Putting “multiforme” back into glioblastoma: intratumoral transcriptome heterogeneity is a consequence of its complex morphology

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Key words: genomics | glioblastoma | heterogeneity | histology | transcriptome

See the article by Prabhu et al on pages 1599–1606.

Modern-day neuro-oncology can be traced back to the pioneering work of Bailey and Cushing in the 1920s.¹ Diagnosis and treatment of gliomas, the most common and deadly primary brain tumor, continue to be based on one of their core principles: Morphological features evident under the microscope correlate with clinical outcomes.² They termed one particularly aggressive tumor glioblastoma “multiforme” due to its highly variable microscopic appearance. Then as today, its defining morphological features included diffuse brain invasion, increased tumor cell proliferation, microvascular proliferation, and necrosis.

While “multiforme” has fallen out of favor in subsequent definitions of glioblastoma (GBM), it remains no less relevant to its biology today.² Indeed, in this issue of *Neuro-Oncology*, Prabhu et al refocus attention on the “multiforme” nature of GBM and reestablish the relevance of its diverse and complex morphology in the context of contemporary genomics-based molecular classification.³

The NCI Director’s Challenge issued in 1999 prodded “the scientific community to harness the power of comprehensive molecular analysis technologies to make the classification of tumors vastly more informative.” Its fruit was realized over the ensuing decade by The Cancer Genome Atlas (TCGA) and others. TCGA defined significant *intertumoral* heterogeneity of GBM by identifying mutational drivers and the core pathways in which they functioned, dividing the disease into 4 subtypes based on transcriptome profiles (proneural, neural, classical, and mesenchymal) and identifying molecular features that correlated with clinical outcomes and therapeutic response.^{4,5}

Although this “vastly more informative” molecular classification went far beyond histology, it was based on studies with

one common design flaw. Only a single, random sample of each tumor was analyzed, assuming that each GBM was a molecularly homogeneous entity. Subsequent studies have demonstrated the folly of this assumption in a tumor where *intratumoral* heterogeneity has proven definitional for nearly a century. Indeed, analysis of multiple spatially distinct fragments from single tumors has established *intratumoral* heterogeneity on multiple genomic levels. These fragments harbor multiple expression subtypes and evolve into unique subclones with divergent mutational drivers.⁶ Longitudinal sampling of recurrent tumors over time has revealed mutational profiles that drive subclonal tumor evolution in the presence and absence of therapy.⁷ Single cell sequencing has revealed that the extent of *intratumoral* heterogeneity may adversely impact survival, as bulk tumors harbor individual tumor cells with different expression subtypes and divergent oncogenic signaling, proliferation, and hypoxic signatures.⁸

Despite the insights provided by these studies, it remained unclear whether the diverse morphological features of GBM impacted its *intratumoral* genomic heterogeneity. To answer this question, Prabhu and colleagues interrogated a publicly available RNA sequencing (RNA-seq) dataset from the Ivy Foundation Glioblastoma Atlas Project (Ivy GAP, <http://glioblastoma.alleninstitute.org/>) whereby multiple regions of a single tumor were purified by laser capture microdissection. This comprehensive RNA-seq dataset presented the unique opportunity to examine the genomic profiles of 119 individually isolated morphological features from 37 different GBMs. Features identified using a semi-automated machine learning algorithm trained against ~12,000 hematoxylin and eosin stained images of GBM included:

1. Infiltrative tumor—leading edge (LE) and infiltrative tumor (IT) components where tumor cell density increased progressively from ~1%–3% to 10%–20%.
2. Cellular tumor (CT), where tumor cells outnumbered normal cells ~100–500-fold. CT was further subdivided into the morphological hallmarks of GBM:
 - a. microvascular proliferation (MVP) and hyperplastic blood vessels (HBV); and
 - b. pseudopalisading cells around necrosis (PAN) and the perinecrotic zone (PNZ).

After validating that vascular (MVP, HBV) and hypoxic (PAN, PNZ) regions were enriched for endothelial and hypoxic markers, respectively, the authors examined *intratumoral* heterogeneity by subtyping 90 nonvascular regions using the 4 gene expression signatures defined by TCGA.⁴ What they found was striking. Infiltrative tumor components (LE, IT) subtyped as proneural. Perinecrotic regions (PAN, PNZ) were almost exclusively mesenchymal. Central tumor (CT) regions were either classical or neural subtype. Importantly, similar structural components from different tumors clustered together, suggesting a striking lack of *intertumoral* genomic heterogeneity after accounting for *morphological intratumoral* heterogeneity.

Prabhu and colleagues then proposed a framework to define molecular events associated with each stage of morphological tumor progression. Their model assumed that continued proliferation of infiltrative tumor cells (IT/LE) results in a highly cellular tumor core (CT), parts of which ultimately become hypoxic, then necrotic (PAN/PNZ). They used gene enrichment analysis of IT/LE versus CT and CT versus PAN/PNZ regions to identify pathways important for each stage of tumor progression. The authors found cell cycle and growth factor signaling pathways critical for development of the cellular tumor component. Apoptosis inhibition, angiogenesis, immune modulation, and altered metabolism were important in perinecrotic tumor regions.

Their data suggest that *intratumoral* heterogeneity of GBM is conserved, largely attributable to its “multiforme” nature—the diverse morphological features that define its complex microenvironment.³ The authors propose that targeting these morphologically unique tumor “biomes” may lead to therapeutic gains that have so far eluded genomics-guided precision medicine efforts in glioma neuro-oncology.

Yet some questions remain. How diverse are the mutational profiles of individual tumor cells in the morphologically distinct tumor biomes? Do subclones arise within each? What is their subclonal architecture? Perhaps particular subclones are driven by programs that could allow for targeting their unique vulnerabilities. What genetic mechanisms do they utilize to effect the hallmarks of cancer?⁹ Temozolomide has been shown to act as an evolutionary stressor, leading to alternative pathways of escape and altering the phylogenetic branching of subclones in recurrent disease.⁷ Thus, how do surgery, radiation, cytotoxic and targeted chemotherapy, and immunotherapy influence the subclonal architecture within each biome? Do lower-grade gliomas that lack the morphological hallmarks of GBM also have unique tumor biomes?

Regardless of the answers to these questions, the work of Prabhu and others further emphasize a central tenet of contemporary neuro-oncology—in the era of molecular diagnostics and precision medicine, caution must be exercised when interpreting results of *genomic* biomarkers obtained from a single tumor fragment. Accurate molecular classification and therapeutic stratification of patients will likely require taking multiple samples from the same tumor to account for multiple forms of *intratumoral* heterogeneity.

Funding

C.R.M. is supported by the University Cancer Research Fund from the UNC Lineberger Comprehensive Cancer Center, the NIH National Center for Advancing Translational Sciences (550KR21202), and NCI (R01CA204136).

Conflict of interest statement. None.

References

1. Bailey P, Cushing H. *A Classification of the Tumors of the Glioma Subgroup on a Histogenetic Basis with a Correlated Study of Prognosis*. Philadelphia: J. B. Lippincott; 1926.
2. Louis DN, Ohgaki H, Wiestler OD, Cavenee WK, eds. *WHO Classification of Tumours of the Central Nervous System*. Revised 4th ed. Lyon: IARC; 2016.
3. Prabhu A, Kesarwani P, Kant S, Graham SF, Chinnaiyan P. Histologically defined intratumoral sequencing uncovers evolutionary cues into conserved molecular events driving gliomagenesis. *Neuro Oncol*. 2017;19(12):1599–1606.
4. Verhaak RG, Hoadley KA, Purdom E, et al.; Cancer Genome Atlas Research Network. Integrated genomic analysis identifies clinically relevant subtypes of glioblastoma characterized by abnormalities in PDGFRA, IDH1, EGFR, and NF1. *Cancer Cell*. 2010;17(1):98–110.
5. Brennan CW, Verhaak RG, McKenna A, et al.; TCGA Research Network. The somatic genomic landscape of glioblastoma. *Cell*. 2013;155(2):462–477.
6. Sottoriva A, Spiteri I, Piccirillo SG, et al. Intratumor heterogeneity in human glioblastoma reflects cancer evolutionary dynamics. *Proc Natl Acad Sci U S A*. 2013;110(10):4009–4014.
7. Johnson BE, Mazar T, Hong C, et al. Mutational analysis reveals the origin and therapy-driven evolution of recurrent glioma. *Science*. 2014;343(6167):189–193.
8. Patel AP, Tirosh I, Trombetta JJ, et al. Single-cell RNA-seq highlights intratumoral heterogeneity in primary glioblastoma. *Science*. 2014;344(6190):1396–1401.
9. Hanahan D, Weinberg RA. Hallmarks of cancer: the next generation. *Cell*. 2011;144(5):646–674.