Trends in Cancer

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Review

Glioblastoma Stem Cells: Driving Resilience through Chaos

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Glioblastoma is an aggressive and heterogeneous tumor in which glioblastoma stem cells (GSCs) are at the apex of an entropic hierarchy and impart devastating therapy resistance. The high entropy of GSCs is driven by a permissive epigenetic landscape and a mutational landscape that revokes crucial cellular checkpoints. The GSC population encompasses a complex array of diverse microstates that are defined and maintained by a wide variety of attractors including the complex tumor ecosystem and therapeutic intervention. Constant dynamic transcriptional fluctuations result in a highly adaptable and heterogeneous entity primed for therapy evasion and survival. Analyzing the transcriptional, epigenetic, and metabolic landscapes of GSC dynamics in the context of a stochastically fluctuating tumor network will provide novel strategies to target resistant populations of GSCs in glioblastoma.

Highlights

Glioblastoma contains a dynamic cellular hierarchy in which stem cell-like tumor cells (GSCs) occupy positions of highest entropy.

GSCs are crucial drivers of treatment resistance and recurrence in glioblastoma.

Heterogeneity and chaotic fluctuations in GSC populations prime tumors for adaptation and evolution.

Therapeutic strategies targeting cellular potential will be essential for the development of effective treatments.

Glioblastoma Overview

Glioblastoma (see Glossary; World Health Organization grade IV glioma) is a universally lethal disease for which there is no effective therapy. Current standard-of-care includes maximal surgical resection, concurrent radiotherapy, and treatment with the orally available alkylating agent temozolomide, followed by adjuvant temozolomide, a treatment regimen which extends survival to a median of only 14.6 months [1]. Glioblastoma is a heterogeneous tumor, as reflected by its previous designation, glioblastoma multiforme (GBM), in which multiple subclonal driver mutations create a highly adaptable entity that is resistant to all therapeutic approaches [2,3]. Glioblastomas are complex ecosystems that rapidly evolve in response to harsh environmental conditions. Because tumors have been characterized as 'wounds that do not heal', tumor cells can coopt stem-like features to survive and thrive [4]. Further, tumors actively remodel their microenvironments through modulation of the immune system, stroma, and vasculature [5]. Thus, numerous drugs showing promising results in preclinical studies have failed to demonstrate efficacy in clinical trials.

Intratumoral heterogeneity and therapy resistance that characterize glioblastomas are thought to be promoted by **glioblastoma stem cells** (GSCs) which demonstrate two principal features of stem cells: self-renewal and differentiation [6–8]. GSCs recapitulate the heterogeneity of the parental tumor *in vivo*, and their biological relevance is demonstrated by their functional role in tumor growth and recurrence [8–10]. GSCs drive resistance to pharmacology, radiation, and surgery, and are thus a key therapeutic target [9–13]. GSCs thrive in harsh, complex microenvironmental niches, unencumbered by stringent checkpoints on proliferation and survival that constrain their normal counterparts [14–18]. Several markers, including CD133 (PROM1), CD15 (stage-specific embryonic antigen-1, SSEA1), L1CAM, and SOX2 are enriched in GSCs, although, similarly to normal stem cells, no marker or set of markers (i.e., immunophenotype) has been identified that exclusively and comprehensively mark GSCs [8,19,20]. Intertwined with this question of classification is the ongoing controversy regarding the structure, immutability, and linearity of the cellular hierarchy in glioblastoma [21–23]. Although specific pathways that

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contribute to the augmented aggressiveness and resilience of GSCs have been described, effective therapies remain elusive. Successfully targeting glioblastoma heterogeneity, driven by subclonal variation, regional features (e.g., vasculature, hypoxia, inflammation, etc.), and the repopulating ability of GSCs, will require a shift away from the binary characterizations of cell state and linear gene interactions and towards an understanding of the molecular landscape of cellular potential as well as of its relationship to the microenvironmental, epigenetic, and metabolic characteristics of GSCs.

Characterization of intratumoral heterogeneity, tumor evolution, and single-cell transcriptomes suggests that the glioblastoma hierarchy represents a dynamic network in which GSCs occupy a spectrum of multipotent microstates at positions of highest entropy [3,24–28]. In this context, chaotic oscillations of cell states create heterogeneity, adaptability, and therapy resistance [29–34]. As cells differentiate, they are constrained to a limited transcriptional program [31]. This model predicts greater therapy resistance for cells at higher entropy owing to a greater diversity of available escape routes [31,34]. By applying current knowledge and the growing library of omic data to this scaffold, we can derive a more nuanced understanding of tumor evolution and therapy resistance as emergent properties of cellular potential.

GSCs – The Apex of a Dynamic Network

Hierarchical models of cellular differentiation, such as that proposed for glioblastoma, are characterized by predominantly unidirectional progression from founder stem populations to more differentiated progeny, a phenomenon eponymously visualized as Waddington's landscape [22,23,35,36]. Research into the apparent thermodynamic favorability proceeding towards differentiation has drawn from principles of dynamic network theory and statistical mechanics [33,37]. GSCs, similarly to normal stem cells, appear to be poised in a crucial state of maximal entropy relative to their more differentiated counterparts [29,38]. This state is maintained by a permissive epigenetic landscape and the low and oscillating expression of a large number of genes [29,38]. As a result, individual stem cells shift stochastically within a potential landscape through transcriptional fluctuations, enhancing the diversity of the overall population [33,38]. Multipotency may therefore be defined as an emergent property of a cell population in constant dynamic flux [30]. Although entropically primed for adaptation and differentiation, the stem cell population remains relatively resilient to large, random state changes. Instead, state changes are driven by non-stochastic forces or events – known as attractors – generated by the microenvironment, therapeutic intervention, or cell–cell interactions [33,39]. Thus, cancers cells removed from their environment may inhabit a potential landscape distinct from that of the original tumor, and treatment may not only select for but also generate new subpopulations. This hypothesis is supported by observations that genetic discovery efforts performed in parallel *in vivo* and *in vitro* yield largely non-overlapping results, where a greater number of molecular dependencies are seen *in vivo*, suggesting that there are additional attractor states *in vivo* [40]. The attractor state model is depicted in Figure 1. As stem cells differentiate, they are drawn down into an energy valley, becoming locked into a transcriptional profile that expresses fewer genes [31]. Thus, as a whole, the stem cell population is poised to respond to a wide variety of stimuli, whereas differentiated cells exhibit fewer degrees of freedom and have more stringently regulated genetic programs. Greater therapy resistance of the **cancer stem cell** population derives both intrinsically from chaotic fluctuations in gene expression and extrinsically from the complexity of interactions with a variety of attractor states.

The network dynamics that govern tumor cell fate are shared with normal stem cells, but without the constraints that direct normal multicellular development. Somatic mutations and genetic instability appear to redefine the potential space occupied by stem and non-stem tumor cells,

Glossary

Cancer stem cell (CSC): a cell that is capable of recapitulating a tumor and exhibits the two defining properties of stem cells: self-renewal and differentiation.
Glioblastoma: grade IV glioma, the most common malignant brain tumor.
Glioblastoma cancer stem cell (GSC): tumorigenic cancer stem cells in glioblastoma.
Neural stem cell (NSC): a multipotent progenitor cell that gives rise to multiple cell types in the central nervous system.

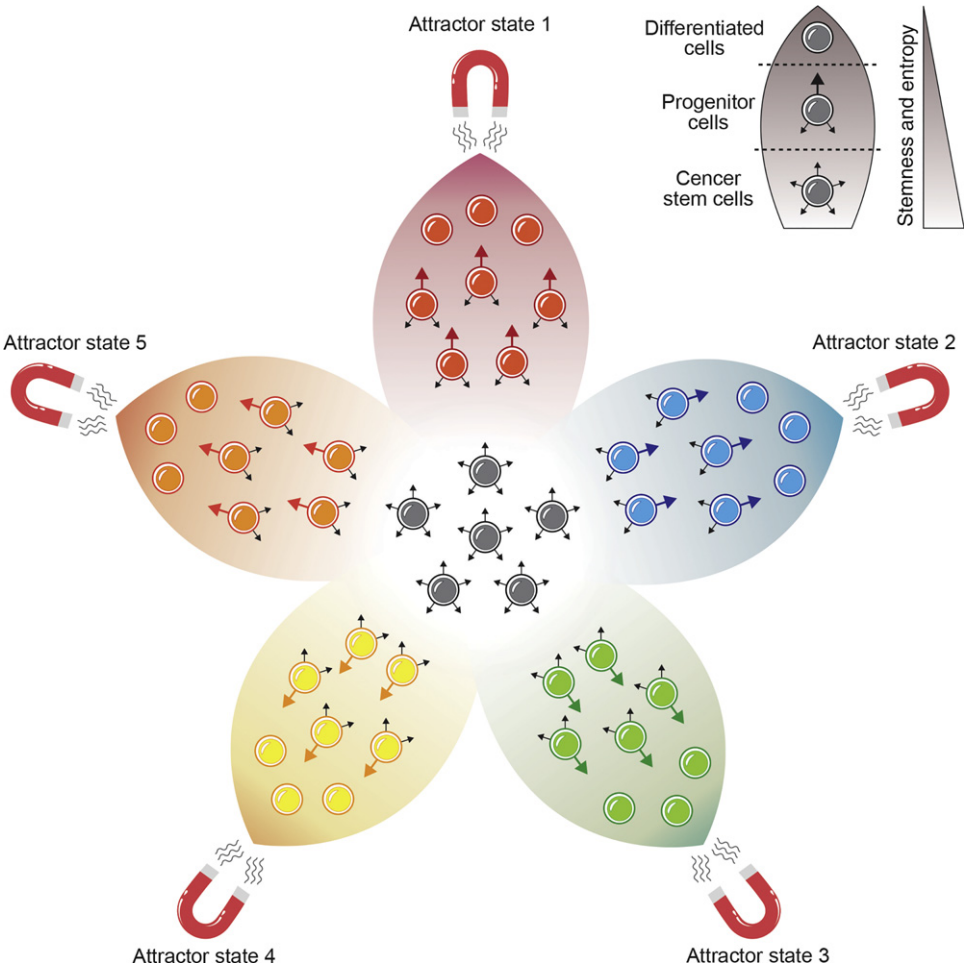

Figure 1. Attractor State Model of Glioblastoma. Glioblastoma stem cells at the center of the tumor hierarchy have the highest entropy and capacity for adaptation. Attractor states (e.g., microenvironmental niches, genetic mutations, therapeutic intervention) drive the development of different tumor cell populations. Each colored petal depicts different attractor states which drive the proportion of each cellular state. Arrows on the different cellular state represent the directionality of the attractor state.

making cancer networks distinct from their normal counterparts [27,29,41]. Although conventional tumor genetics categorize mutations into oncogenes and tumor-suppressor genes, recent genetic analyses of gliomas and other malignancies have detected dysregulation of chromatin regulators, which may lead to plasticity in the epigenetic cell state [42–45]. When analyzed in this context, cancer networks reside at a net higher entropy than do corresponding normal tissues, and display distinct energy relationships between stem and non-stem populations [29]. Differences in entropy between stem and non-stem cancer cells are smaller than in those of a normal tissue, particularly in GBM [29]. Thus, prospective delineation of clear, binary transcriptional distinctions between stem and non-stem glioblastoma cells at a single-cell level has proved to be challenging [25]. GSCs have also proved to be resistant to differentiation strategies, suggesting that terminal differentiation may have different connotations in the context of a perturbed cancer network [46].

The conceptual framework of network dynamics underlying the glioblastoma hierarchy has several concrete implications for the modeling and treatment of glioblastoma. GSCs sit atop a

hierarchy of entropy and are defined by noisy transcriptional fluctuations. Probabilistically, the population will therefore expand to dynamically occupy all available microstates, reconstituting lost populations and regenerating heterogeneity [38,47]. In essence, the significance of GSCs lies not solely in the capacity of a single cell but in the chaotic resilience of the network [31]. GSCs move dynamically through this space in response to perturbations, such as therapeutic intervention. One challenge for the neuro-oncology research community, indeed for the entire cancer stem cell community, is that measuring cell states is limited by the lack of strong immunophenotypes for cancer stem cells and limited functional assays to measure tumor biology. Therapy development targeted at resistant populations must therefore address not only cell state or defined cell fate but also cellular potential. This potential space of GSCs is defined intrinsically by genetic and epigenetic landscapes, and externally by complex tumor microenvironments acting as attractors. The variable metabolic, inflammatory, and cell–cell cues within these environmental niches serve to maintain heterogeneity, which is further reinforced by feedback as GSCs generate and remodel their environments [5,28]. Interactions between GSCs and their environmental niches represent important attractor states and are crucial for generating tumor heterogeneity, thereby promoting development of treatment-resistant populations. GSCs in different tumor niches are summarized in Figure 2.

GSCs and the Tumor Microenvironment – A Tangled Hierarchy

Three major microenvironments have been described in glioblastoma – the hypoxic-necrotic core, the perivascular niche, and the invasive edge [48]. Each biome serves as a unique attractor, activating a variety of cellular programs in GSCs, which in turn serve as architects to actively remodel the microenvironmental architecture. Niche interactions may, therefore, be crucial for maintaining the breadth of states that the GSC population can occupy, promoting both heterogeneity and robust maintenance of stem properties.

The perivascular niche provides crucial cues for maintenance of stemness and induces pathways that enrich for GSCs capable of migration and DNA repair [49–51]. Endothelial cells (ECs) promote a stemness phenotype through NOTCH, sonic hedgehog, and nitric oxide signaling pathways, among many others, whereas other perivascular cell populations, such as tumor-associated macrophages, secrete chemokines that promote GSC growth and expansion [51–54]. Signaling within the perivascular niche also generates GSCs that may be adapted to some types of therapy resistance. TGF-β is highly expressed around the tumor vasculature and promotes stem cell maintenance as well as activation of DNA repair pathways and the expression of matrix metalloproteinase 9 (MMP9), an important mediator of invasion [49,53]. CXCL12, a ligand expressed by ECs, provides a chemotactic signal and positive regulator of MMP expression, thus priming a population of GSCs for invasion [50,73]. GSCs remodel and maintain the perivascular niche and produce high levels of proangiogenic factors, such as VEGF, that drive EC proliferation, survival, migration, and blood vessel permeability [56]. GSCs can give rise to pericyte-like cells, key regulators of vascular remodeling and stabilization, whereas differentiation of GSCs into tumor ECs remains controversial [57–60]. Conflicting results may reflect the challenges of applying immunophenotypic and functional definitions derived from normal cellular hierarchies to the cancer cell hierarchy, which may not achieve classical differentiation. Despite the importance of the perivascular niche for tumor growth, antiangiogenic factors have not performed well in clinical trials [61]. Although some resistant tumors retain high levels of vascularity, presumably through angiogenic pathways that circumvent VEGF targeting, in others the hypoxic niche expands and becomes predominant [62,63].

Hypoxic and necrotic regions are a hallmark of glioblastoma, and support GSC maintenance, proliferation, and therapy resistance [15,64]. Hypoxic stress generates a subpopulation of cells

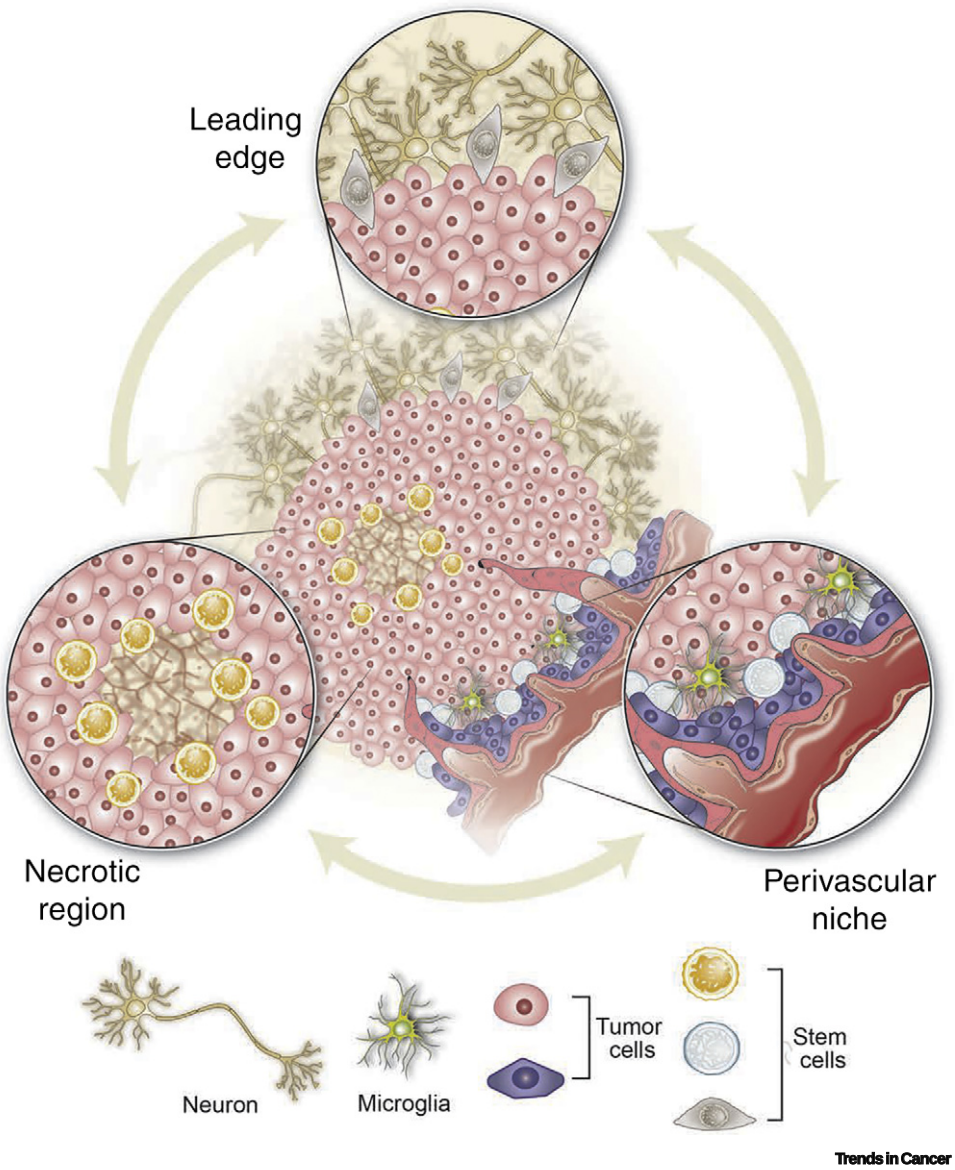

Figure 2. Glioblastoma Stem Cells (GSCs) across Tumor Niches. GSCs are found in the tumor microenvironment and maintain heterogeneity through unique cell–cell interactions and niche properties throughout the tumor. These niches are not stable and independent, but instead are dynamic drivers of cellular adaptation and resistance that communicate and interconvert as the tumor grows and adapts.

that are adapted to survive in nutrient-restricted conditions, and promotes shifts towards aerobic glycolysis and glutamine-mediated fatty acid production [65]. Furthermore, as in normal **neural stem cell** niches, hypoxia is hypothesized to promote quiescence, a phenotype that could significantly contribute to the enrichment of chemo- and radioresistant populations [17]. The effects of hypoxia are mediated in large part through hypoxia-inducible factor 1 (HIF-1) and HIF-2 [48]. HIF-2α remains elevated under chronic hypoxia and is involved in the activation of signaling pathways regulating stem cell maintenance, including KLF4, SOX2, and OCT4 [14,66]. HIF-1α, a key player in the acute hypoxic response, regulates metabolic adaptation to nutrient deprivation

and promotes a mesenchymal shift in hypoxia-treated GBM cells and the expression of prosurvival factors such as ERK [65,67,68]. HIF-1α also promotes VEGF expression, thereby inducing angiogenesis in hypoxic regions [16,69]. Thus, the hypoxic niche primes cells to regenerate the perivascular niche, a prime example of how the multiplicity of subpopulations and signaling pathways induced by different attractor states promotes dynamic heterogeneity within the tumor.

The third major glioblastoma microenvironment is the invasive niche. GSCs are enriched for their invasive potential, a finding consistent with ability of leading edge cells to drive tumor recurrence following surgical resection [12]. These 'surgically resistant' populations migrate along the vasculature and white matter tracts utilizing cadherins and integrins, cleaving their way through extracellular matrix using matrix metalloproteinases such as MMP2, MMP9 and ADAMT2 [11,70]. Invasion is facilitated by several signaling pathways that are upregulated in GSCs, including L1CAM and ephrin-B2 [55,71]. GSCs also express multiple mediators of the epithelial–mesenchymal transition, during which cancer cells convert to a more invasive, metastatic phenotype, including the TWIST1–SOX2 signaling axis, N-cadherin, STAT3, NF-κB, and periostin [12,72–75]. The invasive and migratory phenotype is promoted by signaling in the hypoxic and perivascular niches, and is modulated by the differential tissue mechanics and matrix stiffness of blood vessels and white matter tracts [48,76]. Therefore, normal brain tissue could be considered to be an attractor in the development of the invasive niche.

These three tumor microenvironments serve as attractors that texturize and stretch the fabric of the GBM landscape, generating a spectrum of GSC subpopulations and increasing the probability that any one population will survive a therapeutic challenge to reconstitute the others [47]. Efforts to comprehensively target the heterogeneous GSC population must therefore incorporate the diversity of the tumor ecosystem to fully appreciate the complex landscape of cellular potential. We recently showed that GSCs residing in separate niches express distinct GSC markers, transcriptional profiles, and reciprocal dependencies on core epigenetic regulators, the polycomb repressive complexes [43]. Differences in epigenetic regulation were reflected in differential sensitivity to BMI1 and EZH2 antagonists. The interconversion between GSCs in different niches remains unresolved, but recent evidence supports multiple stem/progenitor populations in normal tissues (e.g., bone marrow and gut) that can repopulate other depleted populations. In cancer, recapitulating cancer stem cell niches in homogenous, nutrient-rich *in vitro* cultures and in animal models is an ongoing challenge. To address this issue, several culture systems have attempted to mimic the heterogeneity of the tumor microenvironment *in vitro* through 3D culture or microfluidic approaches [77–79]. Replacing convenient but simplistic models with more accurate and complex models will be crucial in developing effective therapies. Microenvironments both rely upon and maintain the inherent heterogeneity in the GSC population that allows dynamic transitions and flexible adaptation.

The interaction between the microenvironment and tumor genetics in shaping GSC cellular states remains an open area of investigation. Mouse modeling studies have demonstrated that specific mutational events such as NF1 loss or PDGFB overexpression shift the tumor ecosystem towards macrophage infiltration or vascular dysfunction, respectively [80]. Consistent with human studies demonstrating NF1 loss following temozolomide treatment in recurrent tumors [5], NF1 silencing correlated with temozolomide resistance [80]. Single-cell RNA-sequencing studies have begun to characterize the changing profile of GBM cells in the context of different genetic and microenvironmental attractors [24]. Additional multiregional and single-cell studies may further elucidate the dynamic interplay between attractors and their role in shaping the landscape of tumor heterogeneity, adaptation, and resistance.

Intratumoral Heterogeneity and Tumor Evolution

Glioblastoma exhibits significant intratumoral molecular and phenotypic heterogeneity, and targeting any one component has proved to be minimally effective. IDH1 or IDH2 mutant glioblastomas are fundamentally distinct from IDH wild-type tumors, and have relatively better prognosis and response to therapy. Classification of IDH wild-type glioblastoma has divided their transcriptional profiles into three major subtypes – proneural, classical (or proliferative), and mesenchymal – that are distinguished by distinct prognostic significance, molecular signatures, biologic phenotype, and stemness signatures [81]. However, multiregional sampling and single-cell RNA sequencing have revealed the presence of multiple subtypes within a single tumor [25]. Intratumoral heterogeneity not only increases the likelihood of the emergence of resistant subclones, a phenomenon that characterizes the inevitable tumor recurrence of glioblastoma, but has also been shown to facilitate tumor growth [3,26]. For example, simultaneous implantation of cells with high or inhibited HIF-1α expression led to more rapid tumor growth, whereas coimplantation of GSCs with senescent, differentiated glioblastoma cells can promote tumorigenesis [82,83].

Subtype conversions (e.g., proneural-to-mesenchymal transition) occur frequently during tumor recurrence, presumably driven by both cell-autonomous and shifting tumor microenvironments to add new attractor states secondary to a therapeutic intervention [5]. The pervasive heterogeneity at the apex of the tumor hierarchy has implications for overall tumor architecture because the emergence of resistant subclones likely originates in part from the diversity of the GSC population, which then propagates changes to the whole tumor [25,26]. As noted above, a single tumor can harbor different subtypes of GSCs, whereas GSC-derived subclones from a single patient tumor exhibit significantly different growth patterns [84–86]. Ongoing efforts to characterize an exclusive, comprehensive population of GSCs have revealed the difficulty of finding a binary marker of stemness. CD133 (prominin 1) is a commonly used marker with a high specificity, but low sensitivity, for GSCs in that subsets of $CD133^{-}$ cells demonstrate stemness characteristics and are tumorigenic [8,87]. Several other markers, including CD15 (SSEA-1) [20], integrin α6 [88], ALDH [89], NESTIN [90], SOX2 [91], OLIG2 [25,92], and NANOG [93], are also enriched in populations with stem cell properties. However, a single comprehensive GSC marker remains be identified, and thus the nature of the cellular hierarchy (immutable vs adaptable) in GBM remains controversial [21,23,24]. It is possible that there is a factor or combination of markers that can definitively mark stem versus non-stem cells, but which has not yet been identified. A more likely scenario is that there is a hierarchy in which the path to differentiation is better defined – not as a transition between discrete states, but as a series of reversible transitions through many microstates – in which a heterogeneous population of stem cells exhibits reversible, random oscillations until an attractor drives them towards a committed, differentiated state [28,31,94]. Accordingly, single-cell sequencing has revealed a gradient of stem cell markers expressed by individual cells within a tumor [25]. In this context, dedifferentiation is more likely to occur on a small scale, as a series of microstate transitions, whereas transitions from the nadir to the apex of the hierarchy are generally improbable (and, thus, unfavorable) in naturally occurring biologic states. However, reprogramming can be externally induced through delivery of transcription factors, both in normal and tumor hierarchies [95,96].

Given their capacity to regenerate a tumor and elevated resistance to therapy, GSCs are thought to drive recurrence and evolution. Modeling of clonal evolution suggests that recurrence is driven by subclones that diverged early from the dominant clone of the primary tumor [3]. However, the details of resistance in tumor evolution remain obscure. Multiple mechanisms related to temozolomide resistance have been reported, including induction of stem cell markers, dedifferentiation towards a stem-like phenotype [97], or differentiation of some GSCs towards endothelial-like cells

which reciprocally support the GSC population [60]. Heterogeneous genetic changes may be selected for or induced in recurrent tumors [5,26,98]. Treatment is often characterized as a Darwinian process that selects existing, adapted subclones within a tumor. Indeed, subtype conversion in tumor recurrence may represent simply an expansion an existing population. Many studies have shown that therapeutic intervention induces adaptive changes in a more Lamarckian process that may even promote elevated aggression and resistance [39]. Accordingly, mutational signatures consistent with treatment paradigms such as alkylating agents are frequently found in recurrent tumors, and likely originate from GSCs that survived treatment [99]. Although not yet explicitly investigated in glioblastoma, this process of evolution depends not on selection of existing clones but on the role of treatment as an attractor state that induces a compensatory shift in tumor cell populations. GSCs are highly enriched for this adaptive potential and exhibit upregulation of the DNA damage response, an ability to preferentially utilize nutrients in response to harsh environmental conditions, and a general phenotype of resilience to therapy [9,10,100,101].

GSC-Targeted Therapeutics and Mechanisms of Resistance

Treatment options for glioblastoma remain limited and prognosis is dismal [2]. GSCs inhabit the entropic peaks within the tumor, predicting that they will have the greatest diversity of escape routes in the face of therapeutic intervention owing to less restricted genetic programs and constant flux within the population. Three characteristics of the GSC population exemplify and drive this model of resistance: genetic instability, signaling promiscuity, and population heterogeneity.

As previously described, although they follow the same entropic pattern, cancer networks are distinct from normal cell networks [27,41,47]. In GSCs, genetic instability is a significant driver of this phenotype and promotes DNA repair, aberrant tumor cell survival, and mutation tolerance. Crucial replicative checkpoints are commonly mutated in glioblastoma, including p53, TERT, ATRX, NF1, CDKN2A, and RB1 [102,103]. Building upon these founder mutations, and seemingly paradoxically, GSCs excel at DNA damage repair, and upregulate key players in recognition and repair such as damage detection and checkpoint kinases Chk1, Chk2, ATR, ATM, and RAD17, and the repair enzymes PARP1 and TIE2 [9,104,105]. Many of the pathways that are crucial for maintaining stemness in GSCs also facilitate DNA damage repair. For example, NOTCH signaling, which is important for GSC survival, mediates radioresistance in GSCs through upregulation of the prosurvival pathways PI3K/AKT and Bcl-2 [106]. This balance of checkpoint regulators and repair enzymes in mutation tolerance and repair efficiency in GSCs represents a likely mechanism that drives rapid evolution and plasticity in the context of environmental stress, and is thus a potential target for radio- and chemosensitization of glioblastoma [105,107].

Signaling promiscuity characterizes GSCs both on a global epigenetic level and in specific pathways. Compared to differentiated glioblastoma cells, chromatin profiling reveals that GSCs demonstrate widespread loss of repressive histone marks compared to normal human astrocytes, as well as broad activation of multiple transcription factors networks that do not normally coincide [108]. This pattern would allow greater noise in gene expression, thus generating a dynamically fluctuating population with greater access to alternative pathways and state transitions in response to therapy. GSCs are also more metabolically flexible than their differentiated counterparts. Although differentiated glioblastoma cells rely on the well-known Warburg effect for glucose metabolism, GSCs can more adeptly switch between aerobic glycolysis and oxidative phosphorylation [109]. Differentiated tumor cells and most other cancer cells predominantly express pyruvate kinase isozyme 2 (PKM2), which promotes aerobic glycolysis and is primarily found in proliferating cells. However, GSCs also express the PKM isozyme PKM1, which facilitates oxidative phosphorylation, providing a potential mechanism for the higher mitochondrial utilization of GSCs and the greater flexibility of GSC metabolic regulation [109]. Instead of eliminating

the tumor, targeting any single aspect of metabolic regulation may merely induce a metabolic switch in GSCs.

Finally, the potential space of cellular states available to glioblastoma is diverse. The noise inherent in the genetic and epigenetic landscape of GSCs, enhanced by the heterogeneity afforded by multiple complex attractor states, generates a redundant system that is able to tolerate failure of any one component, such as arises from targeting by a particular treatment regimen [24,28,110]. Current therapeutic modalities target specific cell states, simply selecting for or

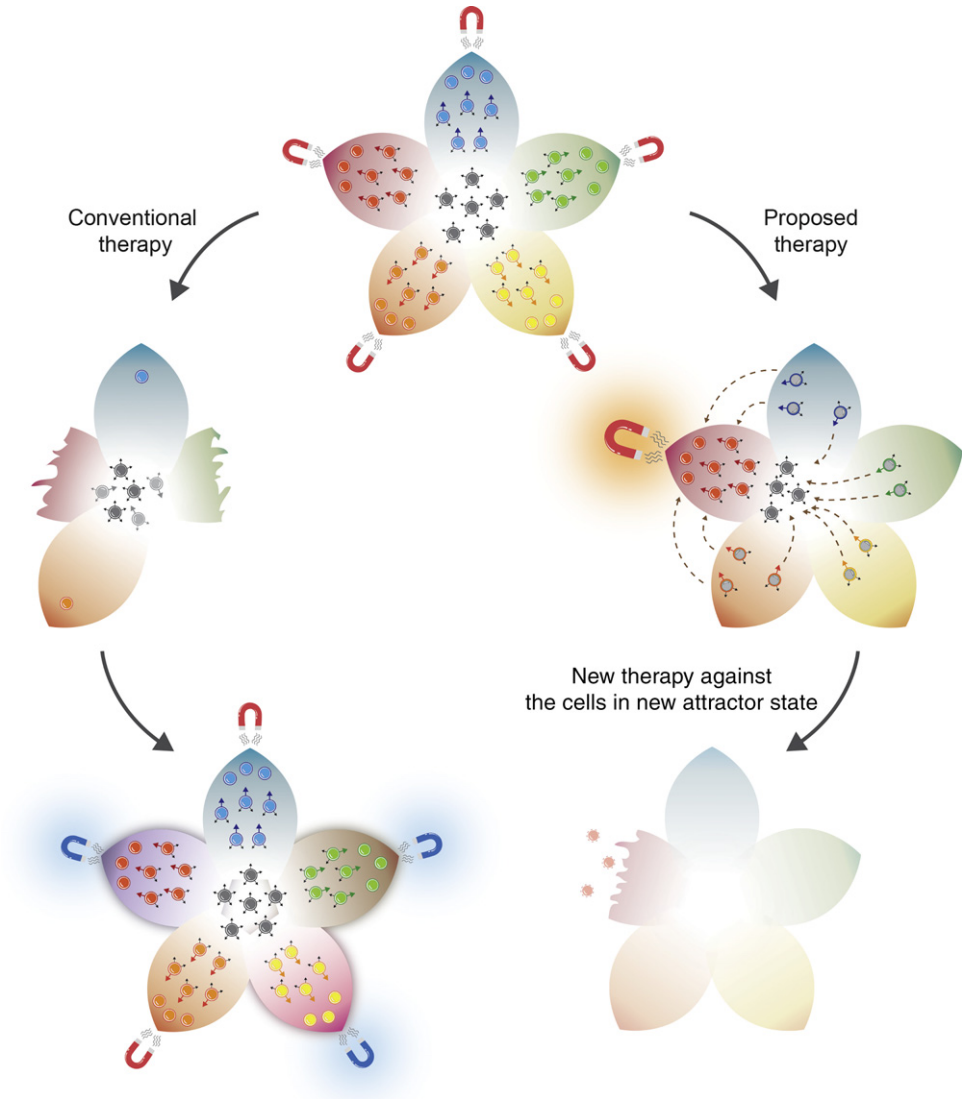

Figure 3. Therapeutic Approach to Glioblastoma Stem Cell (GSC) Adaptation and Heterogeneity. (i) Classical therapeutic approaches often spare the GSC population or target individual components of the tumor landscape, for example the tumor vasculature or rapidly dividing cell populations. This generates new attractor states, together with the older untreated states, that allow the tumor to evolve and repopulate. (ii) The attractor state model implies that effective therapy will require a combinatorial approach. The first treatment bottlenecks tumor adaptation by applying an initial stimulus that drives cells towards one state, and the second intervention is targeted at the resulting specific cellular state.

generating adaptive subclones instead of eliminating the tumor. For example, radiation induces DNA damage and triggers GSC-dependent, NF-κB-driven interconversion between subtypes towards the mesenchymal signature [111]. Radiotherapy may therefore select for or induce GSCs adapted to rapid repair with a high apoptotic threshold. Both radiation and temozolomide also preferentially target proliferating cells. Single-cell sequencing studies have revealed high overlap between stem cell signatures and proliferative markers, suggesting that GSCs are more proliferative than was previously thought [24,25]. Quiescent GSCs may exist as a subpopulation that is maintained by signaling from the microenvironmental niche [112], giving rise to (or existing in parallel with) proliferative GSCs [23]. Although the relationship between proliferative and quiescent GSCs remains an open area of investigation, multiple studies have demonstrated that GSCs maintained in a quiescent state are more resistant to treatment [109,112,113]. As previously noted, antiangiogenic agents such as bevacizumab have not been effective in clinical trials, despite demonstrating initial promise in preclinical models [110,114,115]. Research into mechanisms of resistance to antiangiogenic therapy suggest that it can be circumvented either as an intrinsic property of cellular heterogeneity in the perivascular population, which precludes reliance on a single pathway for angiogenesis (VEGF), or by a global shift in tumor constitution to rely on another major tumor microenvironment, the hypoxic niche [62,63,116]. In addition to underscoring the importance of accurate tumor modeling, this result may highlight the futility of targeting a single component of the tumor ecosystem.

When tumors are modeled as dynamic networks, higher entropy correlates with greater drug resistance and cellular potential becomes a key mediator of resistance [34]. This framework favors several therapeutic strategies. Studies targeting markers of the cell state with highest potential are already underway utilizing immunotherapeutic peptide- and nanoparticle-targeting strategies. Although unlikely to eliminate the entire population of potential GSCs, these approaches may be able to check the recurrent or adaptive potential of the tumor, making it more susceptible to other therapeutic interventions. Another potential avenue is to direct GSCs cells into particular valleys using drugs or other interventions as attractors [41]. Perhaps the most obvious application of this strategy occurs in the context of differentiation therapy. However, the challenge of terminally differentiating cancer stem cells *in vitro* suggests that the genetic profile of these cells is resistant to complete differentiation [46]. Attempts to bottleneck a tumor by driving GSCs into a predictable valley, not necessarily of terminal differentiation, but of restricted transcriptional options will require a more integrated understanding of network potential in the context of both epigenetic and genetic driving forces. Different therapeutic approaches to combat GSC adaptation and heterogeneity are summarized in Figure 3.

Concluding Remarks

Glioblastoma is a complex and diverse entity that has largely thwarted attempts at therapeutic intervention (see Outstanding Questions). The resilience of glioblastoma is founded on heterogeneity and adaptability, characteristics that are enriched in GSCs [6]. The GSC population is defined by chaotic state fluctuations in a perturbed genetic landscape that is stripped of the natural checkpoints that constrain cellular differentiation and sculpted by a permissive epigenetic profile and a complex array of attractor states [27,29,41]. Conceptualizing GSCs in the context of their thermodynamic potential suggests an alternative interpretation of the complexity of the observed tumor hierarchy, one in which the transition between apparently irreversible states occurs through many stochastic shifts and reversions. In modeling these systems as analog states with linear dynamics, potentially crucial nuances may be lost. This theory also further emphasizes the importance of contextualizing GSC function and accounting for cellular potential. Drug discovery in neuro-oncology has been characterized by a narrowing funnel of efficacy, from *in vitro* systems to preclinical animal models and finally therapeutic trials. By removing the complexity of attractors

Outstanding Questions

Tumor cells in different niches express specific transcriptional signatures. Do microenvironmental interactions drive distinct dependencies in GSCs?

Epigenetic dynamics regulate the expression of important oncogenic pathways in GBM. How does the epigenetic landscape in GSCs differ versus their differentiated progeny? What role do histone modifications play in maintaining the stem state?

GSCs adapt rapidly and effectively to therapeutic intervention. Can integration of GSC epigenetic and genomic networks predict likely avenues of cellular adaptation?

from the equation in preclinical studies, the cellular landscape becomes constrained and the adaptive potential of the tumor becomes handicapped. Furthermore, high-throughput strategies to identify nodes for precision therapy must account for the fact that they are not targeting a static population but a dynamically adaptive population. Defining the multifaceted dependencies of the GSC population by mapping the complex intratumoral interactions that facilitate resilience may inform novel therapeutic strategies targeted not only at a snapshot of cellular state but at a topographic landscape of cellular potential.

Acknowledgments

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