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Diffuse midline gliomas with subclonal *H3F3A* K27M mutation and mosaic H3.3 K27M mutant protein expression

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Diffuse midline gliomas are aggressive tumors centered in midline structures of the brain that most commonly occur in children and young adults [6]. They are genetically defined by the presence of K27M mutation in either the *H3F3A* or *HIST1H3B* genes, which encode the histone H3 variants H3.3 and H3.1, respectively [1]. Given their poor prognosis, the 2016 WHO Classification includes “Diffuse midline glioma, H3 K27M-mutant” as a WHO grade IV entity, even in cases where only lower-grade histologic features are present [4]. In all cases reported to date, H3 K27M mutation has been a clonal alteration in all regions of tumors assessed by sequencing, and immunostaining with antibodies against H3 K27M mutant protein has revealed uniform expression in all tumor cells [2, 5, 6]. Thus, it has been

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Scanned image files of the entire slides from which representative images are presented in this report for both patients are available for downloading and viewing at the following link: https://figshare.com/projects/Diffuse_midline_gliomas_with_subclonal_H3F3A_K27M_mutation_and_mosaic_H3_3_K27M_mutant_protein_expression/25507

Competing interests

The authors declare that they have no competing interests related to this case report.

hypothesized that H3 K27M mutation is the earliest or initiating genetic alteration during gliomagenesis in these tumors. Here we describe two cases of diffuse midline glioma with subclonal *H3F3A* K27M mutation and mosaic expression of H3.3 K27M mutant protein, and discuss the implications with regards to classification and grading.

The first patient is a 30-year-old woman who presented with headaches and symptoms of increased intracranial pressure. Imaging demonstrated a heterogeneously enhancing mass centered in the right thalamus (Fig. 1a and Supplemental Fig. 1), and subtotal resection was performed. Sections demonstrated a highly cellular infiltrative astrocytic neoplasm with WHO grade IV histologic features including frequent mitoses, marked nuclear pleomorphism, palisading necrosis, and microvascular proliferation (Fig. 1c and Supplemental Fig. 2a–b). The tumor was negative for IDH1 R132H mutant protein immunostaining and showed somatic loss of ATRX expression diffusely in all tumor nuclei (Supplemental Fig. 2c). Immunohistochemistry for histone H3 K27M mutant protein revealed a mosaic staining pattern with expression seen in some but not all tumor cells (Fig. 1e and Supplemental Fig. 2d). While the majority of tumor cells lacked H3 K27M mutant protein expression, there were scattered large groups, small clusters, and admixed single cells that were positive. Immunohistochemistry for trimethylation of lysine-27 of histone H3 (H3K27me3) revealed an inverse staining pattern relative to H3 K27M mutant protein, with those areas of the tumor negative for H3 K27M mutant protein having robust H3K27me3 expression, and those areas positive for H3 K27M mutant protein having reduced to absent H3K27me3 expression (Supplemental Fig. 2e and f). Given this unusual mosaic pattern, next-generation sequencing was performed on the UCSF500 Cancer Panel as previously described [3]. This testing demonstrated a nonsense mutation in the chromatin remodeling gene *ATRX*, a frameshift mutation in the transcriptional corepressor gene *BCORL1*, a hotspot missense mutation in the receptor tyrosine kinase gene *FGFR1* located within the kinase domain, and *H3F3A* K27M mutation (Fig. 1g). Relative to the *ATRX*, *BCORL1*, and *FGFR1* mutations that were all at clonal allele frequencies suggesting that they were present in all tumor cells, the *H3F3A* K27M mutation was at a subclonal allele frequency. This confirms the immunohistochemical finding that the *H3F3A* K27M mutation was only present in a subset of the tumor cells, and suggests that either: 1) the mutation was acquired as a late event during tumor progression, or 2) the mutation was an initiating event in the tumor but was subsequently lost in a subset of cells during tumor progression. Chromosomal copy number assessment of this tumor revealed loss of distal chromosome 1q containing the *H3F3A* locus, resulting in only one copy of the *H3F3A* gene in most tumor cells (Supplemental Fig. 4). It cannot be reliably determined if this 1q loss occurred before or after acquisition of the *H3F3A* K27M mutation. One possibility is that *H3F3A* K27M mutation was an initiating event in the tumor and that subsequently during tumor progression there was loss of chromosome 1q containing the mutant *H3F3A* allele. Alternatively, chromosome 1q loss may have been an early event in the tumor, and then *H3F3A* K27M mutation occurred later during tumor progression. Additional copy number changes in the tumor included multiple other segmental gains and losses, as well as amplification on 12q15 containing the *MDM2* gene. Following subtotal resection, this patient with diffuse midline glioma with WHO grade IV histologic features and subclonal *H3F3A* K27M mutation declined additional therapy and died six months after diagnosis.

The second patient is a 69-year-old man who presented with progressively increasing confusion, word-finding difficulties, and changes in vision. Imaging revealed a heterogeneously enhancing expansile mass centered in the left thalamus (Fig. 1b and Supplemental Fig. 1), and a biopsy was performed. Sections demonstrated an infiltrative astrocytic neoplasm with 4 mitoses per 10 high power fields, but no discernible necrosis or microvascular proliferation (Fig. 1d and Supplemental Fig. 3a). The tumor was negative for IDH1 R132H mutant protein and had intact ATRX protein expression. Immunohistochemistry for histone H3 K27M mutant protein revealed a mosaic staining pattern with expression seen in some but not all tumor cells (Fig. 1f and Supplemental Fig. 3b). Given this unusual mosaic pattern, next-generation sequencing was performed on the UCSF500 Cancer Panel that demonstrated an *ATRX* missense mutation, *H3F3A* K27M mutation, a frameshift mutation in the *NF1* tumor suppressor gene, a frameshift mutation in exon 6 of *PPM1D*, and a hotspot missense mutation in *PTPN11* (Fig. 1g). Relative to the *ATRX*, *NF1*, *PPM1D*, and *PTPN11* mutations that were all at clonal allele frequencies, the *H3F3A* K27M mutation was at a subclonal allele frequency, suggesting that it was present in only a subset of tumor cells while the other mutations were present in most, if not all, tumor cells. Few chromosomal copy alterations were present in this tumor, and the tumor was diploid for chromosome 1q containing the *H3F3A* locus (Supplemental Fig. 4). This suggests that the most likely explanation for the subclonal *H3F3A* mutation in this tumor was acquisition during tumor progression. While the histologic features were consistent with an anaplastic astrocytoma (WHO grade III), there was uncertainty regarding the prognostic significance of the subclonal *H3F3A* K27M mutation and whether such a tumor qualifies for the entity “Diffuse midline glioma, H3 K27M-mutant” that the 2016 WHO Classification designates as grade IV. The patient, one of the oldest with an H3 K27M-mutant diffuse midline glioma reported to date, subsequently received radiation and chemotherapy with temozolomide but experienced rapid tumor progression and passed away six months after diagnosis.

In our collective experience with pathologic assessment of approximately 300 cases of diffuse midline gliomas, H3 K27M-mutant, these represent the only two such cases in which immunostaining has revealed unequivocal mosaic expression of H3 K27M mutant protein. While these results support the hypothesis that H3 K27M serves as an early driver mutation in most diffuse midline gliomas, the two cases presented here support that this mutation may also be selected for during tumor progression in some instances. Alternatively, in the first patient, we cannot exclude the possibility that the *H3F3A* K27M mutation was instead an initiating event in the tumor and was subsequently lost during tumor progression, a finding which has significant treatment implications for the ongoing clinical trials utilizing peptide vaccines against H3 K27M mutant protein (e.g. ClinicalTrials.gov ID NCT02960230). As the biologic consequences of a subclonal oncogenic mutation acquired during tumor progression are certainly distinct from a clonal mutation functioning as a tumor initiating genetic driver, caution is warranted with grouping such diffuse midline gliomas with subclonal H3 K27M mutation into the entity “Diffuse midline glioma, H3 K27M-mutant” defined as grade IV in the 2016 WHO Classification. However, in this series of two cases, both had high-grade histologic features and aggressive biologic behavior.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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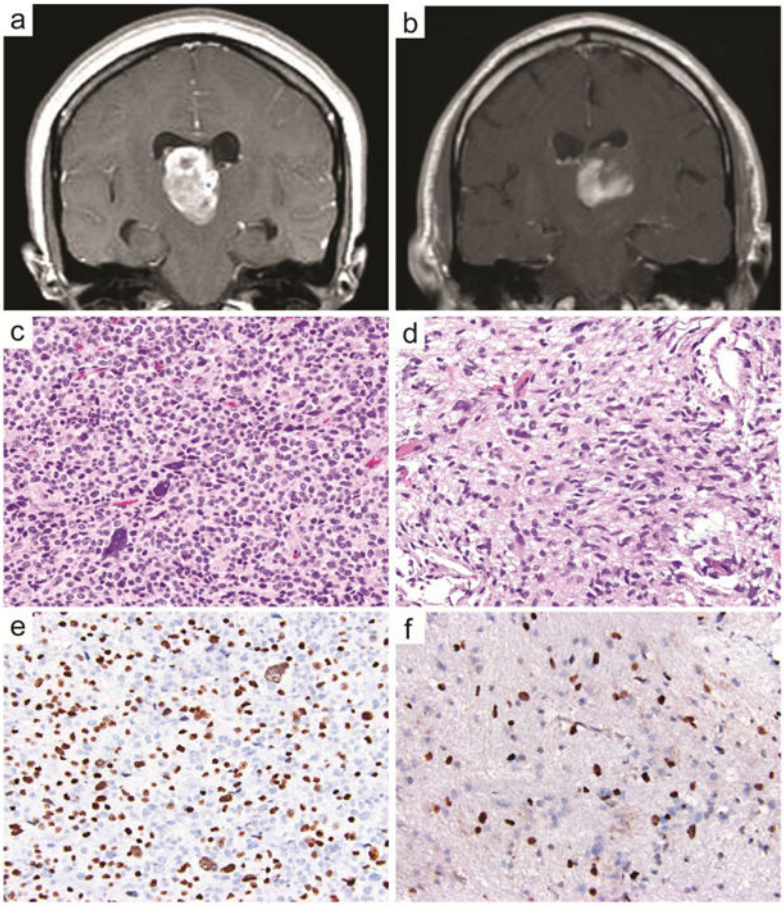

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	Gene	Variant	Reference transcript	Classification	Variant allele frequency	Predicted clonality
Patient #1	ATRX	p.R808*	NM_000489	Pathogenic	52%	Heterozygous
	BCORL1	p.G767fs	NM_021946	Pathogenic	80%	Hemizygous
	FGFR1	p.N546K	NM_023110	Pathogenic	93%	Hemizygous
	H3F3A	p.K27M	NM_002107	Pathogenic	8%	Subclonal
	MDM2	amplification	all	Pathogenic	N/A	ND
Patient #2	ATRX	p.N1753S	NM_000489	Likely pathogenic	29%	Hemizygous
	H3F3A	p.K27M	NM_002107	Pathogenic	3%	Subclonal
	NF1	p.V1971fs	NM_001042492	Pathogenic	28%	Homozygous
	PPM1D	p.N448fs	NM_003620	Pathogenic	15%	Heterozygous
	PTPN11	p.D61Y	NM_002834	Pathogenic	11%	Heterozygous

Fig. 1.

Radiographic, histologic, and genetic features of the two diffuse midline gliomas with subclonal *H3F3A* K27M mutation and mosaic H3.3 K27M mutant protein expression (first patient on the left, second patient on the right). **a,b** Coronal T1 post-contrast magnetic resonance imaging showing heterogeneously enhancing expansile masses centered in the thalamus. **c,d** H&E stained sections showing infiltrative astrocytic neoplasms with high grade histologic features. **e,f** Immunohistochemistry for histone H3 K27M mutant protein revealing mosaic expression. **g** Genetic alterations identified by next-generation sequencing that include subclonal *H3F3A* K27M mutation. N/A, not applicable. ND, cannot be determined.