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REVIEW

A reignited debate over the cell(s) of origin for glioblastoma and its clinical implications

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Abstract **Glioblastoma (GBM) is the most common and lethal primary neoplasm in the central nervous system. Despite intensive treatment, the prognosis for patients with GBM remains poor, with a median survival of 14–16 months. 90% of GBMs are primary GBMs that are full-blown at diagnosis without evidences of a pre-existing less-malignant precursor lesion. Therefore, identification of the cell(s) of origin for GBM—the normal cell or cell type that acquires the initial GBM-promoting genetic hit(s)—is the key to the understanding of the disease etiology and the development of novel therapies. Neural stem cells and oligodendrocyte precursor cells are the two major candidates for the cell(s) of origin for GBM. Latest data from human samples have reignited the longstanding debate over which cells are the clinically more relevant origin for GBMs. By critically analyzing evidences for or against the candidacy of each cell type, we highlight the most recent progress and debate in the field, explore the clinical implications, and propose future directions toward early diagnosis and preventive treatment of GBMs.**

Keywords glioblastoma; cell(s) of origin; neural stem cells; oligodendrocyte precursor cells; subventricular zone; early diagnosis

Introduction

Glioblastoma (also termed glioblastoma multiforme, GBM) is the most common and lethal primary neoplasm in the central nervous system [1]. According to World Health Organization standards, GBM is the highest grade (Grade IV) astrocytoma, characterized by extreme morphological, genomic and transcriptional heterogeneity [2,3]. Highly infiltrative to surrounding normal brain tissues, GBM cannot be completely resected by surgery, and is resistant to conventional radiation and chemotherapy. Consequently, the prognosis for patients with GBM remains poor, with a median survival of 14–16 months even after intensive treatment [1–3].

Targeting pre-neoplastic cells to prevent their transformation toward the malignant state is a promising therapeutic strategy to circumvent the great challenges of treating end-stage cancers. However, the vast majority of GBMs (90%) are primary GBMs that are fully manifested at diagnosis without radiological or histological evidences of a pre-existing less-malignant precursor lesion, while only 10% are secondary GBMs that develop from lower grade astrocytomas (Grade II or III) [3]. Thus, identification of the cell(s) of origin for GBM (the normal cell or cell type that acquires the initial GBM-promoting genetic hits) is the key to the understanding of the disease etiology [4,5]. More importantly, detailed molecular profiling of these cell(s) of origin could potentially lead to the development of early prognostic markers and preventive therapies for this deadly brain cancer.

In this review, we focus on the two major candidates for the cell(s) of origin for GBM: neural stem cells (NSCs) and oligodendrocyte precursor cells (OPCs). Latest data from human samples have reignited the longstanding scientific debate over which cells are the clinically more relevant origin for GBM [6–8]. By critically analyzing evidences for or against the candidacy of each cell type (Table 1), we highlight the most recent progress and debate in the field, explore the clinical implications, and propose future directions toward early diagnosis and preventive treatment of GBM.

Two major candidates

NSCs are self-renewing, multipotent cells that during

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embryonic development generate all the major cell types in the central nervous system: neurons, astrocytes, and oligodendrocyte [9,10]. The latter two are collectively called glial cells. A subset of NSCs persist into the adulthood and primarily reside in restricted regions of the brain: the subventricular zone (SVZ) of the lateral ventricle and the subgranular zone (SGZ) of the hippocampal dentate gyrus [9]. These adult NSCs retain remarkable self-renewal potential and differentiation plasticity, which continue to produce restricted types of interneurons and/or glial cells in the adult brain [9]. In contrast, OPCs are generally considered as lineage-restricted precursor cells that specifically give rise to oligodendrocytes in both the developing and adult brain [11,12]. In the adulthood, OPCs are a slowly proliferating cell population widely distributed throughout the brain, accounting for approximately 3%–4% of cells in the gray matter and 7%–8% in the white matter [11]. While it is still debatable whether terminally differentiated, post-mitotic astrocytes and neurons can be transformed into GBM [5,13,14], the replicative capacity of both NSCs and OPCs single them out as the more likely candidates for the cell of origin for GBM.

Evidences for or against NSCs as the cell of origin

Aside from correlative evidences such as similarities in marker expression between NSCs and GBM cells (e.g., Nestin, GFAP, and Sox2) [1,15], the strongest evidences to support NSCs as the cell of origin come from xenograft and genetically engineered mouse (GEM) models for GBM. In these models, the general approach to induce GBM is to knockdown/knockout driver tumor suppressor genes (e.g., *p53*, *Nf1*, *Cdkn2a*, *Pten*, and *Rb1*) and/or overexpress driver oncogenes (e.g., *Ras, EGFR, PDGF* receptors and ligands) [2,16]. By targeting these driver genes, cultured mouse NSCs and immortalized human NSC lines can be directly transformed to form GBMs after intracranial transplantation into host mice [17–19]. Human embryonic stem cell (hESC)-derived, *PTEN*-deficient NSCs also exhibit glioma-like neoplastic features after transplantation [20]. These studies on xenograft models have established the competency of NSCs to be transformed *in vitro*.

In vivo, GEM models provide direct evidences that mouse NSCs in the SVZ stem cell niche can give rise to GBM in a *de novo* setting. Zhu *et al*. reported the first true genetic mouse model for GBM with high penetrance [21]. Using Cre/loxP system, they achieved targeted deletion of a floxed *Nf1* allele in hGFAPCre-expressing embryonic NSCs and their progeny in *p53* null mice. They made an important observation that the majority of tumors arise within the SVZ-associated areas, consistent with the clinical findings that human GBMs are frequently diagnosed in proximity to the SVZ [22]. The susceptibility of neural progenitors to malignant transformation is further confirmed by multiple murine models targeting different combinations of driver genes [17,23–26]. However, there are several common caveats for these studies: (1) Mutations are introduced in embryonic or postnatal NSCs, which is not ideal since GBM is an adult-onset disease mostly diagnosed between the ages of 45 and 70 [27]; (2) Mutations are initially targeted to NSCs, yet as NSCs differentiate, all of their descendants including transit amplifying cells, neuroblasts and OPCs carry the same mutations, making it difficult to pinpoint exactly which progenitors directly give rise to GBM [15]. Alcantara *et al*. addressed the first issue by specifically targeting *p53/Pten/Nf1* mutations into adult NSCs [28]. They used a transgenic mouse strain carrying tamoxifen inducible Cre (*Nestin-CreER^{T2}*) and induced the Cre activity in postnatal/adult SVZ, or directly injected Cre-expressing adenovirus into the adult SVZ. In both scenarios mice develop GBM. In contrast, when Cre-expressing adenovirus were injected into non-neurogenic regions, no GBM formed. Their findings were further confirmed by Jacques *et al*. [29]. For the second issue, Wang *et al*. partially addressed the problem using a mutant p53-driven GBM model [30]. In this model, they used the accumulation of mutant p53 protein, a common feature of human tumors, to specifically label malignant cells at all stages of GBM development. While all the neural progenitor cells carry the same p53 mutation, the earliest cells that accumulate high level of mutant p53 are restricted in the SVZ and express stem cell markers, suggesting NSCs are the earliest cells undergoing malignant transformation. Together, data from GEM models support that NSCs, or more precisely SVZ NSCs, are the cell of origin for GBM.

A recent study from human patients with GBM provides direct genetic evidence that human GBM originates from the SVZ [6]. In this study, Lee *et al*. collected triple-matched samples of normal cortex, tumor and tumor-free SVZ within the same brain from 55 patients. Through deep sequencing and single-cell sequencing, they found that in majority of patients with IDH-wildtype GBM, tumor-free SVZ tissue contained low level GBM driver mutations that are present at high levels in their matching tumors. The shared mutations prove the lineage relationship between SVZ cells and tumors, and the fact that SVZ cells lack tumor-private mutations can be explained by two scenarios: (1) SVZ cells clonally evolve into GBM in the brain parenchyma; or (2) GBM originate in a location distant from the SVZ and an early GBM clone migrates into the SVZ. To test these possibilities, they used a gene-edited, lineage tracing mouse model to show that cells with NSC features carrying driver mutations *p53*, *Pten*, and *EGFR* could migrate out of the tumor-free SVZ and develop into high-grade gliomas in distant brain regions, while cells in

Table 1 Evidences for and aganist NSCs and OPCs as the cells of origin for GBM

Cell of origin	NSC	References (NSC)	OPC	References (OPC)
Makers expression	Nestin, GFAP, Sox2	Stiles and Rowitch, 2008 [15]	Olig2, NG2, PDGFR	Bergles and Richardson, 2015 [11]
		Sturm *et al.*, 2014 [1]		Sturm *et al.*, 2014 [1]
Xenograft models	Driver mutations			
	Olig2	Ligon *et al.*, 2007 [19]		
	Pten	Duan *et al.*, 2015 [20]		
	p53, Akt	Hu *et al.*, 2016 [18]		
	p53, Olig2, EGFRIII	Griveau *et al.*, 2018 [17]		
GEM models	Driver mutations		Driver mutations	
	p53, Nf1	Zhu *et al.*, 2005 [21]	PDGF	Assanah *et al.*, 2006 [47]
	p53, Pten	Zheng *et al.*, 2008 [23]	p53, Nf1	Liu *et al.*, 2011 [41]
	p53, Pten	Jacques *et al.*, 2010 [29]	p53, Nf1	Galvao *et al.*, 2014 [45]
	p53	Wang *et al.*, 2009 [30]	p53, Pten	Lei *et al.*, 2011 [46]
	p53, Pten, Nf1	Alcantara Llaguno *et al.*, 2009 [28]	Nf1, p53, Pten	Alcantara Llaguno *et al.*, 2015 [44]
	p53, Pten, Nf1	Chen *et al.*, 2012 [24]		
	p53, Pten, Rictor	Akgul *et al.*, 2018 [25]		
	PDGFA, p53, Nf1	Ozawa *et al.*, 2014 [26]		
	p53, Olig2	Griveau *et al.*, 2018 [17]		
Self-renewal and proliferation in adult human brains	Hippocampal neurogensis drops to undetectable level during childhood	Sorrells *et al.*, 2018 [7]	White matter OPCs approach stable numbers at around 5 years	Yeung *et al.*, 2014 [48]
	SVZ neurogensis fully disappears at around 18 months	Sanai *et al.*, 2011 [31]	The number of gray matter oligodendrocytes does not plateau until 40 years of age	Yeung *et al.*, 2014 [48]
	Neurogenesis persists throughout life	Boldrini *et al.*, 2018 [8]		
Clinical evidence	GBM frequently diagnosed in SVZ-associated areas	Barami *et al.*, 2009 [22]	GBM frequently diagnosed in the subcortical white matter	Louis *et al.*, 2016 [3]
	Low level GBM driver mutations in tumor-free SVZ tissue and high level driver mutations in their matching tumors	Lee *et al.*, 2018 [6]		

the cortex targeted by the same mutations could not develop tumors nor invade the SVZ. While the mouse data may not be definitive since these phenotypes might be mutation-specific, it strongly favors the first scenario. Together, their findings provide a clinically relevant example showing that NSCs in human SVZ tissue are the cells of origin for GBM harboring initial driver mutations.

Despite all these evidences to support SVZ NSCs as the cell of origin for GBM, there is one unsettled question: do NSCs and neurogenesis actually exist in adult human brains? Through histological analysis of markers for precursor cells and their proliferative status on human brain samples at different ages, Alvarez-buylla and colleagues concluded that hippocampal neurogenesis drops to undetectable level during childhood, and SVZ neurogenesis fully disappears at around 18 months, long before high-grade gliomas are diagnosed [7,31]. These findings challenge the human relevance of mouse NSC-derived GBM models. In contrast to these human data, mouse NSCs undergo proliferation/neurogenesis even in aged animals, so driver mutations in this cell population may easily give rise to tumors. But if this population of cells is proven not undergoing such levels of proliferation in older adult humans, it may be a less relevant model. However, it is at least premature at this stage to conclude whether NSCs and neurogenesis are persistent in adult human brains [32]. Using similar approaches, Boldrini *et al*. reached an opposite conclusion that neurogenesis persists throughout life in humans [8]. Furthermore, adult neurogenesis in human brains has been confirmed by multiple birth dating studies [33–35]. Unlike in rodents where adult neurogenesis from SVZ mainly gives rise to neurons in the olfactory bulb, it is proposed that in humans SVZ-derived neuroblasts could migrate into the striatum, evidenced by continuous striatal neurogenesis [33]. In addition, in light of the findings by Lee *et al*., one could argue that even if wildtype NSCs could not persist into adulthood, NSCs with low-level GBM driver mutations could remain in the SVZ long after the disappearance of wildtype NSCs and neurogenesis, and serve as the cell(s) of origin to initiate GBM [6].

Finally, other than direct gliomagenesis from NSCs, several studies have proposed that dedifferentiation of astrocytes and neurons into a NSC-like state could lead to GBM development [13,14,36]. However, these studies either used cultured early postnatal "astrocytes"which may not represent mature adults astrocytes *in vivo*, or broadly introduced driver mutations/knockdown into many cell types, confounding their conclusions. Indeed, a very recent paper which rigorously tested the susceptibility of restricted neural progenitor cells and neurons using lineage-specific Cre strains found that a combination of *Nf1*, *Trp53*, and *Pten* deletion in these cell populations is insufficient to induce glioma formation [37].

Evidences for or against OPCs as the cell of origin

While adult OPCs are generally considered a unipotent, slowly proliferating population, their sheer number makes them the largest proliferating cell pool in the brain and a natural candidate for the cell of origin of GBM [12]. Like NSCs, OPCs also share marker expressing profile with GBM cells, e.g., Olig2, NG2, and O4 [1,11]. TCGA analysis revealed OPC-signatures in proneural GBMs, in particular the frequent amplification and mutations of PDGFRA, an OPC-specific marker in the normal brain critical for the regulation of OPC proliferation and migration [38]. However, early GEM models targeting OPCs mostly generated oligodendrogliomas [39,40]. Liu *et al*. provided the first convincing evidence to demonstrate OPCs can function as the cell of origin for GBM [41]. In this study, they used a novel lineage tracing model termed mosaic analysis with double markers (MADM) [42], which can simultaneously label wildtype and mutant (*p53* and *Nf1* double-deficient) cells generated from the same NSCs within the same mouse brain with red and green fluorescent reporters, respectively. By comparing the red versus green ratio in different cell type, they were able to show that only in the OPCs, but not in the NSCs, green mutant cells dramatically outnumber red wildtype cells, suggesting that while mutations were introduced in NSCs, their descendant OPCs are the initial transformed cells undergoing clonal expansion. However, one could argue that cells undergoing initial expansion are not equivalent to the cells of origin. Indeed, similar OPC and oligodendrocyte expansion is observed in *Nf1*-single mutant mouse models, yet no GBM develops, suggesting that the expansion could be attributable to non-tumorigenic developmental defects caused by *Nf1* mutation [43]. More direct evidences to support the OPC-origin come from OPC-specific conditional knockout mouse models using NG2-Cre, inducible NG2-CreERT or retrovirus [41,44–47]. After targeted inactivation of *p53*, *Nf1*, and/or *Pten* in OPCs during development or in adulthood, mutant OPCs could readily generate GBM in these models.

While these studies provide compelling evidences to support that OPCs can be transformed *in vivo*, there are issues against the hypothesis of OPC-origin. First, despite *in vitro* evidences showing the plasticity of OPCs *in vitro*, adult OPCs are generally considered unipotent and lack the ability to self-renew *in vivo* [12]. Thus, it is difficult for an individual adult OPC to accumulate multiple driver mutations *de novo* and initiate GBM. Second, while OPCs are distributed throughout brain regions including both gray and white matter, GBMs are found most commonly in the subcortical white matter of the frontal lobes [3]. Such location preference cannot be explained by the possibility that white matter OPCs proliferate more than gray matter OPCs. In fact, birth dating experiments on

human brain samples found the opposite [48]. While the number of gray matter oligodendrocytes does not plateau until 40 years of age, the number of white matte oligodendrocytes reaches its peak at 9 years of age and stays remarkably stable throughout the rest of the human lifespan. A counter-argument is that GBM may initiate from gray matter OPCs and expand or migrate into the white matter/SVZ to form radiologically evident tumors, as suggested by the MADM study [41]. To rigorously test this hypothesis, it requires specific targeting and lineage tracing of mutant OPCs in the gray matter, more thorough histopathological analysis, along with deep genomic sequencing to establish the lineage relationship.

A possible unifying model

Is there a way to reconcile all the existing data on the cell of origin for GBM? Alcantara Llaguno *et al*. present an interesting case that Ascl1^{+} neural progenitors with targeted mutations can generate two histologically and molecularly distinct GBM subtypes [44]. Gene expression analysis reveals that one subtype resembles NSC-derived GBMs and the other resembles OPC-derived GBMs. Similarly, they reported that hGFAP-Cre-driven NSC glioma models also develop both types of gliomas, while NG2-Cre-driven OPC glioma models could only generate one subtype. This study, along with others summarized in this review, point to a possible unifying model for the cell(s) of origin for GBM (Fig. 1): (1) Given the right combination of driver mutations, NSCs, NPCs, and OPCs all can be transformed; (2) NSCs in the SVZ are more susceptible to transformation and are more likely the cells that accumulate multiple oncogenic mutations during their self-renewal; (3) Mutant NSCs could directly give rise to GBMs in SVZ-associated areas, or indirectly through their descendant progenitors including OPCs in distant brain parenchyma, while the initial NSCs carrying low-level driver mutations remain in the histopathologically non-cancerous SVZ. If this model holds true, we face a complicated scenario where there is a fine distinction between "the cell of origin" (cells susceptible to mutations) and "the tumor-propagating cell population" (the cells actually giving rise to the tumor mass). The latter is potentially more clinically relevant and could be a better target for early tumor diagnosis and intervention.

Alternatively, as Alcantara Llaguno *et al*. have suggested in their paper, it is possible that both NSCs and OPCs are the cells of origin for GBMs, and different cells of origin give rise to distinct GBM subtypes, contributing to the intertumor heterogeneity of GBMs [44]. Nevertheless, it has not been experimentally defined which cell type(s) give rise to each of the TCGA-defined human GBM subtypes (proneural, classical and mesenchymal), while there are increasing evidences showing that genetic mutations, epigenetic changes, and the microenvironment are the major determinants of the intertumor diversity [49,50].

Clinical implications and future directions

We have witnessed tremendous progress on our understanding of the GBM cell of origin in the past decade. With the advent of sophisticated technologies such as high-throughput drug screening, deep sequencing, single-cell analysis, induced pluripotent stem cells (iPSCs), organoids and CRISPR-Cas9-based gene editing, it is time for the field to move forward, address key clinical questions regarding gliomagenesis, and provide novel therapeutic strategies.

First of all, the NSC-origin model for gliomagenesis would have distinct clinical implications compared to the OPC-origin model. Adult NSCs, if they are further proven to exist in the older human brain, are anatomically restricted in the SVZ NSCs and in direct contact with the cerebral spinal fluid. For early diagnosis and intervention, the NSC-origin model would mean that with the development of more specific imaging probes, we could in theory perform high-resolution MRI scanning in the SVZ and associated regions for a preventative GBM screening, or test the tumor specific antigen/microRNA/ctDNA in the cerebral spinal fluid as an early diagnostic method. For the treatment of endstage cancer, the NSC-origin model suggests that targeting the SVZ along with the tumor foci could improve the overall survival, as several clinical studies have already shown [51,52]. On the other hand, given the widespread distribution of OPCs in the brain, the OPC-origin model would mean that it will be very difficult to define a specific region of interest and distinguish abnormal OPCs from normal ones, therefore it is necessary to perform scanning and treatment throughout the brain with more sensitive and specific probes or drugs. Taken together, settling the cell-of-origin debate would have significant clinical implications on the diagnosis and therapy.

Secondly, mouse models for the study of GBM cell of origin are genetically and molecularly defined, subtype-specific, and can be provided at large cohorts. These mouse models can be particularly useful for the screening of novel drugs targeting specific mutations or signaling pathways, in conjunction with widely-used patient-derived xenografts models. These mouse models allow us the opportunity to investigate the earliest stages of GBM development. While human tumors are generally considered monoclonal, all of the current models target a large population of tumor initiating cells. Apparently, not all of these cells with driver mutations can give rise to GBMs. Whether, when and how they accumulate additional mutations, undergo clonal selections and exhibit neoplastic

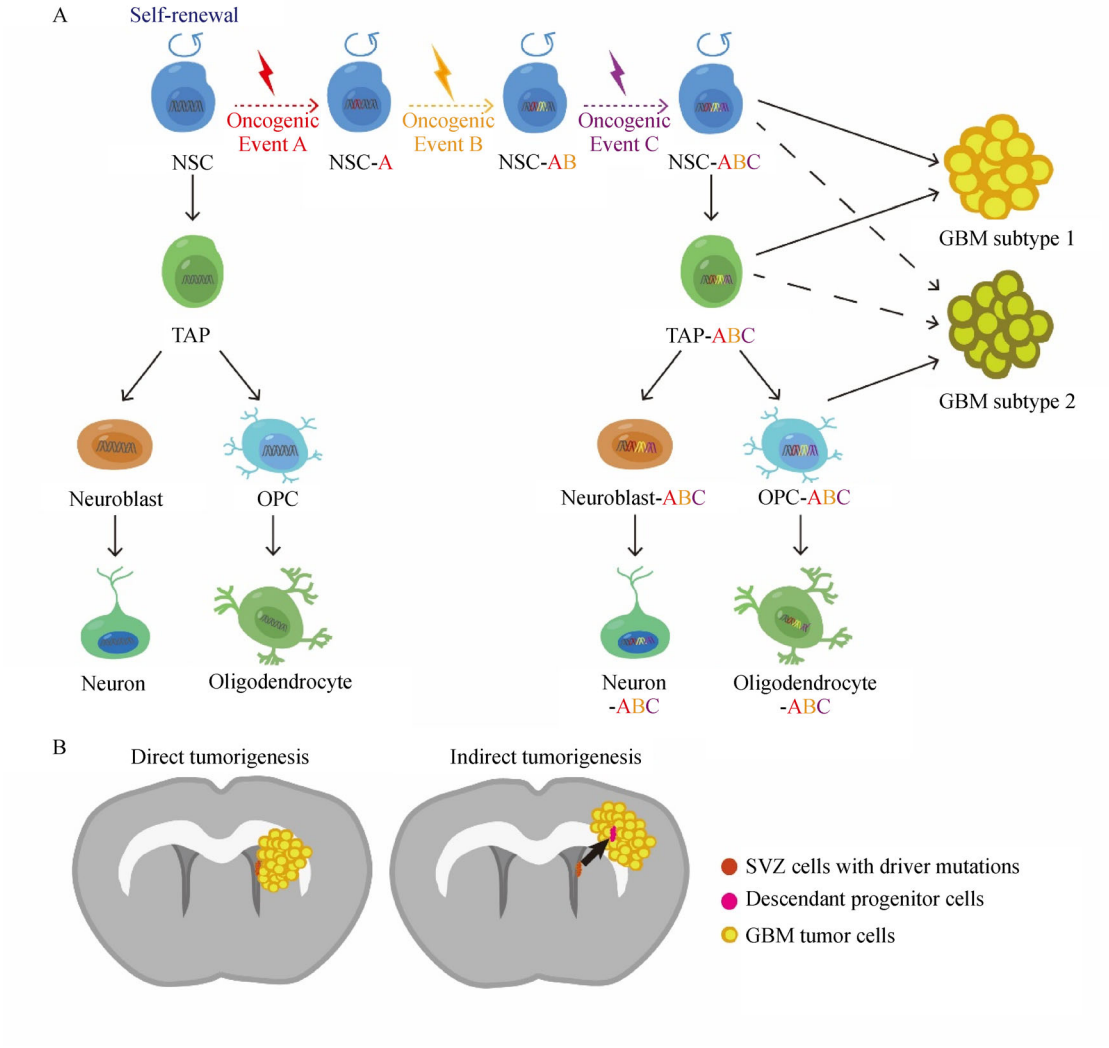

Fig. 1 A possible unifying model for the cell(s) of origin for GBM. (A) NSCs are the cells that accumulate multiple oncogenic mutations to give rise to two subtypes of glioblastoma. Left: The normal adult neurogenesis process from NSCs in the brain. Middle: Normal NSCs sequentially accumulate oncogenic events A (red), B (yellow), and C (purple) and transform into mutant NSCs (NSC-ABC). Right: Mutant NSCs with multiple oncogenic mutations directly generate one subtype of glioblastoma (GBM subtype 1) or pass down the mutations to descendant transit amplifying cells (TAP) and OPCs to generate another subtype of GBM (GBM subtype 2). OPCs can only generate GBM subtype 2. (B) Mutant NSCs could directly give rise to GBMs in SVZ-associated areas (left panel), or indirectly through their descendant progenitors in distant brain regions (right panel), while NSCs carrying low-level driver mutations remain in the non-cancerous SVZ.

features at initial stages of malignant transformation is largely unknown. Simultaneous single-cell RNA and DNA sequencing, deep whole genome/exome sequencing on bulk tissue from different brain regions, along with novel lineage tracing techniques and reporter mouse strains, could potentially address these questions. High-resolution genetic and molecular characterization of incipient cells at different stages of GBM development could make early detection and preventative therapy possible for patients at high risk of GBM development.

Finally, while mouse models are invaluable tools for GBM research and drug testing, scientists need to bear in mind some of the key limitations of these models. Most GEM cancer models typically lack selection for genetic instability or enough time to accumulate enough mutational events mimicking the genetic heterogeneity commonly observed in human cancers [53]. Consequently, tumors arising in GEM models are relatively homogeneous and often polyclonal, in contrast to the monoclonal nature of the majority of human cancers. This could particularly be an issue for modeling complicated cancers like GBM, whose extreme intra- and inter-tumor heterogeneity have

been well documented and further elucidated by bulk and single-cell sequencing analyses [2,16,49,54,55]. It is also worth noting that forced introduction of multiple driver mutations into specific cell populations itself is an artificial setting, which does not mimic the stepwise accumulation of mutations in cancer initiating cells or the clonal selection process. One could argue that while these models can generate tumors, they do not definitively demonstrate a cell of origin, instead it reveals a cell's capacity/vulnerability to give rise to a tumor. As for patient-derived-xenograft models, while they retain many features of human cancers and are widely used for drug testing, they cannot recapitulate the tumorigenesis process, and may undergo mouse-specific evolutions during passaging [56]. Limitations of these existing mouse models call for the development of novel GBM cell-of-origin models using human-derived cells. IPSC and reprogramming technology allows for mass production of patient-derived NSCs, OPCs, and cerebral organoids. Through CRISPR-Cas9 based gene editing, scientists could establish a series of engineered-human-cell-derived xenograft models, directly interrogate the tumorigenic capacity of these human cells/organoids with distinct driver mutations, investigate the stepwise malignant transformation process of targeted human cells, and test novel diagnostic markers for early stage tumor.

In summary, with future advances in basic science, translational and clinical research, it is hopeful that the cell of origin for GBM could be better defined, and this deadly cancer would one day become an early-detectable, preventable, treatable and/or manageable disease.

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Compliance with ethics guidelines

Xiaolin Fan, Yanzhen Xiong, and Yuan Wang declare there is no conflict of interest. This manuscript is a review article and does not involve a research protocol requiring approval by the relevant institutional review board or ethics committee.

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