Letter

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Diffuse astrocytoma with mosaic *IDH1*-R132H-mutant immunophenotype and low subclonal allele frequency

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SUMMARY Molecular alterations found in gliomas are now considered entity-defining features. The World Health Organization (WHO) classification system currently classifies the vast majority of gliomas utilizing an integrated genotype-phenotype approach. We present a case of diffuse astrocytoma with a mosaic isocitrate dehydrogenase (IDH)1-R132H-mutant immunophenotype and low subclonal allele frequency. A 35-year-old patient with a history of *IDH1*-R132H mutated diffuse astrocytoma in 20014 presented to the hospital again in 2019. MRI examination showed a non-enhancing abnormal signal in the periphery of her previous surgical cavity. Histopathological examination revealed that the tumor was hypercellular and without high grade histopathological features. The neoplastic cells were immunohistologically positive for GFAP, Olig2, and ATRX. However, only some scattered tumor cells were positive for *IDH1*-R132H. Cytogenetic studies revealed a lack of chromosomal 1p/19q co-deletion. Further next-generation sequencing (NGS) demonstrated a low-level *IDH1*-R132H mutation and allele frequency. Based on these findings, the diagnosis of diffuse astrocytoma with mosaic *IDH1*-R132H-mutant immunophenotype and low subclonal allele frequency (WHO grade II) was generated. This case indicates that gliomas may have heterogeneous molecular profile and the intra-tumoral molecular heterogeneity highlights the need to further characterize the molecular profile for glioma classification and clinical management.

Keywords diffuse glioma, isocitrate dehydrogenase, *IDH1*, mosaic, intratumoral heterogeneity

Gliomas are the most common primary brain tumor in adults and, despite intensive treatment with surgery and chemoradiation, almost all gliomas relapse (*1,2*). With increasing evidence that molecular markers, such as isocitrate dehydrogenase (*IDH*) 1/2, are more informative than histologic subtype for prediction of tumor response to treatment and prognosis, an integrated genotype-phenotype approach was adopted for the latest World Health Organization (WHO) Classification (*3*). Nowadays, the pathological examination of glial tumors involves immunohistochemical (IHC), cytogenetic, and molecular studies. As a result, rare gliomas with intra-tumoral molecular heterogeneity were identified (*4-6*). We describe a case of recurrent diffuse astrocytoma (WHO grade II) with a mosaic *IDH1* R132H-mutant IHC staining pattern and low subclonal allele frequency to discuss the underlying causes and implications of molecular heterogeneity of gliomas.

A 35-year-old patient presented in March 2014 to the emergency room complaining of long-standing frontal headaches and new onset left-sided paresthesia which became generalized. A magnetic resonance imaging (MRI) examination at that time revealed a non-enhancing T2 signal and FLAIR abnormality within the left superior frontal lobe with no mass effect (Figure 1A). In June 2014, the patient underwent a craniotomy with resection of the tumor. In September 2019, the patient presented to the emergency room after multiple episodes of complex partial seizures. A MRI examination showed a non-enhancing abnormal signal in the periphery of her previous surgical cavity in the left frontal lobe (Figure 1B), consistent with recurrence of a low-grade tumor. The patient underwent a revision craniotomy with total resection of the recurrent tumor. The patient subsequently received radiotherapy and temozolomide with a brain MRI in March 2021 demonstrating no evidence of recurrent enhancement (data not shown). Informed consent was obtained from the patient and the study checked for ethics.

A needle biopsy of the initial tumor performed at an outside institution in 2014 revealed a WHO grade II fibrillary astrocytoma with *IDH1* mutation, and the

total resection specimen from June 2014 confirmed the diagnosis (data not shown). The total resection of the recurrent left frontal tumor in 2020 revealed that the tumor had increased cellularity of infiltrative atypical cells with moderate nuclear pleomorphism and inconspicuous to wispy eosinophilic cytoplasm. There was no necrosis, vascular endothelial hyperplasia, or mitoses identified. The neoplastic cells were diffusely, immunohistologically positive for GFAP, Olig2, and ATRX (Figure 2A-2D). Of note, only scattered tumor cells among other neoplastic cells were immunohistologically positive for the *IDH1*-R132H (Figure 2E and 2F). Ki67 labeling index was approximately 1% in the specimen (data not shown). Fluorescence in situ hybridization (FISH) studies indicated a lack of chromosomal 1p/19q co-deletion. A next generation sequencing (NGS) was performed on microdissected tumor tissue and demonstrated a low-level *IDH1*-R132H mutation (c.395G>A) with an allele frequency of 1.0%. Based on the above findings and the patient's clinical history, the diagnosis of recurrent/residual diffuse astrocytoma with mosaic *IDH1*-R132H-mutant immunophenotype and low subclonal allele frequency was rendered.

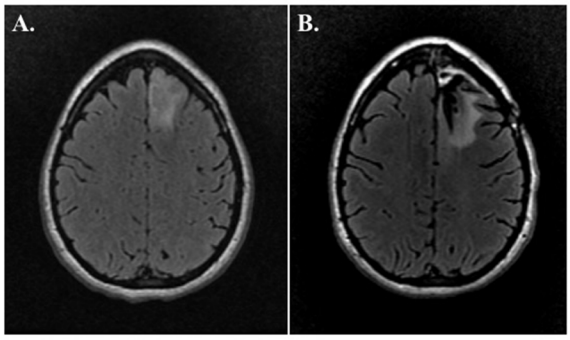

Figure 1. Radiologic images of initial and recurrent tumor. MRI-brain with contrast performed in 2014 **(A)** and 2019 **(B)** revealed a nonenhancing abnormal signal in the left frontal lobe, suggestive of the initial primary glioma and later recurrence, respectively (A, B, Axial FLAIR).

IDH mutation is closely associated with gliomas. *IDH1* encodes a protein located in the cytoplasm and peroxisomes that catalyzes the oxidative decarboxylation of isocitrate to α-ketoglutarate. The most common *IDH1* mutation found in approximately 90% of diffusely infiltrating gliomas is R132H, a missense mutation (c.395G>A) leading to a single amino acid substitution of arginine by histidine at codon 132 in exon 4 of the enzymatic active site (*6-7*). Mutant *IDH1* favors production of 2-hydroxyglutarate, an oncometabolite with multiple downstream effects found to promote tumorigenesis (*2,8*). *IDH2*, localized to the mitochondria, may be mutated at an analogous residue with R172K (c.515G>A) being the most common missense substitution. Oncogenic *IDH* mutations are believed to alter DNA and histone methylation and inhibit normal differentiation processes in gliomas (*2*).

Gliomas are a diverse group of brain tumors (*3,9*). They are among the most difficult cancers to treat, owing to their intra- and inter-tumoral heterogeneity and invasive nature, as well as the inherent challenge of central nervous system (CNS) pharmacokinetics and blood-brain barrier therapy penetration. First-line treatment is limited to a combination of maximally-allowed surgical resection, radiotherapy, and/or chemotherapy with few, if any, effective targeted therapies (*1,9-10*).

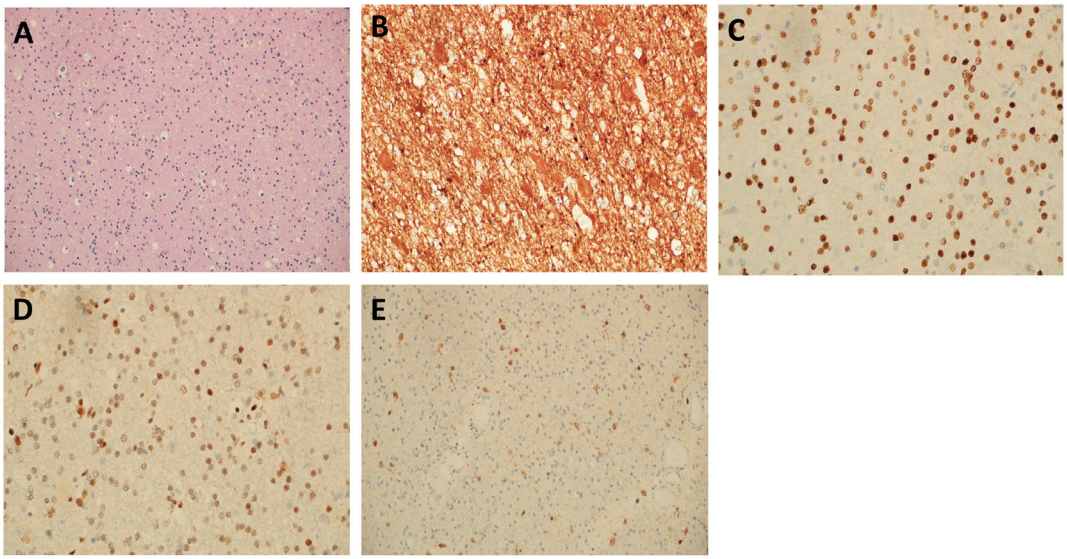

Figure 2. Microscopic findings of tumor recurrence. (A) Hypercellularity with nuclear atypia; no necrosis, vascular endothelial hyperplasia, or mitoses was identified (H&E, A: 100×). **(B, C, D)** The immunophenotype was positive for GFAP (B: 400×) and Olig2 (C: 200×) with retention of ATRX nuclear staining (D: 200×). **(E)** Mosaic staining was noted for the mutant IDH1-R132H epitope with nuclear and cytoplasmic positivity in a subset of scattered tumor cells (100×).

In general, cancer is associated with progressive genomic instability, and the interaction of acquired somatic mutations with environmental selection pressures drives tumor evolution and emergence of genetically distinct subclones (*10*). In particular, it has been found that gliomas undergo significant cellular and molecular evolution during disease progression. Resultant intratumoral heterogeneity such as in our case ultimately confounds diagnosis, creates challenges for the design of effective therapeutics, and acts as a determinant of resistance and recurrence (*1*). These warrant the need for a comprehensive molecular workup and classification of gliomas.

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References

1. Aldape K, Amin SB, Ashley DM, *et al*. Glioma through the looking GLASS: molecular evolution of diffuse gliomas and the glioma longitudinal analysis consortium. Neuro Oncol. 2018; 20:873-884.
2. Cohen AL, Holmen SL, Colman H. *IDH1* and *IDH2* mutations in gliomas. Curr Neurol Neurosci Rep. 2013; 13:345.
3. Louis DN, Perry A, Reifenberger G, von Deimling A, Figarella-Branger D, Cavenee WK, Ohgaki H, Wiestler OD, Kleihues P, Ellison DW. The 2016 World Health Organization Classification of Tumors of the Central Nervous System: a summary. Acta Neuropathol. 2016; 131:803-820.
4. Purkait S, Miller CA, Kumar A, Sharma V, Pathak P, Jha P, Sharma MC, Suri V, Suri A, Sharma BS, Fulton RS, Kale SS, Dahiya S, Sarkar C. ATRX in diffuse gliomas with its mosaic/heterogeneous expression in a subset. Brain Pathol. 2017; 27:138-145.
5. Lopez GY, Oberheim Bush NA, Phillips JJ, Bouffard JP, Moshel YA, Jaeckle K, Kleinschmidt-DeMasters BK, Rosenblum MK, Perry A, Solomon DA. Diffuse midline gliomas with subclonal H3F3A K27M mutation and mosaic H3.3 K27M mutant protein expression. Acta Neuropathol. 2017; 134:961-963.
6. Cai J, Zhu P, Zhang C, Li Q, Wang Z, Li G, Wang G, Yang P, Li J, Han B, Jiang C, Sun Y, Jiang T. Detection of ATRX and *IDH1*-R132H immunohistochemistry in the progression of 211 paired gliomas. Oncotarget. 2016; 7:16384-16395.
7. Pisapia DJ. The updated World Health Organization Glioma Classification: cellular and molecular origins of adult infiltrating gliomas. Arch Pathol Lab Med. 2017; 141:1633-1645.
8. Bruce-Brand C, Govender D. Gene of the month: *IDH1*. J Clin Pathol. 2020; 73:611-615.
9. Brat DJ, Verhaak RG, Aldape KD, *et al*. Comprehensive, integrative genomic analysis of diffuse lower-grade gliomas. N Engl J Med. 2015; 372:2481-2498.
10. Nicholson JG, Fine HA. Diffuse glioma heterogeneity and its therapeutic implications. Cancer Discov. 2021; 11:575-590.

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