Neuro-Oncology

Neuro-Oncology 18(8), 1037–1039, 2016
doi:10.1093/neuonc/now064
Advance Access date 28 May 2016

Longitudinal genomic characterization of brain tumors for identification of therapeutic vulnerabilities

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Tumor recurrence presents substantial clinical challenges and is a leading cause of cancer mortality. Comprehensive genomic profiling initiatives, including the coordinated efforts of The Cancer Genome Atlas (TCGA), have enabled the identification and cataloging of genomic alterations across the primary tumor of all major cancers; yet, these tumors, including the highly malignant brain tumor glioblastoma (GBM), are not static entities but instead display spatiotemporal evolution and dynamic heterogeneity. Meeting the challenges, consequently, demands expansion of cancer characterization initiatives towards longitudinal profiling that holds promise of (1) elucidating mechanisms of tumor evolution and (2) exposing therapeutic vulnerabilities.

Gliomas represent the majority of malignant brain tumors, and the most comprehensive multisector sampling study to date revealed that the most common and highest grade—GBM—presents extensive tumor heterogeneity that conceivably underlies treatment response variation.[1] While patient cohorts have thus far been limited to a few dozen, a number of recent longitudinal studies aimed at elucidating these phenomena have begun generating important initial insights (Table 1).

In an early report featured in *Science*, Johnson et al. presented data revealing driver gene evolution in glioma therapy resistance.[2] By analyzing the genomic landscape of low-grade gliomas (LGGs) and their matched recurrent tumors, the authors could infer a connection among temozolomide treatment, driver mutations in fundamental oncogenic signaling pathways including the retinoblastoma and Akt-mTOR pathways, and malignant progression. We observed a similar scenario in the recurrent GBM setting and discovered that genetic alteration of the p53 pathway is a fundamental molecular event predisposing GBMs to subclonal mutations at recurrence.[3] Our analysis also revealed heterogeneity in GBM evolution, with some recurrent tumors showing profound deviation from their primary disease, while others showed a linear evolutionary pattern. Back-to-back publications in *Cancer Cell* corroborated branched evolution in distal recurrence of GBM[4] (reminiscent of our deviant tumor pairs) and further revealed that epigenetic and genomic events converge during malignant progression.[5] Epigenetics are broadly implicated in cancer, and incriminating epigenetic events in malignant progression of glioma add another dimension to the model in which progression is largely driven by genetic alterations. A fifth report, by Bai et al., compared the genomic landscape of isocitrate dehydrogenase (IDH) mutant LGGs with corresponding progressed tumors.[6] The authors identified a number of specific convergent alterations including activation of the MYC and RTK-RAS-PI3K signaling pathways, upregulation of the FOXM1- and E2F2-mediated cell cycle transitions, alterations in cell cycle regulators including CDKN2A-CDKN2B, and epigenetic silencing of developmental transcription factors. Given the cohort size of 41 primary and recurrent sample pairs, of which 21 were without a matching germline sample, these findings require validation in a cohort sized to provide statistically sound results.

The presence of IDH mutations is the most important molecular marker for adult glioma classification.[7,8] In our recent multiplatform genomic analysis performed on adult diffuse glioma, we found that dissection of glioma based on the extent of genome-wide DNA methylation fully coincides with IDH mutation status and has important biological implications and clinical relevance.[9] We also observed that demethylation is an important marker for poor prognosis and a marker for disease progression in IDH-mutant glioma recurrence, perhaps in contrast with a recent analysis that suggested hypomethylating agents may be able to disrupt topological domain boundaries in glioma cells.[10] Increased understanding of the evolutionary dynamics of glioma may identify therapeutic vulnerabilities with the potential to provide meaningful improvement in patient outcome.

Longitudinal initiatives are gaining momentum for other (neuro-)oncological indications. Like glioma in adults, medulloblastoma is the most common malignant brain tumor in children, and recurrent medulloblastoma is nearly universally fatal.[11] In a recent research article published in *Nature*, Morrissey et al. presented evidence from 33 pairs of human

Received 17 March 2016; accepted 20 March 2016

Table 1. Overview of datasets analyzed in recent longitudinal glioma characterization studies

Citation	Exome	Transcriptome	DNA copy number	Methylome
Johnson BE, et al[2]	23	8	0	0
Kim H, et al[3]	23	22*	23	13*
Kim J, et al[4]	27 (22 w/germline)	30	16	0
Mazor T, et al[5]	27	13	0	19
Bai H, et al[6]	41 (20 w/germline)	28	21 (15 w/germline)	24

*Not discussed in publication.

diagnostic and posttherapy medulloblastomas suggesting that divergent clonal selection of a pre-existing minor clone facilitates tumor recurrence.[12] As in GBM, the authors recognized a tendency for p53 pathway alteration in a subset of recurrent medulloblastomas. Neuroblastoma is a pediatric neoplasm that typically arises extracranially. Few neuroblastoma patients survive upon recurrence, and recent independent studies of primary-relapsed neuroblastoma pairs published in *Nature Genetics* demonstrate that the neuroblastoma genome at diagnosis is different from that at relapse and that clonal selection occurs during this process.[13,14] Interestingly, despite small sample sizes, both studies identified recurrent events that converge on RAS-MAPK pathway activation at relapse. Furthermore, in a departure from that observed in gliomas, Schramm et al. found that global promoter methylation patterns were consistent over the course of disease,[13] which may indicate that the same epigenetic events do not appreciably influence disease recurrence in this patient subset.

Complex genomic heterogeneity likely accompanies branched evolution throughout tumorigenesis, whereby divergent clones emerge with distinct genotypes that confer unique selection advantages and facilitate therapy resistance. Small cohort assessment has revealed both important insight into the biology and effectively achieved proof of principle for expanded longitudinal analysis initiatives in neuro-oncology. While procuring and analyzing longitudinal samples involves a spectrum of logistical and scientific challenges in part due to patient mobility, comprehensive longitudinal assessment of tumor evolution based on cohorts powered to make statistically significant discoveries has the potential to elucidate the intricate convergent alterations and expose accompanying therapeutic vulnerabilities. The Glioma Longitudinal AnalySiS (GLASS) consortium (www.glass-consortium.org), comprising neuropathologists, clinicians, scientists, and bioinformaticians from leading institutions across the globe, was recently inaugurated to combine resources and accelerate the objective. Participation is based on availability of paired high-quality tumor samples and/or molecular characterization of such pairs. Additional sites are actively being recruited, and interested parties are encouraged to participate. The consortium was developed according to the principles of TCGA with a focus on data availability, open access, and collaborative team science.

GLASS is guided by prospective opportunities to both understand glioma tumor evolution and expose therapeutic vulnerabilities. Accordingly, GLASS intends to generate a longitudinal genomic/molecular dataset representing a large cohort of glioma patients across 3 specified diffuse glioma genomic subtypes: IDH-wild-type, IDH-mutant, and IDH-mutant 1p/19q co-deletion. Initial efforts are underway to combine both published and unpublished datasets. Moving forward, newly generated data will be added. While identification of first-line therapeutic vulnerabilities is fundamental, remaining mindful of potential anticipatory targets[15] that have the potential to yield meaningful clinical impact is no less a priority. Global collaboration is becoming increasingly important across research disciplines, and collaboration among researchers in the neuro-oncology community particularly has much to offer. We anticipate that GLASS initiatives have the potential for identification of practice-changing new insights and will provide a highly useful reference dataset to the glioma community.

Funding

This work was supported by the Oligo Research Fund from the National Brain Tumor Society.

Acknowledgment

The text is the sole product of the authors, and no third party had input or gave support to its writing.

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