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Mutational landscape of glioblastoma under therapy

The mutational landscape of glioblastoma has emerged in recent years and includes frequently observed genomic alterations that are drivers of the tumor, including TP53, PTEN, EGFR, PIK3CA, ATRX, IDH1, PIK3R1, NF1, and PDGFRA. The authors of this study examined the clonal evolution of mutations by evaluating 293 whole exomes and 141 transcriptomes from tumor-matched normal samples in 114 patients with glioblastoma. They sequenced the whole exome triplets comprising the initial tumor, recurrence tumor, as well as normal genomic DNA for 93 patients and the transcriptomes and recurrence tumor pairs for 65 patients. The results of the study recapitulated the known driver mutations for glioblastoma. However, some interesting insights emerged. First, a subset of 17 patients, representing 17 percent of patients with temozolomide-treated glioblastoma, had tumors that were hypermutated (an approximately 100 times higher mutation rate than for nonhypermutated tumors); the hypermutated recurrence tumors were highly enriched for C>T (G>A) transitions; and 16 of the 17 hypermutated tumors had mutations in DNA mismatch-repair genes. Interestingly, *MGMT* promoter methylation was associated with hypermutation. None of the non-temozolomide-treated patients had hypermutated tumors. Furthermore, the gene LTBP4 was found to be significantly more often mutated in the recurrence-only tumor group. The recurrent glioblastoma tumors with wild-type IDH1 and high *LTBP4* expression were associated with worse prognosis. *LTBP4* is involved with regulating the TGF-β pathway and is an activator of TGF-β signaling. The activation of TGF-β is known to be a driver of aggressiveness in malignant gliomas. The association of high *LTBP4* expression with recurrence tumors and poor prognosis could yield potential therapeutic targets in the TGF-β pathway for glioblastoma. Finally, the study looked at tumor evolution in the presence of clonal heterogeneity. The analysis showed a highly branched evolutionary pattern. Based on mathematical modeling, the authors estimate that the relapse-associated clones had been present in patients for years before diagnosis. The median estimate was that divergent clones were present for more than 12 years before diagnosis for the subset of 46 patients whose disease fit the authors' mathematical model. Based on the overall comparison of mutations in untreated initial tumors and recurrence tumors and potential evolutionary trajectories, the authors observed that mutations in *IDH1, PIK3CA,* and *ATRX* are early events; mutations in *TP53, NF1,* and *PTEN* occur later; and mutations in mismatch repair genes (most notably *MSH6*) and LTBP4 most often develop in recurrence tumors only. This study provides additional insights into the role of mutations and pathways following treatment for glioblastoma and at relapse, as well as potential therapeutic options.

Wang J, Cazzato E, Ladewig E, et al. Clonal evolution of glioblastoma under therapy. *Nat Genet.* 2016;48(7):768–776.

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Molecular profiling for minimal residual disease in standard-risk AML

Treatment decisions for acute myelogenous leukemia, particularly with regard to allogenic stem cell transplantation, are guided by morphology-based assessment, cytogenetic analysis, and assessment of molecular

genetic markers. High-risk groups are associated with internal tandem duplications in the gene *FLT3* (*FLT3*-ITD), monosomy chromosome 5 or 7, and deletion of 5q. Low-risk groups are associated with t(8;21) and inv(16), as well as mutations in *NPM1* and *CEBPA*. Mutations in *NPM1* are the most common molecular finding in adults with standard-risk acute myelogenous leukemia (AML), occurring in 60 percent of patients with cytogenetically normal AML. The authors of this study showed that assessment of minimal residual disease in patients with AML and *NPM1* mutations can provide prognostic information independent of other risk factors. Minimal residual disease was assessed by reverse-transcriptase quantitative polymerase chain reaction (RT-qPCR) using a mutation-specific primer with a common primer and probe. The development phase of the study assessed 346 patients with *NPM1* mutations (median age, 50 years; range, 6–68 years) with follow-up bone marrow or peripheral blood samples (median, six samples per patient). Molecular remission was defined as absence of detectable *NPM1*-mutated transcripts on RT-qPCR in a bone marrow sample, while molecular relapse was defined as detection of increasing levels of *NPM1*-mutated transcripts in two successive samples in the absence of hematologic relapse. Persistence of *NPM1*-mutated transcripts in blood was present in 15 percent of patients after the second chemotherapy cycle and was associated with a greater risk of relapse after three years of follow-up than was an absence of such transcripts (82 versus 30 percent; hazard ratio, 4.8; 95 percent confidence interval, 2.95–7.8). These results were replicated in a second set of 91 patients with *NPM1* mutations. Using multivariate analysis, the presence of minimal residual disease, as measured by *NPM1* mutations in the peripheral blood of patients after the second chemotherapy cycle, was the only independent prognostic factor. Apart from refining risk stratification, assessment of *NPM1* mutations could be used to sequentially monitor minimal residual disease to identify impending relapse, a strategy that has been widely applied in acute promyelocytic leukemia. This study indicates that *NPM1* mutations are another mechanism for evaluating risk and may be a better prognostic indicator than other diagnostic molecular genetic markers. Monitoring minimal residual disease by quantitative measurement of *NPM1* mutations may help guide treatment decisions.

Ivey A, Hills RK, Simpson MA, et al; UK National Cancer Research Institute AML Working Group. Assessment of minimal residual disease in standard-risk AML. *N Engl J Med.* 2016;374(5):422–433.

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