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CORRESPONDENCE

Evidence of H3 K27M mutations in posterior fossa ependymomas

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Histone 3 (H3) K27M mutations are considered to be a genetic hallmark of diffuse midline gliomas, including high-grade astrocytomas and diffuse intrinsic pontine gliomas (DIPG) [3]. Similar to IDH-mutated gliomas in adults, these mutations are associated with alterations in the epigenetic profile of tumor cells, and are thought to represent a main driving factor in gliomagenesis [2, 8]. In diffuse midline gliomas, H3K27M mutations have been demonstrated to induce de–repression of pro-oncogenic transcription factors by global reduction of histone 3 K27 trimethylation (H3K27me3) [2, 8]. Reportedly, H3K27M mutations are exceedingly rare in tumors other than in diffuse midline gliomas [6, 7, 14]. The possibility of an H3K27M mutation occurring in other brain neoplasms cannot, however, be excluded a priori. We report here the very unexpected finding of H3K27M mutations in two Group A posterior fossa ependymomas (PF-EPN-A), an aggressive subgroup of tumors with relatively stable genomes and no well-characterized oncogenic driving event [9, 10].

The first patient was a 1.5 year-old female. MRI scans revealed a large tumor in the posterior fossa, highly suggestive of an ependymoma (Suppl. Fig. 1a–c). To avoid the high risk of severe morbidity associated with gross total resection (GTR), a biopsy was performed. Neuropathological analysis showed an anaplastic ependymoma (WHO grade III) (Fig. 1a–d). Next-generation sequencing (NGS) of a custom gene panel [12] from the tumor tissue performed within the German Molecular Neuropathology 2.0 study (http://pediatric-neurooncology.dkfz.de/index.php/en/diagnostics/molecular-neuropathology) detected an *H3F3A* K27M mutation (not shown). The presence

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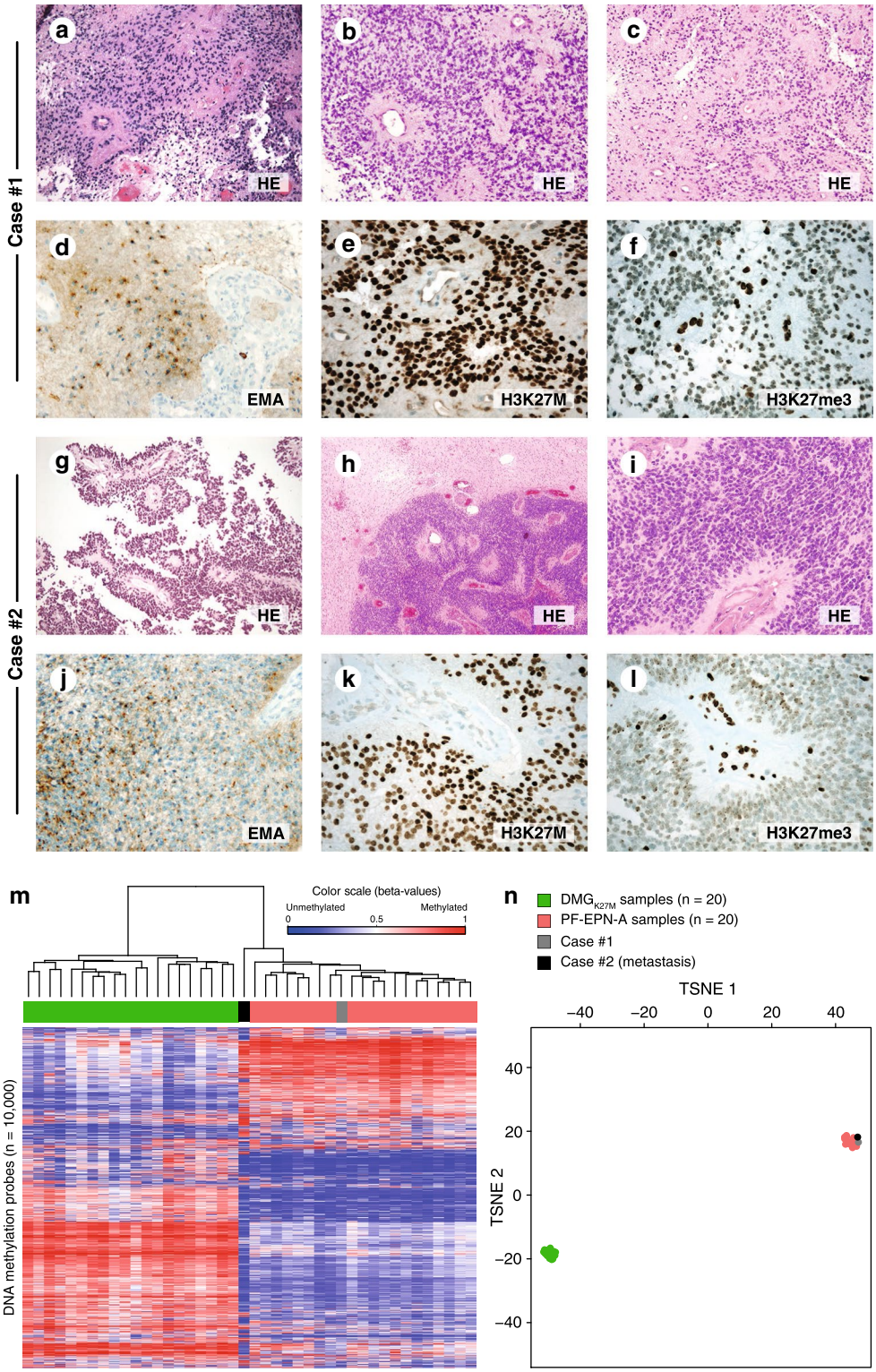

Fig. 1 Neuropathological and molecular features of *H3F3A* K27M- and *HIST1H3C* K27M-mutated ependymomas. The tumor of case #1 displayed classic ependymoma histology (**a**–**c**). EMA immunohistochemistry demonstrated a typical dot like pattern (**d**). Due to the presence of brisk mitotic activity, the tumor was classified as an anaplastic ependymoma (WHO grade III). The IHC analysis for K27M-mutated H3 (Millipore, Darmstadt, Germany) was positive (**e**) and H3K27me3 expression (Cell signaling, Danvers, USA) was reduced (**f**). The primary tumor of case #2 displayed as an anaplastic ependymoma (WHO grade III) with focal papillary features (**g**–**i**) and also demonstrated a typical dot like EMA pattern (**j**). By IHC, the tumor displayed strong nuclear immunoreactivity for K27M–mutated H3 protein and lack of H3K27me3 expression (**k**, **l**, respectively). DNA methylation patterns of H3K27M-mutated ependymomas were indistinguishable of those of PF-EPN-A and did not resemble those of H3K27M-mutated diffuse midline gliomas (DMG_{K27M}) by unsupervised hierarchical clustering analysis (**m**) and t-Distributed Stochastic Neighbor Embedding (t-SNE) (**n**) using the 10,000 most variable DNA methylation probes from the Illumina Infinium HumanMethylation450 BeadChip (Illumina, San Diego, USA)

of this mutation was confirmed by immunohistochemistry (IHC), by which the tumor homogeneously displayed strong nuclear immunoreactivity for K27M-mutated H3 protein and a strong reduction of H3K27me3 (Fig. 1e, f). The patient received chemotherapy according to the E-HIT 2000-R protocol, consisting of alternating cyclophosphamide/vincristine and carboplatinum/etoposide. After two cycles and radiological improvement, re-surgery was still not considered possible and a third cycle was administered. At last follow-up 6 months after diagnosis,

the patient was in partial remission and still improving. At that time, re-evaluation of re-surgery was scheduled, followed by local radiotherapy.

The second patient was a six year-old female, presenting with a tumor in the brain stem with infiltration of the 4th ventricle (Suppl. Fig. 1d, e). Histological examination revealed an anaplastic ependymoma (WHO grade III) (Fig. 1g–j). After GTR and chemo-/radiotherapy according to the E-HIT 2000-AB4 protocol, an isolated intraspinal metastasis (Th 11–12) was detected 19 months after initial diagnosis (Suppl. Fig. 1f). It was subtotally resected after three blocks of temozolomide according to the E-HIT-REZ-2005 protocol. NGS analysis performed on the metastasis as part of the INFORM study [13] revealed a *HIST1H3C* K27M mutation (not shown). IHC analysis of the primary tumor homogeneously showed nuclear expression of K27M-mutated H3 protein and loss of H3K27me3 expression (Fig. 1k, l). After craniospinal radiotherapy (total dose: 35.2 Gy) with a boost to the spinal tumor bed (total dose: 53.2 Gy), a second intraspinal lesion (L 2) was detected 24 months after the first relapse (43 months after initial diagnosis). After slight progression over 3 months it was completely resected. At last follow-up, the patient was in complete remission 49 months after initial diagnosis.

In both cases, genome-wide DNA methylation patterns closely resembled those of PF-EPN-A, with no similarity to those of K27M-mutated diffuse midline gliomas (or any other molecular brain tumor class) (Fig. 1m, n).

This is the first report of H3K27M mutations in ependymoma. No mutations were found in 224 cases of ependymoma included in published series with analysis of H3K27 mutational status [1, 4, 5, 9, 11]. Although such mutations likely represent a very rare event in these neoplasms, assumptions about the incidence of H3K27M in ependymoma may be biased by the fact that neither H3K27M IHC nor mutational analysis are usually required in standard neuropathological diagnostics of ependymomas.

Biologically, the possible functional effect of H3K27M in PF-EPN-A is difficult to interpret. The increased DNA methylation reported in PF-EPN-A is rather thought to depend on over-activity of the PRC2 complex [9], while the H3K27M mutation has been shown to interfere with PRC2 functions and hinder its activity [2, 8].

Because H3K27M mutations have rarely been reported in other brain tumor entities (the exception being a small number of low-grade gliomas and anaplastic gangliogliomas [6, 7, 14]), its determination has become a reliable tool for the identification of diffuse midline gliomas. Along with molecular methods, specific antibodies [1] able to recognize the mutated protein are now widely used in neuropathology laboratories. Although the presence of H3K27M mutation as detected by IHC or sequencing remains a strong argument for the diagnosis of a diffuse midline glioma, the cases reported here suggest prudence in the interpretation of a positive result, especially in the absence of congruent histology, immunohistochemistry and/or clinical data.

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