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Doomed from the TERT? A two-stage model of tumorigenesis in IDH-wildtype glioblastoma.

Lucy. F. Stead[1¥] and Roel G.W Verhaak[2¥]

[1]Leeds Institute of Medical Research at St James's, University of Leeds, Leeds, LS9 7TF, UK.
[2]The Jackson Laboratory for Genomic Medicine, Farmington, CT, 06032, USA.
¥Correspondence to l.f.stead@leeds.ac.uk and roel.verhaak@jax.org

Using longitudinal molecular profiling, Körber et al. propose in this issue of Cancer Cell that IDH-wildtype glioblastomas initiate years pre-diagnosis with chromosome-level alterations that drives cell proliferation but require survival-promoting mutations, commonly in the TERT promoter, to form a detectable tumor. Multiple subclones drive disease progression, creating a therapeutic challenge.

Main Text

Glioblastoma (GBM) is the most common and most malignant adult brain cancer. Median survival is just 15 months for the 90% of patients with an IDH-wildtype GBM, which lacks mutations in both IDH1 and IDH2 that encode isocitrate dehydrogenases. This dismal prognosis is due to the infiltration of GBM cells into the surrounding brain parenchyma, making full eradication of the disease impossible despite aggressive standard treatment with surgery, radiotherapy and temozolomide (TMZ). Large-scale sequencing efforts like The Cancer Genome Atlas attempted to identify genomic features common to GBMs that may represent therapeutic vulnerabilities (Brennan et al., 2013). However, targeting these features has not yet resulted in prolonged patient survival. GBMs are marked by a large degree of genomic intratumor heterogeneity (ITH), which may affect treatment response by therapy-driven selection of treatment resist cells and promoting survival via subclonal cooperation (Amirouchene-Angelozzi et al., 2017). Analysis of paired pre- and post-therapy GBMs to characterize how treatment affects the tumor's evolutionary trajectory may reveal new clues on the process of clonal selection under therapy.

In this issue, Körber et al. present the results of characterizing pairs of primary and first recurrent IDH-wildtype GBM using whole-genome sequencing (WGS, n = 21), DNA methylation profiling (n = 50), transcriptome sequencing (n = 21) and targeted sequencing (n = 43) (Körber et al., 2019). At the core of their findings is the set of whole genome sequencing profiles, sequenced to a depth that enables subclonal deconvolution (median coverage 149x). The subtype of each GBM was assigned from its DNA methylation profile (Capper et al., 2018). Inspecting subtype assignment alongside the WGS and transcriptional

profiles confirmed prior observations that the mesenchymal (MES) subtype associates with low tumor purity and evidence of immune cell infiltration, masking the true tumor subtype (Wang et al., 2017). Discounting eight of 50 pairs where either GBM was classified MES, subtype was stable through therapy in 90% of cases.

Three recurrences and one primary and recurrent pair were hypermutated, with TMZ-treated cases carrying evidence of the mutational signature associated with TMZ exposure (Alexandrov et al., 2013). Barring these, paired tumors harbored comparable numbers of mutations, distributed fairly equally between shared events and those private to either. Clonal events must be shared between time points, whereas private mutations likely occurred after any major selection events with sufficient time before diagnosis or before disease recurrence for the tumor to have accumulated them. Körber et al. investigated whether any clonal events were seen repeatedly across patients and found at least one of three specific structural aberrations in 20 of the 21 subjected to WGS, nominating these as tumor-initiating alterations (**Fig. 1**). These include gain of chromosome 7, affecting EGFR; loss of all or some of chromosome 10, affecting PTEN, and loss of chromosome 9p, affecting CDKN2A/B. These were clonal in isolation or combination; 81% of cases had more than one. In contrast, point mutations within previously reported driver genes were repeated across fewer cases and were often subclonal. The only exception was point mutations in the TERT promoter (pTERT), which were found in 41 of the 42 tumors (all but one primary), suggesting their importance for gliomagenesis. However, in one third of cases the pTERT mutations were subclonal, suggesting that these are required for tumor growth but not present at tumor initiation (**Fig. 1**), in line with their role in overcoming the telomere attrition associated with cellular crisis (Barthel et al., 2018).

Körber et al. used mutation counts, mutation rate estimates, ranges of tumor size upon diagnosis and time between surgeries to model the timing of key events during gliomagenesis. Whilst this approach is parsimonious and affected by chosen parameters, it enabled the proposal of a two-stage evolution model whereby a founder cell acquires one or more of the aforementioned structural variants, triggering aberrant proliferation but with massive cell death with only 8-31% of daughter cells surviving. This is projected to occur two to seven years prior to first diagnosis with subsequent continued cell division but limited overall tumor growth until a pTERT or similar survival-promoting mutation is acquired, reducing cell death by 6-26%, allowing a detectable tumor to form. This provides great insight into the formation of a GBM and raises important questions about possibilities for earlier diagnosis. How a mutation conferring such a large selective advantage would not cause a selective sweep, resulting in a primary tumor with much less ITH than we observe,

requires further investigation. A possibility worth exploration is that pTERT, or ATRX, mutations have a non-autonomous driving effect, as observed from other common genomic events in GBM (Inda et al., 2010).

The large amount of subclonal variation in all tumors suggests that they evolved, most recently, via branched or neutral evolution. 12% of shared mutations were subclonal in both samples indicating a maintenance of genomic heterogeneity that is unlikely if treatment imposed a strong selective pressure (Davis et al., 2017). Körber et al. found that most (15/21) recurrent tumors were of oligoclonal origin i.e. retained multiple subclones from the primary GBM, independent of subtype or MGMT promoter methylation status (predictive TMZ response in GBM). The lack of evidence for oligoclonal origin in the six remaining recurrences is likely owing to limitations imposed by sampling. There were no genes repeatedly mutated privately across recurrences. Altogether this suggests a lack of common evolutionary bottleneck imposed by treatment (**Fig. 1**), traversal through which may have presented a therapeutic opportunity. This suggests several directions for future studies: 1) analysis of the specific subclones that survive treatment to understand whether oligoclonal recurrences result from treatment resistance mechanisms that co-occur within the primary; 2) unsupervised whole-genome analyses to identify genes driving treatment resistance as current drivers were mostly identified based on their prevalence in primary, treatment-naïve, tumors; 3) investigation of subclonal cooperation in treatment resistance; 4) investigation of non-genomic selection; despite DNA methylation profiles remaining stable through therapy, alternative epigenetic phenomena are yet to be inspected; and 5) unbiased characterization, using e.g. single-cell analyses, of the tumor microenvironment to explore the effect of immunosurveillance.

The authors present, for the first time, sufficiently deep whole-genome profiling of longitudinal gliomas to characterize evolutionary trajectories informed by non-exonic events e.g. in the TERT promoter. They show how multi-tumor analysis enables accurate determination of event clonality and tumor growth dynamics in ways that single tumor studies, prone to sampling error, cannot. Körber et al. pave the way for larger longitudinal molecular profiling studies to adequately address the important questions raised and hypotheses presented. Efforts to globally coordinate such studies, such as the Glioma Longitudinal Analysis Consortium (GLASS), are underway and, contrary to providing ‘more of the same’, aim to characterize the response of this deadly tumor to therapeutic assault in sufficiently powered cohorts and in sufficient detail that we can one day stop calling it incurable (Aldape et al., 2018).

References

Aldape, K., Amin, S. B., Ashley, D. M., Barnholtz-Sloan, J. S., Bates, A. J., Beroukhim, R., Bock, C., Brat, D. J., Claus, E. B., Costello, J. F*., et al.* (2018). Glioma Through the Looking GLASS: Molecular Evolution of Diffuse Gliomas and the Glioma Longitudinal AnalySiS Consortium. Neuro-Oncology, noy020-noy020.

Alexandrov, L. B., Nik-Zainal, S., Wedge, D. C., Aparicio, S. A. J. R., Behjati, S., Biankin, A. V., Bignell, G. R., Bolli, N., Borg, A., Børresen-Dale, A.-L*., et al.* (2013). Signatures of mutational processes in human cancer. Nature *500*, 415.

Amirouchene-Angelozzi, N., Swanton, C., and Bardelli, A. (2017). Tumor Evolution as a Therapeutic Target. Cancer discovery.

Barthel, F. P., Wesseling, P., and Verhaak, R. G. W. (2018). Reconstructing the molecular life history of gliomas. Acta neuropathologica *135*, 649-670.

Brennan, C. W., Verhaak, R. G., McKenna, A., Campos, B., Noushmehr, H., Salama, S. R., Zheng, S., Chakravarty, D., Sanborn, J. Z., Berman, S. H*., et al.* (2013). The somatic genomic landscape of glioblastoma. Cell *155*, 462-477.

Capper, D., Jones, D. T. W., Sill, M., Hovestadt, V., Schrimpf, D., Sturm, D., Koelsche, C., Sahm, F., Chavez, L., Reuss, D. E*., et al.* (2018). DNA methylation-based classification of central nervous system tumours. Nature *555*, 469-474.

Davis, A., Gao, R., and Navin, N. (2017). Tumor evolution: Linear, branching, neutral or punctuated? Biochimica et biophysica acta Reviews on cancer *1867*, 151-161.

Inda, M.-d.-M., Bonavia, R., Mukasa, A., Narita, Y., Sah, D. W. Y., Vandenberg, S., Brennan, C., Johns, T. G., Bachoo, R., Hadwiger, P*., et al.* (2010). Tumor heterogeneity is an active process maintained by a mutant EGFR-induced cytokine circuit in glioblastoma. Genes & Development *24*, 1731-1745.

Wang, Q., Hu, B., Hu, X., Kim, H., Squatrito, M., Scarpace, L., deCarvalho, A. C., Lyu, S., Li, P., Li, Y*., et al.* (2017). Tumor Evolution of Glioma-Intrinsic Gene Expression Subtypes Associates with Immunological Changes in the Microenvironment. Cancer Cell *32*, 42-56.e46.

Figure 1. The growth of an IDH-wildtype glioblastoma and the effect of standard treatment. Tumor initiation occurs several years before diagnosis with one of three key chromosome-level mutations, but a detectable GBM does not form until a survival-promoting mutation fuels tumor growth. Standard treatment of surgery, radiotherapy and temozolomide does not produce a clear evolutionary bottleneck causing it to slow progression rather than to provide curative effects. This is analogous to a plant in which the seed is sowed but does not become observable until the conditions are suitable to enable growth above the earth, at which point current therapeutic interventions act only to prune rather than to eradicate.