Almost a decade ago, I wrote a preview feature entitled, “Many Tumors in One: A Daunting Therapeutic Prospect,” in response to the dramatic intratumoral genetic heterogeneity found in glioblastomas and some other human gliomas (1). This genetic/epigenetic heterogeneity undoubtedly plays a role in the paucity of effective treatments and abysmal prognosis of this disease. Now, with the article by Wang and colleagues (2) in this issue of Cancer Discovery, along with a number of related papers, that preview article might more appropriately be renamed “Many Tumors Are One: A Hopeful Therapeutic Prospect.”

At least two major findings over the last 15 years have had a major impact on our understanding of glioblastoma (GBM) ontogeny and biology. The first paradigm change came with the demonstration that GBMs follow a hierarchical developmental process, starting with a primitive tumor/glial “stem-like” cell (GSC), akin to a neural developmental process (3). Like normal neural stem cells (NSC), GSCs were shown to have the ability to self-renew as well as to produce more differentiated progeny that can recapitulate the totality of the tumor phenotype.

The second paradigm change came with the genomic and epigenomic characterization of GBMs, demonstrating that gliomas could largely be divided into tumors with and without mutations in the IDH1/2 genes, and with the demonstration of their dramatic genomic and epigenomic intratumoral and intratumoral heterogeneity (4, 5). Initial hopes for a more manageable framework for understanding the diversity of the GBM mutational landscape came with the demonstration that bulk GBM could be classified into three major transcriptional subgroups (proneural, mesenchymal, and classic; ref. 6). Such analyses from bulk tissue presented a good general picture of the spectrum of GBM biology, but the true cellular architecture of human gliomas. Among the most profoundly important of these are a series of studies by Suva and colleagues, who have evaluated the cellular makeup of the major human glioma subtypes including IDH-wild-type, IDH-mutant, and H3K27M-mutant midline gliomas (7–9). These studies have demonstrated an IDH-mutant and H3K27M-glioma hierarchy consisting of a cycling stem-like subpopulation and two differentiated subpopulations of cells that have transcriptomic programs that resemble differentiated oligodendrocytes and astrocytes; the two tumor types differing largely by the neural developmental state and number of stem cell progenitors (NSC-like versus oligodendrocyte progenitor cell-like).

In contrast, IDH-wild-type GBMs appear to be characterized less by a hierarchical neural developmental-like structure and more by the coexistence of at least four cellular states [astrocyte-like (AC-like), mesenchymal-like (MES-like), oligodendrocyte progenitor cell-like (OPC-like), and neural progenitor cell-like (NPC-like)] that roughly recapitulate the originally described whole-tumor transcriptomic states; each enriched for, but not defined by, specific genomic aberrations (7). Although each of these states coexists in an individual GBM, one state tends to dominate over the other three in any given tumor, possibly accounting for the transcriptomic classification for that tumor when assayed in bulk. Interestingly, it appears that cells from any of the states produce tumors that recapitulate the state configuration of the original tumor, suggesting the genomic/epigenomic landscape of a given tumor may bias it toward that particular distribution of cellular states.

Within this new framework comes the study by Wang and colleagues that addresses the critical question of the interrelationship among the various transcriptional subtypes of glioma as represented by the GSC populations and their overall ontology. Using single-cell RNA sequencing and single-cell assay for transposase-accessible chromatin using sequencing data, Wang and colleagues found that the classic GBM transcriptional subgroups merely represent different proportions of two primary transcriptional subgroups [mesenchymal (mGSC) and proneural (pGSC)]. They find that the GSC population is constituted by individual GSCs that exhibit transcriptional profiles along the axis of mGSCs and pGSCs, consistent with previously published experimental data showing the plasticity of GSC transcriptional profiles in vitro. What’s most novel in this study, however, is the assertion that pGSCs come from a mGSC precursor in a unidirectional...
developmental process and that the varying proportions of these cells (and their progeny) are adequate to explain the genetic and phenotypic heterogeneity observed in GBM.

The findings by Wang and colleagues are generally consistent with those of Suva and others, with a few differences. First, Wang and colleagues identify a developmental axis between two major cellular states, whereas Neftel identifies at least four such states. This discrepancy may largely reflect a difference in the computational methodologies used by each group, for the mGSC and pGSC groups in Wang and colleagues’ article may very well constitute a combination of the MES-like/AC-like and NPC-like/OPC-like Neftel groups, respectively. Publication of the raw data from both laboratories will now allow an unbiased analysis by other investigators to better understand this subtle difference. Subsequent analyses of both datasets may also resolve why Wang and colleagues found almost all...
cellular proliferation within the pGSC cellular state, whereas Neftel found it in all four cellular states (albeit weighted more toward the NPC-like and OPC-like states).

The most important finding of the Wang study, however, is the assertion that the mGSC state is a relatively quiescent founder state, and that the developmental hierarchy moves down toward the rapidly proliferating pGSC state. In contrast, the Neftel study found no evidence of such a hierarchical developmental lineage in IDH-wild-type GBMs (in contrast to IDH-mutant gliomas), and in fact presented indirect data suggesting a robust plasticity among all four cellular states. Which of these models is most accurate is of critical importance not only for understanding the ontology of IDH-wild-type GBMs, but also for the development of novel therapeutic strategies (e.g., how many states need to be therapeutically inhibited at once). Indeed, Wang and colleagues demonstrate that maximal tumor inhibition in vitro occurs following targeted inhibition of both transcriptional states in combination.

Wang and colleagues’ data suggesting a unidirectional axis of differentiation of mGSCs toward pGSCs is strong, but it is entirely based on two computational methodologies: RNA velocity and mitochondrial mutational assays (2). Although both are powerful and validated computational tools for predicting lineage, they are somewhat less well validated for genetically unstable cancer stem cells. Thus, the ultimate proof of hierarchal relationships and plasticity between the different GSC states will rest on experimental verification such as those using single-cell bar coding lineage determination studies.

Despite the still-open question of plasticity between the various GSC cellular states, the study by Wang and colleagues adds important information and data to a growing picture, whereby gliomas represent a family of tumors consisting of subpopulations of individual cells occupying a specific cellular state defined by a gene-regulatory network (GRN). These cellular states occupy stable phenotypes of biological behavior represented as low-energy attractor states on the proverbial Waddington landscape (Fig. 1A; ref. 10). This landscape and the glioma-occupied attractor state are a result of tumor-associated genetic and epigenetic pressures superimposed on a pathway of normal NSC development. The extent to which the developmental hierarchy and tumor attractor states resemble those of normal NSC development is largely dependent on the degree of genetic/epigenetic perturbations, with a lower mutational burden resulting in a more normal-like developmental landscape. Thus, pediatric-predominant gliomas (e.g., NSCs differentiating to AC-like and oligodendrocyte-like cells). In contrast, the higher mutational burdens and/or genomic instability found in IDH-wild-type GBMs push these tumors out of the normal developmental landscape to reside on an alternate (not occupied in normal development) landscape whereby neural development–like lineages and attractors still exist, but on a much flatter landscape allowing easier passage between attractor states. Whether various subpopulations of GBM cells represent “subattractor” states separated by low potential energy, thereby allowing a cell-ready passage from one subattractor state to another within a single GBM attractor (e.g., the Neftel model), or rather one state holds a less differentiated attractor state only allowing spontaneous passage downward to a more differentiated attractor state (the Wang model), awaits further study (Fig. 1).

Not only are these new insights important to the understanding of the ontogeny of human gliomas, but they pose a potentially promising way forward for future therapy. Despite the significant genomic/epigenomic heterogeneity of gliomas, all paths are beginning to appear as though they may coalesce onto a limited number of cellular/attractor states. If true, then theoretically one may need only to identify and target a few key nodes responsible for the stability of those GRNs underlying the GBM attractor states operative in most GBMs, rather than the current paradigm that posits the need to target each major genetic perturbation found in each individual tumor (a clinically implausible goal).

The last 50 years of GBM research have seen us go from the idea of one tumor to many tumors to maybe now, again, just a very few tumors. If so, we may be at a turning point in this biologically fascinating yet clinically devastating disease, whereby we may finally have a rational way forward in the development of real treatment breakthroughs so desperately needed by our patients.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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Malignant Gliomas: Simplifying the Complexity

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