, specifically in embryonic or adult NSCs by the Cre/loxP system, was sufficient to generate GBMs in mice.[22] In addition, conditional knockout of p53, NF1, and PTEN in either adult mouse SVZ, where NSCs reside, or nonneurogenic areas, such as the cortex, using transgenic mice led to tumor formation only in the SVZ.[23,24] Together, these studies suggest that NSCs residing in the SVZ can act as the cells of origin for GBM. Also, it was found that low levels of GBM driver mutations can be detected in SVZ cells, suggesting that tumor cells had originated and migrated

from the SVZ. For directional validation, the authors discovered that while tumor cells exhibited tumor-unique mutations, no SVZ cells contained SVZ-unique mutations, suggesting that the directionality of gliomagenesis was SVZ to the tumor.[25] Upon further analysis, these putative origin cells were NSCs in the astrocytic ribbon. This was confirmed in a mouse model in which p53/PTEN/EGFR mutations were induced in the SVZ, producing tumors in NSC mutant mice; other groups have found similar results.[25,26] Additionally, evidence from human and mouse data suggest that GFAP-positive NSCs may act as the cell of origin. By analyzing TERT promoter mutation enrichment in various SVZ layers, one study found that these mutations were significantly enriched only in GFAP-positive NSCs in the astrocytic ribbon, not in bulk astrocytes. This suggests that GFAP-positive NSCs, rather than mature differentiated astrocytes, may act as the primary cells of origin for GBM.[16]

Furthermore, mutations in the telomerase reverse transcriptase promoter (TERTp) associated with GBM were found in the astrocytic ribbon, where many NSCs reside in the adult brain.[25] TERTp mutations are found in more than 80% of GBMs, suggesting that TERTp may be a critical mutation of GBM. Its presence in the NSC niche further supports NSCs as the cell of origin.[2] Another group showed that human induced pluripotent stem cell lines, upon differentiation into NSCs and deletion of canonical GBM mutations such as PTEN, NF1, TP53, and PDGFRA, could form GBM of the various molecular subtypes when implanted into the brains of immunocompromised mice.[24] CD133 could be a potentially important marker that can help identify both neural stem cells (NSCs) and GBM stem cells. Importantly, however, sharing the same markers does not necessarily imply a lineage relationship between NSCs as a cell of origin and GBM stem cells, a topic that requires further investigation.[27,28] Thus, the ability to malignantly transform human NSCs into tumorigenic GBM cells offers additional evidence that NSCs are a cell of origin for GBM. Secondarily, neurogenic niches that house neural progenitor cells (NPCs) may promote GBM pathogenesis or malignant behaviors. However, to our knowledge, there is no experimental evidence for NPCs per se as cells of origin.

Cells of Origin from Glial Lineage:

Neural stem cells give rise to progenitor cells, which can differentiate into glial cells (Figure 1). Glial cells are the supportive "glue" of the brain and spinal cord, with a ratio of one glial cell to one neuron.[29] Glial cells include astrocytes and oligodendrocyte lineage cells.[30] Their functions involve directing neuronal migration, synaptogenesis, influencing growth, and monitoring the CNS microenvironment.[31] A long history of GBM literature suggests that glial lineage cells may be the cells of origin. Therefore, whether other developmental neural cell states harbor tumorigenic potential remains to be seen.

Oligodendrocyte Progenitor Cells as a Cell of Origin:

Oligodendrocyte progenitor cells (OPCs) are lineage-restricted progenitor cells that arise from the asymmetrical division of NSCs during development and reside in the parenchyma of adult brains.[32] OPCs are more abundant than NSCs and more widely distributed, constituting 70% of the dividing cells in the brain.[9,32,33] They can also arise from the differentiation of glial progenitor cells (GPCs) and constitute one of the specific populations of progenitor cells within the heterogeneous glial progenitor population. OPC-specific markers include neural/glial antigen 2 (NG2), a type of cell membrane glycoprotein or proteoglycan, the PDGF receptor alpha (PDGFRA), and Olig1.[34] Histopathologic analysis of human GBM shows expression of these markers,[10] suggesting that GBM may derive from OPCs as a cell of origin. However, NSCs are progenitor cells for most of the population of cells within the brain. Thus, we could expect that NSCs with tumorigenic potential may go down a developmental route, leading them to express markers found in OPCs instead of the tumor arising in OPCs themselves. Thus, a method to confirm and trace lineage would be important in supporting the OPC theory for the cell-of-origin.

In GBM, PDGFRA mutations are the second most common tyrosine kinase receptor mutation, observed in around 30% of patients.[35] Exogenous PDGFA infusion into the adult SVZ has been shown to induce OPCs to form GBM lesions.[36] In mice, PDGFB transfer via a Ctv-a transgenic mouse model induced gliomas in 33% of the cases.[37] Furthermore, other growth factors, such as epidermal growth factor receptor (EGFR) overexpression, are common in about 60% of primary glioblastomas.[38] The overexpression of growth factors like EGFR and PDGF is catalyzed by mutations in tumor suppressor genes like p53 and PTEN, thus suggesting that p53 and PTEN mutations cannot generate GBM independently but work in coordination with growth factor mutations.[34] These observations have been confirmed in murine models, showing successful tumor formation.[39] Injection of a PDGF growth factor-expressing retrovirus with GFP radiolabeling into the subcortical white matter formed GBM tumors in 100% of animals (86/86). At the same time, none of the GFP-only control mice showed any signs of tumor growth. The replication incompetent retrovirus selectively infects the cycling glial progenitors, and their identity was confirmed by immunohistochemistry staining showing the presence of markers like NG2, OLIG2, and CC1, which are associated with OPCs.[40] The successful tumor formation of OPCs in mice provides evidence that they may represent a possible cell of origin. In summary, successful tumor formation with specific growth factors and tumor suppressor mutations in OPCs provides evidence that OPCs can be the cell of origin in these models. In summary, the same mutations in OPCs generate a molecularly distinct GBM from that of NSCs. The difference in tumorigenesis from NSCs and OPCs highlights how the cell of origin in GBM may determine the specific subtype of GBM and may help to stratify GBM tumors for distinct therapeutic strategies.

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Astrocytes as a cell of origin:

-Astrocyte dedifferentiation theory and mutation dependency:

Astrocytes are a type of glial cell. They are the most abundant glial cells and link neurons to the blood supply, forming a critical blood-brain-barrier (BBB) component.[30] Embryologically, they develop from outer radial glia (oRG) cells and subpallial cells near the basal ganglia.[41] Astrocytes develop from neural progenitors in the SVZ in adults and proliferate through local mitotic division. At the end of embryonic development, oRG cells give rise to astrocytes. Early on, it was shown that mature mouse astrocytes can dedifferentiate to radial glia (which don't exist in adult brains) through *in vivo* induction of the tyrosine kinase receptor ErbB2. When cultured, these dedifferentiated glial progenitors can give rise to mature glial cells like astrocytes. These dedifferentiated astrocytes share similar properties with embryonic oRG cells and are less lineage-restricted than typical adult NSCs.[42]

Additionally, astrocytes cultured with TGFα were converted to radial glial cells and subsequently differentiated into NSCs that formed self-renewing neurospheres.[43] These unique properties of astrocytes offer insight into the astrocyte dedifferentiation theory and lay a foundation for studies of astrocyte gliomagenesis. One study showed that upregulation in growth factor receptors like PDGF contributes to the activation of autocrine loops that lead to high-grade astrocytomas.[44] Similarly, the combined loss of p16INK4a and p19ARF triggered the dedifferentiation of astrocytes in response to EGFR activation, forming GBM-like tumors upon orthotopic implantation into mice.[38] A mouse model with Tp53/PTEN deletion mutations induced in the SVZ targeting GFAP (a marker for astrocytes) positive cells found that over 20% of tumors were formed in regions outside of the SVZ. Thus, it is difficult to distinguish between astrocytes and NSCs in the SVZ. Still, it was hypothesized that the tumors formed in the cortex, brain stem, cerebellum, and spinal cords originated from astrocytes.[45] One study found that sequential mutation of NF1 and p53 in isolated astrocyte cultures had weak transforming abilities. But, when the same mutant astrocytes were treated with PDGF and EGF growth factor, they could form tumors when injected into mouse brains. Thus, astrocytes can transform into tumor cells, but this phenomenon may depend on the mutation and growth factor exposure.[46] Astrocytes with mutations in the retinoblastoma protein family initiated grade II gliomas. However, additional mutations in PTEN caused grade progression, suggesting that gliomagenesis depends not only on the cell of origin but also on the mutation.[9,47] Importantly, modeling with genetically engineered mice (GEMM) is not error-proof, and there are limitations to inducing mutations in specific cell populations. In summary, evidence for the astrocyte dedifferentiation theory and successful GBM formation in mice suggest that astrocytes are a possible cell-of-origin.

- Debate over biomarkers and astrocytes as cells of origin:

Dedifferentiation of astrocytes or other terminal cell populations has been a controversial theory since evidence for this is derived primarily from *in vitro* and *in vivo* mouse studies rather than human studies.[12,48] In particular, in the transgenic mouse experiments, it is challenging to ensure that only terminal astrocyte populations are targeted (*e.g.*, GFAP-driven transgenes are expressed in both astrocytes and NSCs). In studying the cell of origin for GBM, Glial Fibrillary Acid Protein (GFAP) is generally used as a promoter or marker for astrocytes. However, it has been difficult to find markers that account for the heterogeneity of the astrocytic population uniquely in the astrocyte population. This is the case for GFAP expressed by all astrocytes and NSCs.[41] Additionally, GFAP+ cells don't always account for all astrocytes, as more than 40% of astrocytes in mice were GFAP-. [49,50] There have also been difficulties in tracing the lineage of astrocytes through biomarkers, most recently with limited successes in determining molecular markers for the astrocyte precursor cell (APC). In conclusion, the shared biomarkers make it challenging to create transgenic mice that target astrocytes specifically. Furthermore, findings from studies that use a GFAP promoter are thus subject to uncertainties of whether astrocytes or NSCs induced tumorigenesis.

Thus, the non-specificity of transgenic mouse experiments and the difficulty in distinguishing mature astrocytes from NSCs and even earlier neural progenitors using cellular markers are all reasons that astrocytes have remained controversial as a cell of origin for GBM.

However, astrocyte progenitor cells may still have potential as cells of origin. According to Lee *et al*, GBM cells of origin are a GFAP-positive NSC or neural progenitor in the astrocytic ribbon of the SVZ.[16] The non-specificity of GFAP expression and uncertainty of the identity of this cell of origin opens the possibility of a glial progenitor as a potential cell of origin (which can form both OPCs and astrocyte progenitor cells). Thus, while the dedifferentiation theory is controversial and there is no direct evidence for astrocytes as cells of origin *in vivo* and in human tumors, it is possible that astrocytes or other glial progenitors may act as a cell of origin. Future research is needed to elucidate the identity of these cells.

Mesenchymal Stem Cells:

Mesenchymal stem cells (MSCs) are a type of multipotent adult stem cells. They can differentiate into various tissue cell precursors like adipocytes, chondrocytes, and osteoblasts. In GBM, MSCs can arise locally or differentiate from glioma stem cells (GSCs). They exhibit tumor-promoting effects such as suppressing immune responses and supporting tumor growth. Single-cell RNA sequencing has provided evidence for MSCs as a possible cell of origin for the GBM mesenchymal subtype.[51]

Despite this evidence, the study of MSCs as a candidate cell of origin for GBM is still in its infancy. The mesenchymal subtype is also defined by other factors such as the influence of the tumor microenvironment, accumulating mutations like NF1, and treatment-induced mesenchymal transition (wherein treated tumors tend to shift towards the more resistant mesenchymal phenotype.[51] Nevertheless, recent transcriptomic and lineage-tracing studies have begun to reveal the potential of MSCs as cells of origin.

Additional Challenges in Defining Cell of Origin Studies:

- Plasticity:

The plasticity of cell lineages creates another nuance in the research on the cell of origin of GBM by revealing how tumors can evolve between origination, progression, and recurrence. Research on the role of epigenetics (reversible modifications that affect gene expression without altering the DNA sequence) in GBM recurrence has shown that the cell of origin may be more dynamic and fluid and less unchangeable and anchored, as previously thought. For example, it was demonstrated that culturing OPCs with fetal calf serum (FCS), cytokines, and basic fibroblast growth factor (bFGF) could revert OPCs to multipotential NSCs that could differentiate successfully into oligodendrocytes, neurons, and astrocytes.[52] Additionally, OPCs were found to be able to turn into NSCs through reactivation of SOX2 and chromatin remodeling on histone H3.[53] Plasticity thus represent a potential challenge in identifying the cell of origin.

- Cell-autonomous vs. Non-Cell-autonomous:

In transgenic mice models of the cell of origin, mutations cannot be induced in a singular cell but rather in a general population. Thus, these models cannot differentiate between the cell of origin and their neighbors. As such, phenotypic changes cannot be exclusively attributed to mutations in the cell of origin. The effects of a driver mutation depend not only on the mutation itself (cell-autonomous) but also on the genetic background and the tumor microenvironment. These non-cell autonomous effects can affect the cancer cell phenotype, leading to further heterogeneity.[54] Liu *et al*. induced p53/NF1 mutations under the Mosaic Analysis with Double Markers (MADM) system and found that although mutations were initiated in NSCs, only the OPC cell type proliferated and formed GBM tumors.[11] The conclusion was that the cell of mutation (NSC) is distinct from the cell of origin (OPCs). This suggests that OPCs can be the cell of origin, at least with p53/NF1 mutation. The technique of MADM allows for tracing green fluorescent protein-tagged single cells prior to malignant transformation and continuous comparison to a wild-type red fluorescent protein-tagged sibling cell throughout cell cycles and evolution.[55] This addresses the issue of cell-autonomous vs. non-cell-autonomous effects. Thus, additional studies should be conducted with different mutations.[56] The results of these studies would shed light on the relationship between driver mutation and cell of origin in GBM.

- Tumor Heterogeneity:

One major obstacle in developing effective treatments for GBM is its highly heterogeneous nature, both intertumorally and intratumorally. There have been various approaches to studying GBM subtypes. In 2008, the Cancer Genome Atlas created a transcription-based classification system to stratify GBM into four subtypes (Proneural, Neural, Classical, and Mesenchymal), which were also associated with PDGFRA and TP53 mutations for Proneural, EGFR for Classical, and NF1 for Mesenchymal subtypes.[57,58] Verhaak and colleagues found that treatment susceptibility may vary amongst these subtypes.[56] In 2017, Verhakk's group updated their subtyping system, removing the neural subtype after showing it was primarily composed of normal brain elements and thus did not represent an individual subtype.[59]

Despite classification attempts, there are limitations in the current molecular classification system.[60] For example, subtypes can change with recurrence. One study found that 63% of patients were observed with a different transcriptional subtype after recurrence.[61] Furthermore, characterization of the post-treatment proneural-to-mesenchymal transition, or PMT, shows increased aggressiveness, tumor-associated macrophages (TAMs), and therapy resistance.[62,63] Additionally, Single-cell RNA sequencing (scRNA-seq) of tumor cells demonstrated a mixture of subtypes within one tumor, i.e., intratumoral heterogeneity.[64,65] These subpopulations of tumor cells vary in function, type, and lineage. While previous classification based on bulk tumors accounted for intertumoral heterogeneity, developing sensitive single-cell sequencing provides insights into defining a high-resolution classification of GBM subpopulations. Neftel *et al.* used scRNA-seq to identify four distinct tumor cell states, which were also associated with mutations in CDK4, EGFR, PDGFRA, and NF1, respectively.[19,66] Not only does GBM exhibit molecular heterogeneity, but developmental heterogeneity as well. Single-cell techniques have also opened further discussion on the existence of a self-renewing glioma stem cell population (GSC), which may also play a role in GBM recurrence.[65,67] Efforts to more comprehensively characterize the intratumoral heterogeneity of GBMs raise the possibility of stratifying patients based on predicted similarities in response to treatment and targeting specific subcellular populations. For GBM, intratumoral heterogeneity reflects and accounts for intertumoral heterogeneity, so recent focus has been shifted to tumor genetic and transcriptomic classification.[68] In the future, subpopulation-based classification could guide precision therapy.

Therapeutic Implications of the Cell of Origin in GBM:

Currently, standard GBM treatment includes surgical resection followed by radiation and chemotherapy with temozolomide.[69] Clinical trials of Optune, a cancer division prevention device, and the EGFRV3 vaccine have improved survival time.[70,71] However, patients survive only around 17-20 months, even with this novel treatment. Additionally, GBM is well known for its near 100% recurrence rate, which occurs at least in part due to the heterogeneous nature of the tumor and the natural selection that occurs when using drugs like temozolomide that don't target specific tumoral cell populations.[72,73] GBM derived from different cells of origin have shown corresponding differences in drug/treatment sensitivities. Targeting specific cells of origin offers a possible therapy route, such as Dasatinib (an ERBB2 inhibitor), which effectively targets OPC-derived tumors.[58] Furthermore, NSC-originated tumors are less sensitive to temozolomide therapy than OPC-derived tumors.[74] Stratifying glioblastoma in patients may be an important way to determine the most specific treatment possible to improve patient outcomes and prevent recurrence and drug resistance in the progression of the disease (Figure 2).

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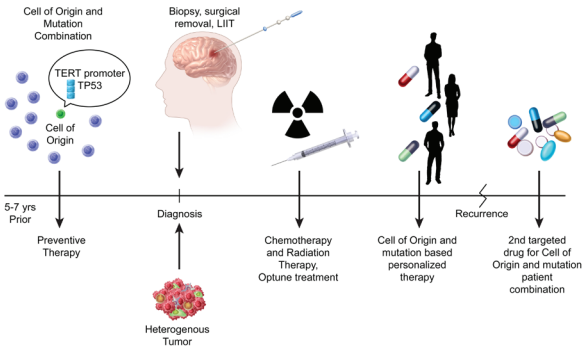

Figure 2: Timeline of standard treatment for GBM and how cell-of-origin-based treatments may be integrated into routine treatment. With a combination of early preventive therapy, personalized therapy, and drugs, GBM treatment may improve patient outcomes and survival.

- Personalized Therapy:

In the future, GBM therapy may target heterogeneous populations with various treatments for each population, including astrocytes, oligodendrocyte progenitors, neural stem cells, glial progenitors, and cancer stem cells. This therapy could be personalized per patient based on the various combinations of cell populations defined by the cell of origin and mutation combinations unique to each patient. Research has shown from patient-derived samples that NSC clustered primary GBM cells were highly malignant and more sensitive to drugs.[75] For example, to target NSC-derived tumors, radiation therapy has been targeted to the SVZ region as a blockade against further gliomagenesis, with significant increases in patient progression-free and total survival in Glioblastoma patients post-total resection.[22] Since GBM tumors tend to be radiation resistant, the use of the chemokine CXCL12, which has been shown to increase the radiosensitivity of the SVZ, offers a possible combination therapy.[76] There have also been suggestions on using DNA technology like CRISPR/Cas9 to correct driver GBM mutations, such as tumor suppressor mutations in mutated NSCs within the SVZ.[18] PTEN deficiency, a common mutation in GBM, has also been shown to allow NSCs to reprogram to a more stem-cell-like state through the indirect effects of increasing PAX7.[24] Thus, upregulating PTEN in the SVZ region offers another possible therapy. Immunotherapy and small-molecule inhibitors can inhibit TERT mutations in the SVZ, which usually produce pro-tumor effects by creating telomerase.[77]

Current clinical trials harness the immune system's ability to specifically target GBM mutations like EGFR. A phase II trial using rindopepimut (a vaccine targeting EGFRvIII) showed potential for immunotherapeutic targeting of specific tumor mutations. 80% of patients had prolonged survival.[78] However, a follow-up phase III trial of rindopepimut showed no significant survival benefit.[79] Current early-stage studies targeting EGFR or its mutations are also underway, including a phase I study targeting EGFR-amplified GBM, which intends to treat patients with CART-EGFR cell therapy, and a phase I study employing combinatorial T-cell and standard-of-care therapy for EGFR-mutant GBM patients (Clinical Trial ID NCT05168423, NCT03344250). Additionally, a study of three participants treated with CARv3-TEAM-E T-cells (engineered CAR-T cells targeting EGFRvIII as well as a T-cell-engaging antibody targeting wild-type EGFR) showed rapid (but transient) tumor regression in two of three patients.[80]

There may be a biological basis for why EGFR mutation targeting may not be effective. Firstly, EGFR mutations are heterogeneous and subclonal.[81] Additionally, experiments suggest that EGFR overexpression causes preferential proliferation of mouse astrocytes vs. NSCs, suggesting that EGFR primarily impacts astrocyte-like tumor cell states rather than NSC-like states.[66] This evidence motivates research on mutational targets that also affect other cell states and potentially, other cells of origin.

These therapies may all be targeted to the SVZ region, where NSCs can be identified as the cell of origin. They offer insight into how identifying the cell of origin and mutation combination may play an important role in the future of GBM personalized therapy.

- Preventive Therapy:

Research has shown that glial progenitors (NSCs, astrocytes, OPCs) migrate from the SVZ in a predictable path that follows the developmental pathway.[34] Additionally, Korber *et al*. predicted a distant origin of GBM - up to 7 years before diagnosis – with an accumulation of specific milestone mutations, such as chromosome 7 gain, 9p loss, or 10 loss, and eventual TERT promoter mutation.[82] Targeting GBM based on precancerous indicative mutations offers a preventive treatment method for further brain infiltration and tumorigenesis. GBM's plasticity could be prevented with early detection by tracing mutations specific to the possible cells of origin.[83] Developing novel technology and methods to access precancerous mutational data through non-invasive procedures are important frontiers in research. There have already been investigations on epigenetic MRIs, which would allow imaging of the brain and its epigenetic landscape without *in vivo* sampling.[84] Identifying the patient-specific cell of origin and mutation combination may also aid in the early detection of developing tumors and allow for preventative therapies for patients at high risk of developing GBM, such as neurofibromatosis type 1 (NF1) patients.[85]

- The Tumor Microenvironment:

Mutations in growth factor genes have also been shown to be significant predictors of tumor aggressiveness, especially in the case of OPCs as the cell of origin.[86] While research has shown that a monoclonal antibody that inhibits Vascular Endothelial Growth Factor (VEGF) decreases angiogenesis, these improvements came with significant side effects.[87] Additional research and therapy must be developed to target growth factors that promote OPC self-renewal, such as PDGF inhibitors. Furthermore, depending on the cell of origin and mutation combination, the cell may interact with specific cells in the TME. For example, the PTEN mutation in GBM has been shown to recruit more macrophages.[88] Understanding these interactions could have implications for personalized therapies as well.

Conclusion

In summary, multiple lines of evidence debate the cell of origin for Glioblastoma, an aggressive brain cancer. While astrocytes have been especially controversial due to their complete differentiation and common cell markers, Neural Stem Cells and Oligodendrocyte Progenitor Cells have various convincing lines of reasoning. Liu *et al*. suggest that the cell of origin is related to the mutations accumulated and show that initial p53/NF1 mutations may occur in NSCs. Still, tumors contain OPC markers and transcriptome, thus separating the cell of mutation (the NSC in this case) from the cell of origin (OPC). Additional intricacies occur when following the mutation-dependent theory, as the cell of origin may differ by mutation, as shown by Kim *et al*., showing that GBM arises from the accumulation of driver mutations in cells of origin (NSCs and progenitor cells) and IDH mut might arise from different cells of origin that may or may not have been discovered. We conclude that the cell of mutation may differ from the cell of origin. Furthermore, we must consider differences in mutations and tumor microenvironment (growth factor, anatomical location, proximity to blood vessels).

In the future, we must determine non-invasive methods to determine each patient's cell of origin and molecular profiles to offer personalized treatment for this highly heterogeneous tumor. Additionally, logistical issues arise when catching GBM in its early stage, as it is incredibly difficult to find the singular mutated cell of origin in a sea of millions of other cells. Furthermore, it's important to consider what is realistically possible in terms of delivering treatment due to the restrictive blood-brain barrier. There are also potential limitations of targeting cells of origin; namely the possibility of off-target effects, unintended consequences of modifying progenitor cells, and the impact of GBM heterogeneity on cell-of-origin-targeted therapeutic strategies. In the future, cells of origin could be studied by directly comparing OPCs and NSCs, differentiating human induced pluripotent stem cells (hiPSCs) into OPCs and NSCs, allowing us to control the mutations introduced in an experimental setting.

Determining the cell(s) of origin of GBM is important in its potential applications to GBM treatment to improve the length and quality of life for GBM patients. In addition, identifying the cell of origin may also lead to intriguing novel strategies in the future, such as, potentially, preventing tumor cell pathogenesis, for instance, if pre-malignant cells with only a few early mutations could be targeted or prevented from acquiring further mutations

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Authors

Grace Qian is a current Stanford undergraduate student interested in neuroscience, immunology, and oncology. She is studying human biology on the pre-med track.