

Perspective

The Glioma Stem Cell Model in the Era of Single-Cell Genomics

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Glioma stem cells (GSCs) are thought to underlie glioma initiation, evolution, and resistance to existing therapies. Although functional evidence for GSCs is abundant, tumor heterogeneity and intrinsic limitations in GSC assays have represented barriers for the field. In this perspective, we revisit the GSC model in light of recent single-cell expression profiling studies. We highlight how classes of glioma differ in their cellular architecture and relate the observed cellular states to established GSC markers. We additionally propose a set of single-cell informed definitions as a framework for our understanding of the cellular architecture of gliomas and a potential therapeutic outlook.

Introduction

Cellular hierarchies related to the developmental programs of tissue stem cell and their lineage differentiation play a central role in many cancers (Shibue and Weinberg, 2017). Subpopulations of cells endowed with stem cell properties, such as self-renewing capacity, tumor-propagating potential, and expression of embryonic or tissue stem cell genes, have been identified in a number of malignancies and termed cancer stem cells (CSCs). CSCs are thought to be preserved as a small population through self-renewal, and to generate more differentiated progenies that constitute the bulk of the tumor mass (Kreso and Dick, 2014). In addition to providing the driving force for tumor growth and maintenance, CSCs have been shown to be more resistant to existing anticancer therapies, consistent with their role in relapse after therapy (Bao et al., 2006; Chen et al., 2012). Accordingly, transcriptional signatures of CSCs are predictive of overall patient outcome, supporting their clinical relevance (Ben-Porath et al., 2008; Shibue and Weinberg, 2017). CSC programs are sustained by master transcription factors (TFs), chromatin regulators, and associated cellular networks, and it is generally believed that their defining properties are rooted in their epigenetic state (Kreso and Dick, 2014; Shibue and Weinberg, 2017). Accordingly, regulators of developmental decisions (e.g., TFs) often function as oncogenes by promoting the reacquisition of programs required for dedifferentiation (Flavahan et al., 2017; Suva et al., 2013).

In the field of diffuse gliomas, glioma stem cells (GSCs) have been functionally identified in isocitrate dehydrogenase (IDH) wild-type glioblastoma (GBM) using single-surface marker approaches, with markers, such as CD133, CD44, SSEA1, L1CAM, CD49f, A2B5, PDGFRA, and EGFR (Anido et al., 2010; Gimple et al., 2019; Lathia et al., 2010; Piccirillo et al., 2006; Singh et al., 2004; Son et al., 2009). GSCs have been functionally defined as cells possessing tumor-propagating potential *in vivo* and sustained self-renewal potential (Gimple et al., 2019; Lathia et al., 2015). In addition, common (but not required) features

of GSCs include the ability to differentiate into multiple cellular lineages, expression of defined markers, low frequency in a tumor sample, and drug resistance. Beyond the body of work prospectively identifying GSCs by their defining functional properties, the contribution of the stemness-to-differentiation axis to cellular properties in gliomas has additional experimental support: (1) triggering GSC differentiation reduces their tumor-propagating potential (Piccirillo et al., 2006); (2) reprogramming GSCs to induced pluripotent stem cells alters their capacity to propagate tumors (Stricker et al., 2013); and (3) tumorigenic GSCs can be generated from non-tumorigenic differentiated glioma cells by overexpression of defined neurodevelopmental TFs (Liu et al., 2015; Suva et al., 2014). Together these studies suggest that developmental programs super-imposed over genetic alterations determine key features of malignant cells in gliomas.

However, several aspects of GSC definitions have generated controversy. First, the core GSC functional properties—self-renewal and tumor propagation—are hallmarks shared by many cancer cells, which blurs the distinction between malignancy and GSC features. Second, these definitions are traditionally tested in animal models, and hence depend on xenogeneic environments that are very different from the native human tumor milieu. Third, functional approaches do not distinguish between the relative contribution of genetic alterations and epigenetic states to the observed phenotypes. Fourth, the plethora of surface markers used to isolate GSCs might identify subsets of cells in different cellular states (and with different underlying genotypes). Thus, different groups and studies may use the term GSC to refer to completely different populations of cells. Moreover, GSCs are often contrasted with all other “non-GSCs” with diminished tumor-propagating potential, although these likely contain a variety of distinct cellular states. Finally, GSC models vary with regard to the relationship between GSCs and cell cycle. Traditionally, GSCs have been associated with a slow rate of proliferation, or in other words with “quiescent stem cells,” giving rise to the notion that GSCs may be less



proliferative than other malignant cells. However, alternative models have suggested that GSCs may be more proliferative than the quiescent populations they are derived from (Gimple et al., 2019; Lathia et al., 2015). These caveats all highlight the limitations of the GSC model and call for a more granular definition that encompasses the continuum of states that can be found in tumors, their functional properties, their relation to developmental cell types, cell-cycle programs, and underlying genotypes. Notably, different forms of stem/progenitor cells might drive different classes of glioma and we might not expect that “one model fits all” but rather that for any cell, its functional properties result from the integrated output of its developmental state and its underlying genotype.

Here, we discuss the evolution of the GSC model in light of recent single-cell genomic studies. We review the recent literature and attempt to address four outstanding questions: (1) Can we leverage single-cell RNA sequencing (scRNA-seq) to refine the “generic” GSC terminology with genomically informed cellular states that provide enhanced granularity of the cellular programs that drive gliomas? (2) Do different glioma classes (IDH-mutant, histone mutant, and IDH-wild-type GBM) share similar putative hierarchies and GSCs? (3) What is the relationship between functionally characterized GSC markers and genomically informed cellular states? (4) What are the properties of inferred glioma cellular hierarchies compared with standard GSC models? In closing, we discuss some potential therapeutic outlooks.

Inferring Tumor Cellular Architecture from scRNA-Seq

scRNA-seq is facilitating a revolution in our understanding of complex biological systems, by providing a transcriptome-wide characterization for thousands of individual cells that are profiled from a single biological sample (Tanay and Regev, 2017). This approach is being applied to diverse healthy and diseased tissues, and gliomas have been among the tumor types at the forefront of the application to cancer samples (Tirosch and Suva, 2019). Analysis of scRNA-seq datasets from tumor samples should roughly follow three main steps that are further described elsewhere (Suva and Tirosch, 2019). First, after initial quality control and normalization, cells are classified into broadly distinct cell types (malignant, myeloid, glial, endothelial, etc.) based on their expression of sets of marker genes and the identification of genomic aberrations. Second, cellular heterogeneity is analyzed within each of those cell types to identify subpopulations with distinct cellular states as well as expression programs associated with continuous variability among cells. Third, cellular states and expression programs identified in the previous step are compared across patients to identify the common (i.e., recurrent) states and these are further analyzed and compared with external datasets to clarify their biological meaning.

The most common patterns of intra-tumoral heterogeneity that have been observed among malignant glioma cells are directly reflecting the cell-cycle program (Filbin et al., 2018; Neftel et al., 2019; Tirosch et al., 2016; Venteicher et al., 2017), as actively cycling cells upregulate a large number of associated genes. Importantly, other common patterns of variability are primarily correlated with neurodevelopmental cell types, such that each glioma harbors multiple subpopulations of cells that each resemble a distinct neural cell type. The neurodevelopmental

cell types of highest similarities include differentiated glial lineages (oligodendrocytes [OCs] and astrocytes [ACs]) as well as their progenitors (e.g., neural progenitor cells [NPCs]). Thus, malignant cells may be annotated as a certain neural cell type, e.g., NPC-like, and also independently classified as cycling or non-cycling. NPC-like refer to a variety of neural progenitor states that may be further refined in future work. However, we note that the similarities of malignant cells to normal cell types are always partial and thus advise to exert caution when assessing similarities to normal development based on narrow gene signatures. Moreover, since cellular states are continuous, one should avoid a strict classification of malignant cells and instead refer to “cell scores” reflecting the degree to which a cell expresses these cell-type programs (Figure 1). In addition to expression programs, genetic events (copy-number aberrations, mutations, rearrangements) may also be inferred from the scRNA-seq data. scRNA-seq analysis allows us to identify the spectrum of cellular states that are consistently found in a certain class of tumors, to relate them to developmental cell types, and to map cycling cells as well as tumor genotypes onto those cellular states. We refer to the resulting scRNA-seq-based models as “putative cellular hierarchies,” which we view as informing the next generation of GSC models. Below we discuss in more details the glioma scRNA-seq findings, their inferred properties, and attempt to relate this body of work to functionally defined GSC markers.

Cellular Hierarchies in IDH-Mutant Gliomas

The first study using scRNA-seq to define a putative cellular hierarchy in glioma examined oligodendroglioma, a class of IDH-mutant glioma characterized by chromosome 1p/19q co-deletion (Tirosch et al., 2016) (Figure 1A). Subsequently, analysis of the second class of IDH-mutant glioma (astrocytoma, *ATRX* and *TP53* mutant) identified a similar cellular architecture (Figure 1A) (Venteicher et al., 2017), suggesting that this hierarchy may be present across IDH-mutant glioma subsets, and demonstrating how single-cell genomics may help revise textbook views on the difference between classes of glioma (see Box 1). This “IDH-mutant hierarchy” comprised three main subpopulations: stem/progenitor-like cells, specifically resembling NPC-like cells, and two subpopulations of differentiated glial-like cells, specifically resembling oligodendrocytes (OC-like) and astrocytes (AC-like). scRNA-seq analysis additionally demonstrated that, in IDH-mutant gliomas, proliferation is largely restricted to the NPC-like cells, implying that GSCs are more proliferative than more differentiated malignant cells. This pattern, which we term “GSC-restricted proliferation,” may limit the rate of tumor growth and hence may primarily characterize slow-growing tumors, such as IDH-mutant gliomas.

Thus, both neurodevelopmental and proliferation signatures suggest a model that is largely consistent with a standard hierarchical model, with subsets of primitive cells fueling tumor growth. The scRNA-seq data also highlight the notion that cancer hierarchies may resemble differentiation processes in normal development (i.e., NPCs giving rise to both OCs and ACs), which we term “developmental coherence.” However, this hierarchy lacks a third path of expected NPC-related differentiation toward neurons. Thus, IDH-mutant gliomas appear to be driven by NPC-like cells that undergo differentiation into two lineages (OCs and ACs), while differentiation into the third lineage (neurons)

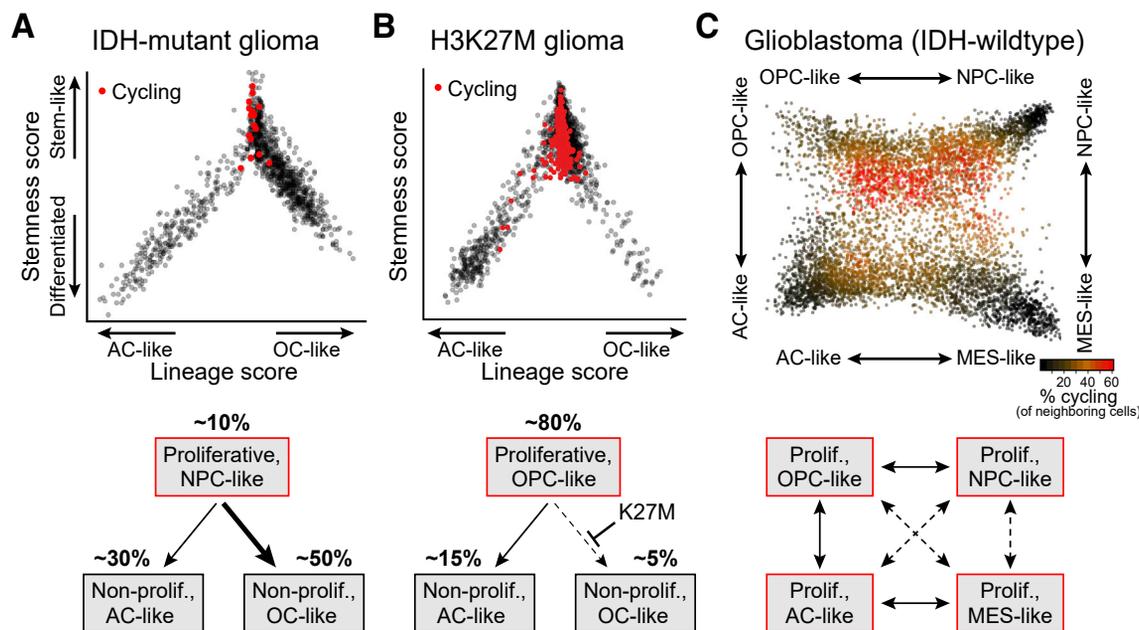


Figure 1. Putative Cellular Hierarchies of Three Classes of Glioma

Top panels show scRNA-seq-based cell scores for the differentiation/stemness programs identified in IDH-mutant glioma (oligodendroglioma and astrocytoma) (A; adapted from [Tirosh et al., 2016](#) and [Venteicher et al., 2017](#)), H3K27M-glioma (B; adapted from [Filbin et al., 2018](#)), and IDH-wild-type glioblastoma (C; adapted from [Nefitel et al., 2019](#)). Cycling cells are shown in red (A and B), or their frequency is shown by black-to-red color scale (C). Bottom panels show the inferred cellular hierarchies, with proliferating cell types highlighted in red, and approximate cellular fraction indicated next to each cell type. Arrows represent cellular plasticity, with full arrows reflecting cellular transitions supported by previous studies and dashed arrows reflecting cellular transitions that are less common (B) or are not supported by previous studies (C).

appears to be blocked by unknown mechanisms, possibly related to the *IDH1/2* mutations ([Lu et al., 2012](#); [Turcan et al., 2018](#)). Differentiation may also be blocked during tumor evolution; indeed, as IDH-mutant gliomas recur with increased grade and aggressiveness, their fraction of differentiated cells decreases, while their proliferation and pool of GSCs increase ([Bai et al., 2016](#); [Shirahata et al., 2018](#); [Venteicher et al., 2017](#)). Such trends are even more pronounced in other classes of gliomas, as described in the next section.

Blocked Hierarchies in H3K27M Gliomas

Following the studies of IDH-mutant glioma, putative cellular hierarchies were also described in histone H3 lysine27-to-methionine mutant (H3K27M) glioma, a class of highly aggressive, pediatric midline gliomas ([Filbin et al., 2018](#)). Although the “H3K27M hierarchy” ([Figure 1B](#)) was overall similar to the IDH-mutant hierarchy ([Figure 1A](#)), it differs in two important ways. First, the fraction of undifferentiated progenitors is dramatically increased (up to ~80%) in H3K27M glioma, suggesting a differentiation block, and mirroring the aggressive nature of this disease. Second, the putative GSCs in H3K27M glioma resemble oligodendrocyte-progenitor cells (OPC-like) rather than NPCs, suggesting that a distinct cell state is driving these tumors compared with IDH-mutant glioma, and possibly suggesting a different cell-of-origin. Interestingly, the OPC-like cells were further demonstrated to have tumor initiation capacity that more differentiated cells in H3K27M glioma lack ([Filbin et al., 2018](#)), providing further experimental support to the potential relevance of stemness and differentiation programs to functional

properties of glioma cells, even in tumor types in which differentiation is limited ([Anastas et al., 2019](#)).

Interestingly, while in normal development OPCs primarily differentiate toward OCs, the few differentiated cells in H3K27M glioma are in fact relatively depleted with OC-like cells and consist primarily of AC-like cells. This is possibly linked to the H3K27M mutation, as OPC to OC differentiation may require the activity of polycomb-repressive complex 2 (PRC2) ([Filbin et al., 2018](#)). Inhibition of PRC2 by H3K27M therefore provides a potential mechanistic model for both the overall differentiation block and the AC lineage skewing ([Lewis et al., 2013](#)). This would suggest that H3K27M results in an aberrant cellular state with enhanced self-renewal and decreased differentiation ([Nagaraja et al., 2017, 2019](#)).

Plasticity in IDH-Wild-Type GBM

As discussed above, the GSC model has extensive experimental support in GBM, but many questions remain unanswered by traditional functional approaches. Recent scRNA-seq efforts provided additional granularity into GBM cellular composition ([Bhaduri et al., 2020](#); [Lan et al., 2017](#); [Muller et al., 2016](#); [Nefitel et al., 2019](#); [Patel et al., 2014](#); [Wang et al., 2019](#); [Weng et al., 2019](#)). An emerging model suggests that GBM primarily consists of four malignant cellular states: three with similarities to those described above for other glioma classes and tightly linked to neurodevelopmental cell types (NPC-like, OPC-like, and AC-like), and a fourth mesenchymal state (MES-like) that is not anchored in neurodevelopment ([Figure 1C](#)) ([Nefitel et al., 2019](#)). Although similarities to programs driving other classes of

Box 1. Historical versus Emerging Views of Glioma Classification**TWO TYPES OF IDH-MUTANT GLIOMA**

Oligodendroglioma and astrocytoma are two types of IDH-mutant glioma that differ in their genetics (1p/19q co-deletion versus *TP53* and *ATRX* mutations, respectively) and their histology. As implied by their names, these glioma types have traditionally been thought to be derived from the two main lineages of glial cells, namely oligodendrocytes and astrocytes, respectively, primarily due to their morphologies and their differential staining by the AC marker GFAP. However, recent scRNA-seq studies concluded that oligodendroglioma and astrocytoma are associated with the same putative cellular hierarchy (Figure 1A), which includes cells that resemble both of the glial lineages, as well as cells that resemble NPCs and that are driving the growth of these gliomas. Thus, scRNA-seq analysis suggested a revised model: that all IDH-mutant gliomas may originate from NPCs that differentiate into both glial lineages, and that morphological differences between oligodendroglioma and astrocytoma primarily reflect the consequences of their distinct genetics. For example, both loss of chromosome 19q and point mutations reduce the activity of *CIC* in oligodendroglioma. *CIC* downregulation is associated with lower levels of GFAP (Tirosh et al., 2016; Venteicher et al., 2017) and thereby may contribute to the distinct morphology and marker staining of these glioma types.

SUBTYPES OF GBM

In 2010, an influential work by The Cancer Genome Atlas defined a classification of GBM tumors into four distinct subtypes: proneural, neural, classical, and mesenchymal (Verhaak et al., 2010). These subtypes became a key concept in GBM and were adopted by a large number of studies, which viewed each tumor as belonging to one specific subtype, and considered the possibility that each subtype might reflect a different biology and ultimately necessitate a different treatment. This view is consistent with multiple other cancer types in which subtypes are treated differently or are thought to have a different etiology or response to treatments. However, recent studies challenge these ideas, as they demonstrate that each individual GBM profiled by scRNA-seq contains multiple subpopulations of cells that map to distinct subtypes, with most tumors containing cells that are representative of at least three distinct subtypes (Nefitel et al., 2019; Patel et al., 2014). Importantly, these subpopulations differ considerably in their relative frequencies in each tumor, such that most GBMs have particularly high frequency of one of their represented subtypes, often related to the tumor's genetics (high-level *EGFR* amplification is associated with tumors that have high frequency of AC-like cells, high-level *PDGFRA* amplification is associated with tumors that have high frequency of OPC-like cells, and high-level *CDK4* amplification is associated with tumors that have high frequency of NPC-like cells). Such high-frequency cellular states are therefore observed as the strongest signal in a standard bulk RNA-seq profiling. Thus, GBM subtypes primarily reflect the abundance of distinct cellular states rather than their exclusive presence, while the four different cellular states are all common across the GBM subtypes.

gliomas are apparent, important differences exist. One of the key differences is that in GBM, unlike in other types of glioma, proliferation signatures were observed in all four malignant states (albeit in different proportions), suggesting that multiple compartments may fuel tumor growth in GBM. Moreover, at least two of those states (MES-like and NPC-like) were demonstrated to have capacity to propagate tumors in mice. Together with a third state (OPC-like) that was shown to propagate tumors in many glioma subsets (including GBM) (Filbin et al., 2018; Liu et al., 2011), these results suggest that at least three GBM states are capable of propagating tumors, while one state (AC-like) appears to have decreased tumor-initiating potential, which might be therapeutically important (see Concluding Remarks). We denote this property as “GSC-multiplicity.”

Notably, the experiments suggesting GSC-multiplicity also highlight another important feature of GBM cells—their “state plasticity.” When cells in a given state are isolated and implanted in mice, they do not propagate tumors of only that particular state, but rather re-establish the diversity of cellular states that was observed in the primary human tumor (Nefitel et al., 2019). Thus, GBM cells seem capable of switching between cellular states, and such transitions appeared to be common in lineage-tracing experiments (Nefitel et al., 2019). Despite these transitions, each GBM appears to have particularly high abundance of one or two of the four cellular states. The identity of such

“common” cellular states is at least partially dictated by tumor genetics, as specific genetic alterations favor the proliferation of particular cellular states while others are kept at lower abundance (Nefitel et al., 2019). Indeed, while most genetic events appear to have little consequence on the distribution of cellular states in GBM (Bhaduri et al., 2020; Nefitel et al., 2019), specific GBM drivers (see Box 1) are associated with enrichment of a particular cellular state. The common cellular states in a tumor are those that define the strongest signal in a bulk RNA-seq profile, which in turn accounts for the assignment of GBMs into three or four “subtypes,” as defined previously (see Box 1).

The combination of GSC-multiplicity with state plasticity and genetic-dependent proliferation rates, creates a conundrum: a number of different cellular states may be isolated and shown to have (1) increased capacity for tumor propagation (compared with other cells combined) and (2) capacity to generate other cellular states. Hence, each of those states could be interpreted as having both a unique self-renewal capacity and a differentiation capacity, and accordingly denoted as GSCs and envisioned as being at the apex of a unidirectional cellular hierarchy. This may lead to a situation in which different groups isolate distinct cellular states under the heading of GSCs, and obtain partially conflicting results. Such situations may be further confounded by the choice of model systems, which is likely to quantitatively influence the relative capacity

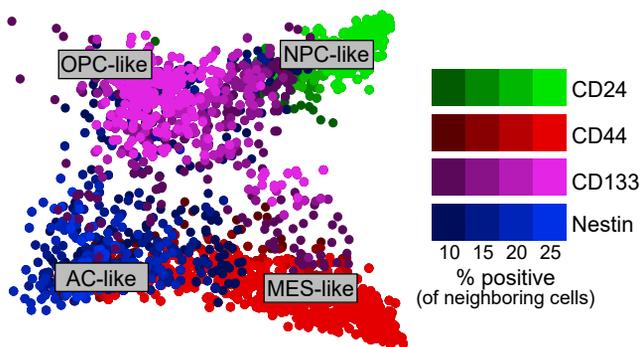


Figure 2. Established GSC Markers Are Associated with Distinct Glioblastoma Cellular States

A 2D representation of the diversity of glioblastoma malignant cells, including four main cellular states represented by the four quadrants (data and 2D presentation derived from Neftel et al., 2019, as shown in Figure 1C). Cells are colored by the density of positive cells for each of four established GSC markers: for each cell we defined the fraction of scRNA-seq-based positive cells (for each GSC marker) among the 100 closest cells by Euclidean distance; if the marker with highest positive fraction was positive in at least 10% of the neighboring cells then the cell was colored by the respective shade (see color map at the right); alternatively (if all markers were positive in less than 10% of the neighboring cells), the cell was not shown. The threshold for “positive” mRNA signal was manually set for each marker in order to define 5%–10% of cells as positive. See also Figure S1.

of distinct cellular states to propagate tumors, partially due to their genetics.

To investigate these hypotheses and relate the functional GSC literature to the scRNA-seq-informed cellular states, we examined the expression of previously proposed GSC markers in a recently published GBM scRNA-seq dataset (Neftel et al., 2019). We investigated both GSC surface markers (*CD133*, *CD24*, *CD44*, *L1CAM*, *EGFR*, and *PDGFRA*) as well as GSC TFs and lineage markers (*SOX2*, *OCT4*, *BRN2*, *OLIG2*, *ID1*, and *NES*) (Anido et al., 2010; Chen et al., 2012; Ikushima et al., 2009; Jin et al., 2016; Suva et al., 2014). Strikingly, most of these GSC markers showed a significant bias toward one of the four GBM cellular states, and each of the four states was enriched by some of the markers (Figures 2 and S1). Among the cell surface markers, *CD24* is the highest in NPC-like cells, *CD133* in OPC-like cells, *EGFR* in AC-like cells, and *CD44* in MES-like cells. Of the TFs and lineage markers, *NES* displays a significant bias toward AC-like cells. Other tested markers showed expression in two of the four states or no discernable pattern (Figure S1). Although this analysis relies on mRNA levels, it is consistent with experiments in which some of those markers were used for antibody-based isolation, demonstrating that sorting cells by anti-*CD24* enriched for NPC-like cells while sorting cells by anti-*CD44* enriched for MES-like cells (Neftel et al., 2019). Taken together, these results suggest that different GSC markers isolate distinct cellular states and call for caution in interpreting traditional GSC experiments. Similar conclusions were reached by a recent study with independent analysis and scRNA-seq datasets (Bhaduri et al., 2020).

We thus propose that future GSC studies should integrate scRNA-seq, genetics, and detailed functional approaches. We suggest to first define all primary cellular states and underlying genotypes in a given tumor, then identify and validate markers for their isolation, test their relative tumor propagation capacity

(with *in vivo* limiting dilution assays), and finally assess by scRNA-seq the spectrum of cellular states recapitulated in a patient-derived xenograft for each tested subpopulation. In addition, exciting developments in single-cell technologies, such as genotyping of transcriptomes (Nam et al., 2019), and combined DNA methylation and RNA profiling (Gaiti et al., 2019), will provide higher-resolution tumor phylogenies and insights into the determinants of cellular states in clinical samples. RNA velocity in single cells (La Manno et al., 2018) and mitochondrial mutational analysis (Wang et al., 2019) might further assist in deciphering GBM dynamics, although additional approaches are needed for validation of their predictions. Recent advances in *ex vivo* organoid models also offer unprecedented opportunities for GBM modeling and for functional interrogation of defined GBM cellular states (Jacob et al., 2020; Linkous et al., 2019). Overall, such new approaches are expected to provide much-needed insights into the rules that govern tumor initiation and cellular transitions in GBM.

Concluding Remarks and Therapeutic Outlook

Research over the last several decades has been demonstrating, in increasing detail, the heterogeneity of cancer cells, thus emphasizing the key challenge associated with attempts at eradicating all cancer cells. Yet, subpopulations of cancer cells might not all be equally important for tumor progression and relapse. Accordingly, considerable efforts have been devoted to the search for CSCs, as their targeted elimination might, in principle, be sufficient for clinical benefit. This approach remains promising, and recent description of CSC programs by scRNA-seq might facilitate the design of CSC-specific therapies. However, the search for CSCs has also generated considerable controversy and discrepancies between studies. As described here for GBM, multiple cellular states may function as GSCs, these states might interconvert, and functionally established GSC markers might isolate distinct cellular states rather than a unique subpopulation.

Given these complexities with defining a unique GSC state to be targeted, we would like to propose a complementary approach: rather than focusing on defining a unique GSC state for elimination, it may be more efficient to identify a cellular state that differentiation therapies should attempt to induce. This approach may be particularly appropriate for tumors with multiple GSC states, thereby reducing their diversity while leveraging their intrinsic plasticity. This cellular state should be both inducible and indolent. By inducible we mean that it may be efficiently induced from multiple GSC states, and by indolent we mean that it should lack proliferation and tumor initiation capacity. Ideally, the induced state should also be targetable by existing therapies, thus allowing a state-inducing plus state-killing drug combination.

In the context of glioma, we suggest that the induction of an AC-like state could potentially be therapeutically interesting. First, this is the only state that is found as part of all three cellular hierarchies described above (Figure 1). Accordingly, we find robust AC-like subpopulations across diverse gliomas, suggesting that AC differentiation is occurring in most gliomas and hence may be induced further. Second, in all cases, AC-like states were associated with low proliferation and, when tested experimentally, with limited tumor-propagating potential. Indeed,

differentiation toward AC-like states has been demonstrated both *in vitro* and *in vivo* (Anastas et al., 2019; Filbin et al., 2018; Piccirillo et al., 2006; Suva et al., 2014). Taken together, inducing the AC-like state in gliomas could represent a potential avenue for differentiation therapies, especially for IDH-mutant and H3K27M gliomas. Future studies are needed to dissect the determinants of glioma cellular states, their transitions, and the impact of therapies on the spectrum of cellular states that drive gliomas.

SUPPLEMENTAL INFORMATION

Supplemental Information can be found online at <https://doi.org/10.1016/j.ccell.2020.04.001>.

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