Diffuse intrinsic pontine glioma-like tumor with EZHIP expression and molecular features of PFA ependymoma

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Diffuse brainstem gliomas, historically termed diffuse intrinsic pontine glioma (DIPG), account for approximately 75% of pediatric brainstem tumors and have a particularly poor prognosis with a median survival of only 10 months [8, 10]. Until recently, the diagnosis of DIPG was principally made by imaging, with biopsy relegated to an ancillary role owing to the delicate anatomic location [1]. However, with improved surgical techniques [4, 14] and the discovery of canonical histone H3 lysine-27-methionine (H3K27M) driver mutations, direct examination of these lesions to distinguish DIPG from radiologic mimics has reemerged as an important component of the diagnostic process [17, 19].

H3K27M mutations in DIPG result in global loss of the repressive H3K27 trimethylation (H3K27me3) through multiple mechanisms including inhibition of PRC2 methyltransferase activity and spread of H3K27me3 [5, 9, 18]. Global reduction in H3K27me3 is also observed in a subset of childhood posterior fossa (PF) ependymomas termed PF-group A ependymomas (PFA) [2, 12]. PFAs show overexpression of EZH inhibitory protein (EZHIP) in most cases, or harbor mutations in EZHIP in ~10% of tumors [11]. Additionally, three independent groups have demonstrated that EZHIP mimics the H3K27M “oncohistone” to cause global H3K27me3 reduction [6, 7, 13]. The genomic distribution of H3K27me3 in H3K27M DIPGS and PFAs show remarkable similarities suggesting that these two tumors may be epigenetically related and share similar pathogenic mechanisms [2, 7]. Indeed, in support of this hypothesis, ~4% of PFAs demonstrate H3K27M mutations that are mutually exclusive from EZHIP mutations [11].

Here, we present an unusual case of a brainstem tumor with diagnostic radiographic and characteristic histopathologic features of DIPG, but demonstrating methylation features of PFA ependymoma. A 5-year-old boy presented to a local hospital with new-onset headache. Exam revealed evidence of cranial nerve deficits and cerebellar dysfunction (dysconjugate gaze and difficulty with tandem walking). MRI disclosed an infiltrating, expansile mass centered within the pons and obstructive hydrocephalus (Fig. 1a). The mass encased the basilar artery and contained a ventral exophytic component within the prepontine and suprasellar cisterns (Fig. 1b). Imaging characteristics included hyperintensity on T2 and fluid attenuated inversion recovery (FLAIR) sequences (Fig. 1c-d) and absence of enhancement following gadolinium administration (Fig. 1e). The mass did not involve the fourth ventricle or the cerebellum. Cystic change was noted. Other atypical features for DIPG, such as circumscription or dorsally exophytic growth, were not seen. The findings met clinicoradiologic criteria for DIPG [6]. Biopsy of the mass was deferred and radiation therapy was commenced.

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The patient was then evaluated at our institution for clinical eligibility in a trial for DIPG that prompted a tissue diagnosis. Biopsy revealed small fragments of densely cellular tumor (Fig. 2a). Scattered entrapped neurons hinted at its infiltrative nature (Supplemental Figure 1a-b). Tumor cells were positive for GFAP and neurofilament highlighted variable patterns of infiltration (Fig. 2b, Supplemental Figure 1c), with involvement of seemingly normal brain parenchyma by single tumor cells (Supplemental Figure 1e-f). No necrosis, microvascular proliferation, true ependymal or perivascular pseudorosettes were noted. Staining for H3K27M mutant protein was negative (Fig. 2c). Initial histologic and immunophenotypic findings suggested an H3-wildtype infiltrating astrocytoma consistent with DIPG. At our institution, pediatric CNS tumors frequently undergo integrative sequencing through the Michigan Oncology Sequencing Project (MI-ONCOSEQ) [16]. Tumors are assayed using whole exome and transcriptome-based techniques (see [15] for a description of the project). Sequencing revealed relatively few genomic alterations (Supplemental Table 1), but was notable for 1q gain (Fig. 2e) and confirmation of its H3-wildtype (H3F3A, HIST1H3B/C, HIST2H3A) status. RNA-seq showed overexpression of EZHIP mRNA that was confirmed by immunohistochemistry (Fig. 2f, Supplemental Figure 1d). Subsequent immunohistochemistry for H3K27me3 and Olig2 showed complete loss of nuclear expression in tumor cells (Fig. 2d, f). The relatively few genomic alterations and EZHIP overexpression in conjunction with loss of H3K27me3 in the absence of H3 mutations did not fit with classic molecular features of a H3-wildtype DIPG and prompted us to perform methylation analyses. Array-based profiling of CpG methylation in brain tumors has recently been shown to result in diagnostic refinements that are highly robust and prognostically meaningful [3]. We profiled our tumor using the Infinium MethylationEPIC BeadChip (interrogating
Fig. 2 (See legend on next page.)
~850,000 CpG sites) in conjunction with the DKFZ Classifier tool recently implemented for CNS tumors (http://www.molecularneuropathology.org) [3]. While the methylation class most closely matched ‘ependymoma, posterior fossa group A’, the calibrated Classifier score was 0.62, below the proposed threshold of 0.9 (potential reasons are discussed below). To further assess the methylation profile of this tumor in relation to other CNS entities, we performed unsupervised clustering on the DKFZ cohort that comprises the 82 tumor methylation classes used in the Classifier (v11b4). Reproduction of the unsupervised clustering (t-SNE) demonstrated that the tumor clusters with the group ‘EPN, PF A’ (Fig. 2g). We next evaluated the tumor in relation to recently defined nine subtypes of PFA ependymoma [11]. Hierarchical clustering analysis revealed clustering within the PFA-1c subtype (Fig. 2h) and this was concordant with the results of t-SNE (Fig. 2i). While the overall Classifier score was 0.62, this may be due to the composition of PFA ependymomas in the current version of the Classifier (v11b4). The ‘EPN, PF A’ tumor class contains tumors arising mostly within the fourth ventricle and/or cerebellum. Thus, the low calibrated score we encountered may reflect a potential subgroup of PFA ependymomas not yet recognized in the current implementation of the Classifier.

In summary, we present an unusual childhood brain tumor arising within the pons that met all clinical criteria for a DIPG but unexpectedly demonstrated H3K27me3 global reduction between H3K27M DIPGs and PFA ependymomas [2, 7], and have biologic implications from both a neurodevelopmental perspective and in the design of targeted epigenetic therapies.

**Supplementary information**

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**Authors’ contributions**
DP conceptualized the project, drafted the manuscript, performed the bioinformatic analyses, and designed the figures. MQ, ZA, and KA performed and interpreted the methylation analysis. DH, FY and ARJ performed and interpreted the histopathologic review, conceptualized and supervised the project, and wrote the manuscript. The authors read and approved the final manuscript.

**Consent for publication**
Obtained.

**Competing interests**
The authors declare that they have no competing interests.

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endogenous inducible gene that antagonizes H3K27 methylation activity of Polycomb repressive complex 2 via an H3K27M-like mechanism. Sci Adv eaax2887. https://doi.org/10.1126/sciadv.aax2887

14. Puget S, Becarra K, Blauwblomme T, Roujeau T, James S, Grill J, Zerah M, Varlet P, Sainte-Rose C (2015) Biopsy in a series of 130 pediatric diffuse intrinsic Pontine gliomas. Childs Nerv Syst 31:1773–1780. https://doi.org/10.1007/s00381-015-2832-1

15. Robinson DR, Wu YM, Lonigro RJ, Vats P, Cobain E, Everett J, Cao X, Rabbai E, Kumar-Sinha C, Raymond V et al (2017) Integrative clinical genomics of metastatic cancer. Nature 548:297–303. https://doi.org/10.1038/nature23306

16. Roychowdhury S, Iyer MK, Robinson DR, Lonigro RJ, Wu YM, Cao X, Kalpana-Sundaram S, Sam L, Balbin OA, Quist MJ et al (2011) Personalized oncology through integrative high-throughput sequencing: a pilot study. Sci Transl Med 3: 111ra121. https://doi.org/10.1126/scitranslmed.3003161

17. Schwartzentruber J, Korshunov A, Liu XY, Jones DT, Pfaff E, Jacob K, Sturm D, Fontebasso AM, Quist DA, Tonjes M et al (2012) Driver mutations in histone H3.3 and chromatin remodelling genes in paediatric glioblastoma. Nature 482:226–231. https://doi.org/10.1038/nature10833

18. Stafford JM, Lee CH, Voigt P, Descostes N, Saldana-Meier R, Yu JR, Leroy G, Okouz O, Chapman JR, Suarez FE et al (2018) Multiple modes of PRC2 inhibition elicit global chromatin alterations in H3K27M pediatric glioma. Sci Adv 4: eaau5935. https://doi.org/10.1126/sciadv.aau5935

19. Wu G, Broniscer A, McEachron TA, Lu C, Paugh BS, Beckford J, Qu C, Ding L, Hurether R, Parker M et al (2012) Somatic histone H3 alterations in pediatric diffuse intrinsic pontine gliomas and non-brainstem glioblastomas. Nat Genet 44:251–253. https://doi.org/10.1038/ng.1102

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