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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*.[1] 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/-,[2] 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,[3,4] 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.[1] 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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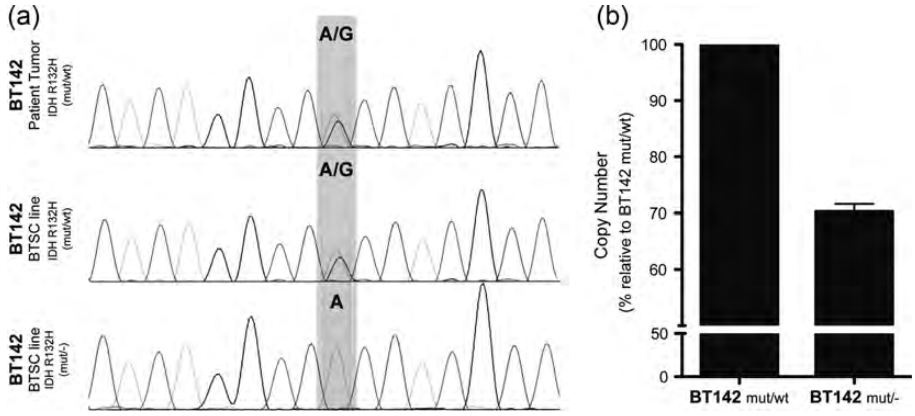

Fig. 1. Loss of heterozygosity in favor of the mutant allele of IDH1 correlates with a decreased copy number of the IDH1 locus. (a) IDH sequencing on the IDH1-mutant anaplastic oligoastrocytoma and derived IDHmt brain tumor stem cell line (BT142). (b) Copy number assay of the IDH1 locus on heterozygote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

References

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