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LETTER TO THE EDITOR

Beyond Midline: Diffuse Hemispheric Glioma, H3 K27M-Mutant with Aggressive Behavior

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To the Editor:

The most recent 2021 World Health Organization (WHO) classification of Tumours of the Central Nervous System introduced the term, "diffuse midline glioma, H3 K27-altered," as an "infiltrative midline glioma with loss of H3 p. K28me3 (H3 K27 trimethylation) and usually either an H3 c.83A>T p. K28M (K27M) substitution in one of the histone H3 isoforms, aberrant overexpression of EZHIP, or an *EGFR* mutation (CNS WHO grade 4)" (1). This modification came to underscore the fact that other changes can define this entity in addition to the already recognized H3 K27 mutations. However, a rare and interesting finding has come to our attention as yet another feature to be assessed when investigating a diffuse glioma, H3 K27-altered, that is the location of the lesion outside the midline. Herein, we illustrate a rare case of a diffuse non-midline glioma with H3-3A (H3F3A) K27M mutation and discuss the approach to this diagnosis using immunohistochemical and molecular analyses.

A 19-year-old man presented with some months of aphasia evolving to the headache that was increasing in frequency. Magnetic resonance imaging revealed an expansile and infiltrative lesion localized entirely in the left temporal lobe without communication with the midline (Fig. 1A). Partial resection of the lesion was performed. Histopathological analysis showed an infiltrative and highly cellular neoplasm composed of cells with relatively large and eosinophilic cytoplasm, round nuclei, prominent nucleoli, and perivascular lymphocytic infiltrate. The mitotic index was high, but neither necrosis nor microvascular proliferation was identified (Fig. 1B, C). The neoplastic cells revealed strong and diffuse GFAP (Fig. 1D) and p53 expression (Fig. 1E). Stain for IDH1 (R132H) mutant protein was negative and Ki-67 labeling index was estimated at 40%. Despite the negativity of the IDH, there was a loss of nuclear ATRX expression (Fig. 1F). Therefore, a preliminary diagnosis of high-grade glioma was rendered.

According to these findings, especially the loss of ATRX and diffuse p53 expression, there were some diagnostic possibilities that we had to explore. One possibility was IDH-mutant anaplastic astrocytoma with non-canonical IDH mutations. Another tumor that could have this association of results was high-grade glioma with H3-3A G34R/V mutation. Lastly, it could have been an epithelioid glioblastoma. However, this is more of a morphological rather than an integrated diagnosis. Next-generation sequencing was performed using a panel of 161 cancer-associated genes. The results demonstrated concomitant mutations in *H3-3A*, *PIK3CA*, and *TP53* genes (Table). Despite ATRX immunohistochemical loss of expression, no mutation was found in the panel employed. However, it is important to pinpoint that amplicon-based panels and the Ion S5 platform used can have limitations and lower sensibility to be detected in primer biding regions or in long homopolymer stretches.

To our surprise, the variant found in *H3*-3A was p. K28M (K27M), not the G34R/V mutation that is more associated with diffuse hemispheric glioma (1–3). Moreover, since the tumor was completely outside the midline, we opted to confirm this result through methylation profiling using the Heidelberg classifier (4). This analysis identified a high-calibrated score for the methylation class "diffuse midline glioma, H3K27M-mutant" (score of 0.99362). We retrospectively performed immunohistochemistry for H3 K27M and H3 K27me3, and, in fact, the glioma cells were positive for H3 K27M mutations (Fig. 1g) with an absence of H3 K27me3 expression (Fig. 1h), further supporting the mo-

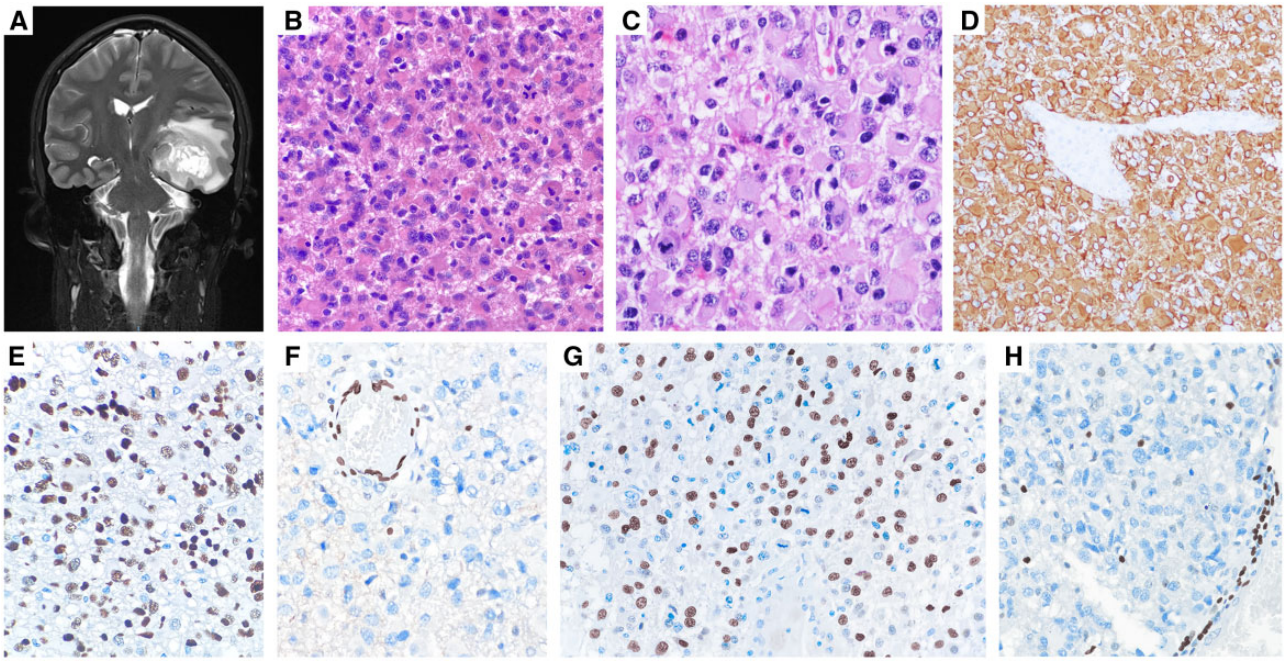

FIGURE 1. Radiologic and pathologic features of a diffuse non-midline glioma with histone H3 K27M mutation. **(A)** Coronal T2 FLAIR magnetic resonance image. **(B)** H&E-stained section of the tumor demonstrating a hypercellular glial neoplasm (×20). **(C)** The tumor cells have large and eosinophilic cytoplasm with peripheral round nuclei, vesicular chromatin, prominent nucleoli and mitotic figures (×40). **(D)** GFAP-positive immunostaining highlights the glial nature of the neoplasm. **(E)** Strong diffuse nuclear immunostaining for p53. **(F)** Loss of nuclear ATRX expression in neoplastic cells, with preserved staining in endothelial cells. **(G)** Nuclear staining for histone H3K27M mutant protein. **(H)** H3 K27me3 loss by immunohistochemistry is also evident.

TABLE. Genetic Alterations Identified in the Tumor by Next-Generation Sequencing

Gene	Exon	cDNA Change	Amino Acid Change	Variant Allele Frequency
H3-3A	2	c.83A>T	p.(Lys28Met)(p.K27M)	664/1748 (38%)
PIK3CA	8	c.1633G>A	p.(Glu545Lys) (p.E545K)	608/2534 (24%)
TP53	10	c.818G>A	p.(Arg273His) (p.R273H)	3650/4474 (82%)

lecular findings. How to report it? We could not call it "diffuse midline glioma" because it was not midline, so the integrated diagnosis was "high-grade glioma with H3K27M mutation (WHO CNS grade 4)." The patient had an unfavorable outcome and died 11 months later after an aggressive tumor recurrence.

Recent molecular studies have revolutionized our knowledge of pediatric high-grade gliomas (1). Since the discovery of somatic mutations of the *H3-3A* and *H3C2 (HIST1H3B)* genes in 2012 (5), some tumor entities have been identified to carry H3 K27M mutations, being the genetic hallmark of diffuse midline gliomas (6). However, non-midline diffuse gliomas with H3 K27M mutation have rarely been described (2, 7, 8). To date, the biology and prognosis for such tumors remain unknown and the current recommendation is to report them as "diffuse hemispheric glioma with H3 p. K28M (K27M) mutation, not elsewhere classified (NEC)" (1).

In conclusion, we propose that immunostaining for H3K27M protein should be taken into account in all pediatric high-grade diffuse gliomas, mainly with lost ATRX expression, regardless of location. This alternative approach should be outlined and discussed in larger series of patients in order to obtain the appropriate diagnosis and classification in such a challenging scenario.

COMPETING INTERESTS

The authors have no duality or conflicts of interest to declare.

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