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Letter to the Editor

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Long survival in a patient with fumarate hydratase mutation-associated glioma

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To the Editor:

Fumarate hydratase (FH) is a crucial enzyme in the tricarboxylic acid (TCA) cycle. In FH deficiency or "loss of function," elevated levels of fumarate can inhibit enzymes involved in DNA and histone demethylation. In particular, ten-eleven translocation (TET)-mediated demethylation is affected, leading to altered DNA methylation patterns and subsequent epigenetic modifications.[1] Elevated levels of the oncometabolite 2-hydroxyglutarate (2-HG) in isocytreat dehydrogenase (IDH) mutated gliomas exerts a similar inhibiting effect on the TET enzyme.[2]

Inactivation of the *FH* gene by genetic alterations on both alleles is well described in tumors arising in the setting of the autosomal dominant hereditary leiomyomatosis and renal cell carcinoma (HLCRCC) syndrome. In the realm of glioma research; however, *FH* mutations stand as a rare but intriguing phenomenon, with only 1 published case.[3]

Here we report the case of a male patient who was diagnosed at the age of 43 years (in 1999) with an intra-axial tumor in the right frontal lobe. The initial scan was performed because of a history of headache; the first epileptic seizures occurred 4 years later at the age of 48 years. At the age of 43 years, the patient underwent gross total tumor resection, which was further confirmed by MRI imaging 6 years later (Figure 1A, left panels). The initial histology revealed a diffuse glioma, with oligodendroglioma-like and astrocytoma-like components and calcifications. There was no mitotic activity and the proliferation index by Ki-67 was below 1% (Figure 1B, left panel). The tumor was diagnosed as "Oligoastrocytoma WHO Grade II" according to the 1993 WHO Classification of Tumors of the Central Nervous System. No further tumor-specific treatment was considered at that time point. Eight years later (at the age of 51 years) the MRI showed suspicion of a new nodular contrast-enhancing tumor at the former resection margin and a second neurosurgical gross total tumor resection was performed (Figure 1A, middle panels). Histologically, the mitotic activity (4 in 10 high-power fields), as well as the proliferation index of 7% were increased compared to the initial tumor (Figure 1B, middle panels). The patient received fractionated radiotherapy (30 times, 2 Gy per fraction). Four years later (at the age of 56 years) the tumor progressed with new contrast-enhancing lesions and the patient subsequently received temozolomide chemotherapy (8 cycles with 5 days treatment out of 28 days). At the time of further progression (patient aged 62 years) (Figure 1A, right panels), a biopsy was performed for a histological and molecular work-up of the current tumor. This analysis demonstrated a pleomorphic fibrillary tumor with few mitotic figures (3 in 10 high power fields, Figure 1B, right panels). The patient again received chemotherapy with temozolomide (8 cycles), and at the time of further recurrence (at the age of 67 years), chemotherapy with lomustine (4 cycles with 1 day treatment out of 42 days). The patient had stable disease at the last follow-up 24 years after the initial diagnosis.

Sanger DNA sequencing of the second recurrence showed wild-type IDH1 and IDH2 and no 1p/19q co-deletion. The

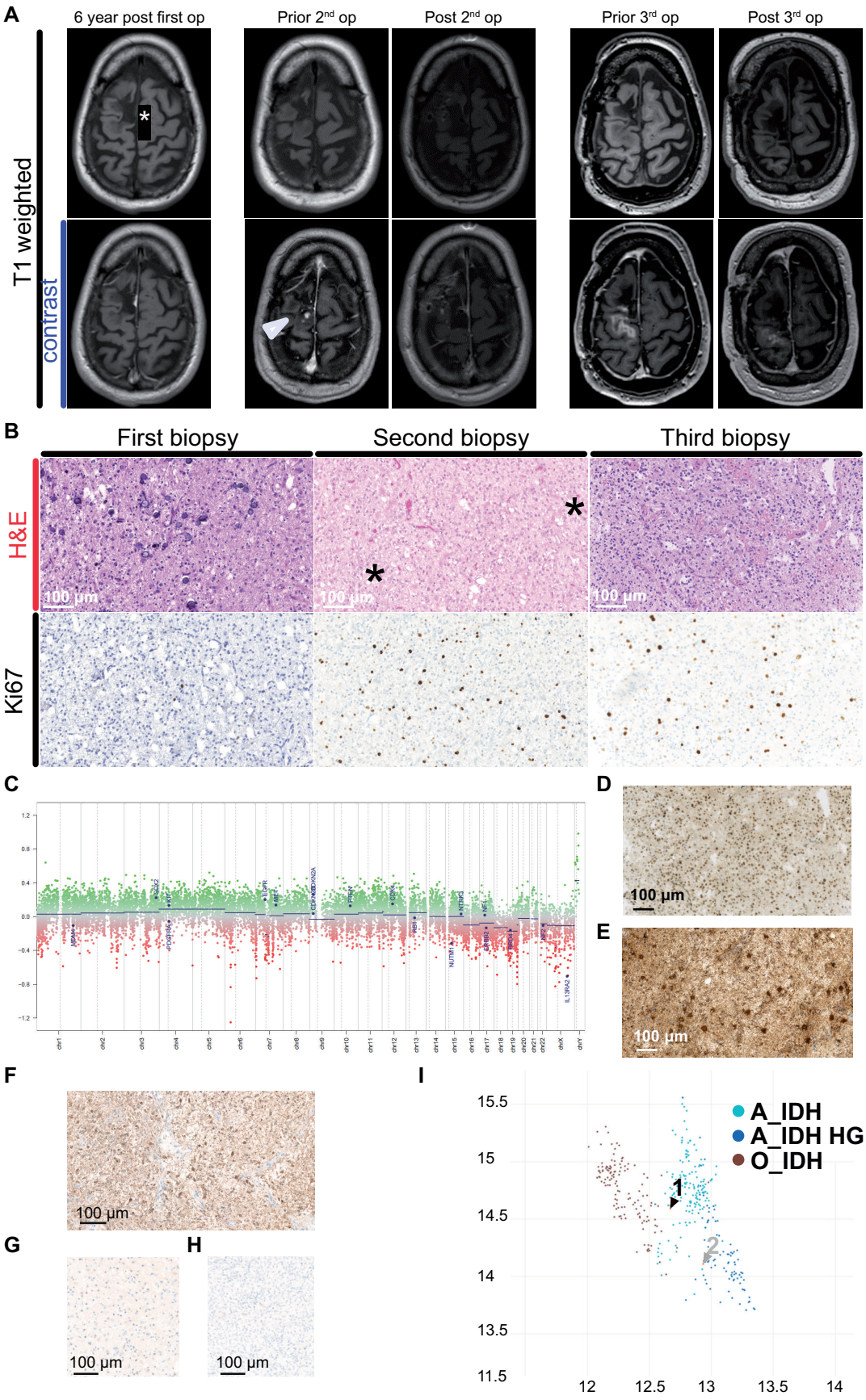

Figure 1. (**A**) T1 weighted (upper row) and contrast-enhanced T1 weighted (lower row) MRI over time. Left panels: first available MRI 6 years after the first operation. The white star indicates the resection cavity in the right superior frontal gyrus. Middle panels: MRI prior to and after the second operation. White arrowhead indicates nodular contrast-enhancement at the former resection margin in the right

DNA methylation classifier v11b4[4] showed the best match with "Glioma, IDH mutant" with a relatively low score of 0.59, subclass "astrocytoma" (score: 0.38). Re-analysis with version 12.8 demonstrated a match with "diffuse glioma IDH mutated" at a score of 0.74 and best match with the subclass "diffuse IDH mutant and 1p19q co-deleted" at a score of 0.58. The copy number plot is flat without major loss or gains (Figure 1C). Unsupervised methylation profile comparison with the dimension reduction technique 'uniform manifold approximation and projection' (UMAP) platting against a pan-cancer reference (REF PMID 38576030) demonstrated the highest similarity with lower-grade IDH-mutant astrocytomas.

We then performed further genetic analysis by FoundationOneCDx (Foundation Medicine, Roche) assay and, among others, identified 2 point mutations in the *FH* gene: p. H318Y with a frequency of 50.6% (tumor content around 50% by morphologic evaluation) and pG326E with an allele frequency of 21.7%, which could be due to a germline mutation and a "second hit." No family history of tumors was reported by the patient. The genetic alterations are summarized in Table 1. Interestingly, there is also a glutamic acid deletion c-terminal at position 2270 in the ATRX protein (allele frequency: 40.5%; variant of unclear significance), while immunohistochemically the ATRX expression was preserved (Figure 1D). Finally, we classified the tumor as "diffuse glioma NEC FHH mutated," as the tumor cannot be classified according to the current 2021 WHO Classification of Tumors of the Central Nervous System.

Immunohistochemical analysis of FH (clone J-13, Santa Cruz Biotechnology, Dallas, TX, United States) demonstrated partially preserved expression (Figure 1E). S-(2-succino)-cysteine (2SC), which is spontaneously generated from excesses of fumarate by succination, accumulated in the cytoplasm and some nuclei of neoplastic cells as shown by immunohistochemistry (crb2005017e, Cambridge Research Biochemicals, Billingham, United Kingdom) (Figure 1F) as a sign of FH-deficiency. In contrast, we observed no S2C accumulation in an "Astrocytoma IDH1 mutated WHO Grade 2" (Figure 1G) or in an "Oligodendroglioma IDH1 mutated and 1p19q-codeleted WHO Grade 2" (Figure 1H). These observations are in concert with the notion that p. H318Y is a loss of function mutation that renders human FH enzymatically inactive via defective oligomerization.[4] Of note, the p. G326E mutation of unknown significance is in close proximity and may have a similar effect.

In the published case from the Mayo Clinic, a p. M195V *FH* missense mutation was found, leading to a loss of FH expression.[3] There were up to 8 mitotic figures in a single high-power field. We mapped the methylation data of the 2 cases on a UMAP plot (REF PMID 38576030) and found both cases matching with astrocytoma IDH-mutant (Figure 1I) with the Mayo clinic case showing the highest similarity to higher-grade IDH-mutant astrocytomas. Indeed, the 2 cases were different regarding the histomorphological features and therefore grade.

The fact that both characterized FH-mutated glioma cases align in the DNA methylation classification analysis with IDH-mutated gliomas supports the hypothesis that the effect of fumarate accumulation on TET-mediated demethylation is similar to that of the accumulation of 2-HG. The case described here shows a long survival, but it remains to be seen whether FH-mutated gliomas generally show a more favorable course than IDH-mutated gliomas. While the historical case presented had a complete loss of FH expression, our case still retained some protein expression with the potential for a minimal enzymatic function. This may have an effect on the tumor behavior, which would need to be investigated in larger case series. The published Mayo Clinic case showed a bi-allelic FH alteration which is probably also present here, as we assume a germline plus second hit situation. Further cases will have to show whether both FH alleles are in general affected in this entity. This second report of an FH mutation-associated glioma with a long survival should encourage others to

Table 1. Complete gene alterations found by Foundation One analysis.

Gene	Protein change	Frequency	Interpretation
CDKN2A	p.Ile49Met	2.30	Pathogenic
FH	p.His318Tyr	50.64	Pathogenic
SF3B1	p.Arg1297His	35.76	Pathogenic
ASXL1	p.Gly646fs	21.28	Likely pathogenic
BCOR	p.Lys175fs	36.11	Likely pathogenic
NBN	p.Ser53fs	9.94	Likely pathogenic
ATRX	p.Glu2270del	40.53	VUS
ERCC4	p.Val178Leu	48.50	VUS
ESR1	p.Ala86Val	52.43	VUS
FH	p.Gly326Glu	21.70	VUS
GNAS	p.Gly297fs	47.99	VUS
MLL2	p.M5135_H5136delinsIY	5.18	VUS
MST1R	p.His277Gln	48.10	VUS
SETD2	p.Asp994Glu	49.34	VUS
SMARCA4	p.Lys689del	13.76	VUS

VUS, variant of uncertain significance.

Figure 1. Continued
superior frontal gyrus, suspicious for tumor recurrence. Third panel: MRI prior to and after the third operation with the postoperative scans showing less pathological contrast-enhancement dorsal to the resection margin. (**B**) Representative hematoxylin and eosin ([H&E], above) and Ki67 (below) micrographs over time. Left panels: initial biopsy with abundant microcalcification and relatively monomorphic neoplastic cells with round nuclei. The Ki-67 index is less than 1% of tumor cells. Middle panels: second biopsy 8 years after the first biopsy showing an increase of mitotic figures (stars) and higher proliferation rate. Right panels: third biopsy taken 19 years after the initial diagnosis. (**C**) A flat copy number profile is seen in the tumor tissue from the third biopsy. Profile generated with www.epidip.org. (**D**) ATRX expression is preserved in the tumor cell nuclei. (**E**) Relative preservation of fumarate hydratase (FH) in the third biopsy. (**F**) Accumulation of S-(2-succinyl) cysteine in the cytoplasm and nuclei of tumor cells in the third biopsy. (**G**, **H**) In contrast, S-(2-succinyl) cysteine is not seen in control cases of an astrocytoma IDH-mutant Grade 2 (G) or an oligodendroglioma IDH-mutant and 1p19q co-deleted (H). (**I**) UMAP plot generated with www.epidip.org displays both cases (1: local case in black, 2: Mayo Clinic case in grey), *x*/*y* = UMAP 0/1, arbitrary scale. A_IDH, lower grade IDH mutant astrocytoma; A_IDH_HG, higher grade astrocytoma; O_IDH, oligodendroglioma; IDH-mutant, 1p/19q-codeleted.

consider this entity in the context of an IDH-wt glioma that matches with the group astrocytoma IDH-mutant on methylation tumor classification.

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CONFLICTS OF INTEREST

None declared.

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