LETTER TO THE EDITOR

Spontaneous loss of heterozygosity leading to homozygous R132H in a patient-derived IDH1 mutant cell line

Dear Editor,

We report a novel follow-up observation pertaining to “An in vivo patient-derived model of endogenous IDH1-mutant glioma,” which was recently published in Neuro-Oncology. Since publication, we have observed the gradual and repeated loss of the wild-type IDH1 allele in vitro with retention of the mutant allele. Sequencing of IDH1 exon 4 from 3 independent late passage cultures showed homozygosity for the R132H allele (mut/−), whereas both mutant and wild-type alleles were present in the original line (mut/wt) and tumor (Fig. 1A). In addition, a decreased copy number was seen at the IDH locus, consistent with loss of the IDH1 wild-type allele (Fig. 1B). The American Type Culture Collection (ATCC) has independently observed this phenomenon in BT142. The ATCC is preparing to distribute the BT142 mut/−, while we test conditions that best preserve the heterozygous phenotype.

The loss of the wild-type allele has been reported in vivo in patients and has been shown to be similar to phenotypically wild-type IDH, resulting in decreased 2-hydroxyglutarate production, also observed in the BT142 mut/− line. This unforeseen change leading to a second cell line will be valuable for comparisons of the implications of mutant and wild-type IDH phenotypes on proliferation, tumorigenicity, and therapeutic resistance in a syngeneic setting.

Methods

Sequencing for IDH1 was performed as previously described. The TaqMan Copy Number Assay (Applied Biosystems) was used to assess copy-number variations. Briefly, genomic DNA was extracted using the DNeasy kit (Qiagen) and quantified using UV absorbance (A260/A280 ratio > 1.7). The genomic DNA samples were diluted to 5 ng/μL in nuclease-free water; 20 ng of genomic DNA was mixed with the IDH1 TaqMan Copy Number Assay and the RNase P Reference Assay in a PCR plate, and quantitative real-time PCR was performed according to the manufacturer’s instructions. The manufacturer’s software, Copy Caller, was used for analysis.

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Fig. 1. Loss of heterozygosity in favor of the mutant allele of IDH1 correlates with a decreased copy number of the IDH1 locus. (a) IDH sequencing on the IDH1-mutant anaplastic oligoastrocytoma and derived IDHmt brain tumor stem cell line (BT142). (b) Copy number assay of the IDH1 locus on heterozygote and homozygote BT142.
References


2. American Type Culture Collection cat#ACS-1018.


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