Supplemental Content

Pooled genetic screens to identify vulnerabilities in TERT-promoter-mutant glioblastoma

Kevin J. Tu; [1,2,3] Connor E. Stewart; [1] Peter G. Hendrickson; [1] Joshua A. Regal; [1] So Young Kim; [4,5] David M Ashley; [5,6,7] Matthew S. Waitkus; [5,6,7] Zachary J. Reitman [1,5,6,7,8]

(1) Department of Radiation Oncology, Duke University School of Medicine, Durham, NC 27710
(2) Department of Cell Biology and Molecular Genetics, University of Maryland, College Park, MD 21044
(3) Department of Radiation Oncology, University of Maryland School of Medicine, Baltimore, MD 21201
(4) Department of Molecular Genetics and Microbiology, Duke University School of Medicine, Durham, NC 27710
(5) Duke Cancer Institute, Duke University School of Medicine, Durham, NC 27710
(6) Department of Neurosurgery, Duke University School of Medicine, Durham, NC 27710
(7) The Preston Robert Tisch Brain Tumor Center at Duke, Durham, NC 27710
(8) Department of Pathology, Duke University School of Medicine, Durham, NC 27710

Supplemental Note 1

We next examined whether Cas9 disruption of TERT occurred in an allele-specific manner because we hypothesized our negative result was due to the lack of selective pressure against disruption of unexpressed TERT alleles. TPMs are known to be expressed in a monoallelic fashion even in the situation of homozygous TPMs (20) such as the T98G line. To explore this possibility, we examined whether a single nucleotide polymorphism (SNP) near a TERT gRNA cut site was preferentially associated with damaging alterations caused by CRIPSR/Cas9, or preferentially selected after long-term passage. We observed SNP at rs28428579 in T98G (at CRCh38/hg38 chr5:1,279,511), which is 40 bp downstream of the TERT-2 gRNA target site in the *TERT* gene (**Supplementary Figure 1a**). T98G is heterozygous for rs28428579 with A/G each appearing in ~50% allele fraction in T98G-TERT-ON cells in the absence of TERT-2 gRNA disruption (**Supplementary Figure 1b**). In T98G-TERT-ON cells disrupted with the TERT-2 gRNA, approximately half of the disrupted alleles still contained the SNP, and this variant allele fraction did not change appreciably even after long-term passage (**Supplementary Figure 1c**). Similar results were found in the mini pooled experiments in the alleles disrupted by TERT-2 gRNA (**Supplementary Figure 1d,e**). This finding excludes the possibility that TERT gRNAs were preferentially targeting a certain allele of TERT, or that a specific allele of TERT underwent positive or negative selection over time after CRISPR/Cas9 disruption.

Supplemental Note 2

CRISPR/Cas9 gene disruption confirmation

PCR primers for GABPB1L and CTRL in green are described in (2). For TERT primers, we searched for the gRNA genomic location in UCSC genome browser. Primer3 was used to identify primer pairs to amplify the genomic location containing the gRNA location plus 30 bp 5' and 3' of that site. 100-300 bp amplicons were targeted. Illumina adapters were added (Illumina F, 5'- ACACTCTTTCCCTACACGACGCTCTTCCGATCT-3' and Illumina R, 5'-GACTGGAGTTCAGACGTGTGCTCTTCCGATCT-3'). PCR products were purified using a PCR purification kit (Qiagen), analyzed by agarose gel electrophoresis to confirm the appropriate size, and subjected to NGS (AmpliconEZ, Genewiz). Gene disruption was confirmed in samples from day 33 post-transduction of LentiCRISPRv2 unless otherwise specified. Wild type, in-frame deletion, and out-of-frame deletions were quantified in Microsoft Excel v16.67. Deletions of a number of base pairs evenly divisible by three were considered in-frame. A small proportion of missense alterations and other alterations consistent with PCR artifacts were removed from the quantification analysis. gRNA target sites were PCR-amplified using primers

in Supplemental Table 1. The proportion of in-frame, out-of-frame, or wild type NGS reads was quantified and summarized in bar graph format. For the pooled screen, barcodes for each gRNA were amplified using primers in Supplemental Table 1. the proportion of NGS reads containing each gRNA barcode was quantified and expressed as a proportion of total interpretable reads