Brainstem pilocytic astrocytoma with H3 K27M mutation: case report

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In this report, the authors present the first case of adult brainstem pilocytic astrocytoma (PA) with the H3 K27M mutation. A 53-year-old man was incidentally found to have a 2.5-cm partially enhanced tumor in the tectum on MRI. The enhancement in the lesion increased over 3 years, and gross-total removal was performed via the occipital transtentorial approach. The resected tissue indicated PA, WHO Grade I, and genetic analysis revealed the H3 K27M mutation. However, although the radiological, surgical, and pathological findings all corresponded to PA, this entity can easily be misdiagnosed as diffuse midline glioma with the H3 K27M mutation, which is classified as a WHO Grade IV tumor according to the updated classification. This case highlights the phenotypic spectrum of PA, as well as the biology of the H3 K27M–mutated gliomas, and may prove to be an exception to the rule that diffuse midline gliomas with the H3 K27M mutation behave in an aggressive manner. Based on the findings of this case, the authors conclude that, in addition to detecting the existence of the H3 K27M mutation, an integrated approach in which a combination of clinical, pathological, and genetic information is used should be applied for accurate diagnosis and determination of the appropriate treatment for diffuse midline gliomas.

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THE 2016 revision of the WHO's classification of tumors of the CNS has newly defined diffuse astrocytic tumors developing in midline structures including the brainstem, thalamus, and spine—and that have a specific mutation in histone 3 (H3) at amino acid 27 resulting in the replacement of lysine by methionine (K27M)—as “diffuse midline glioma, H3 K27M-mutant.”⁹ This new entity is associated with a poor prognosis and a 2-year overall survival rate of < 10%, despite the development of modern therapies.⁶ Accordingly, it is designated a WHO Grade IV tumor, with the existence of the H3 K27M mutation as the sole criterion, regardless of the presence of mitotic activity, microvascular proliferation, or necrosis.⁹

We describe the first case of adult brainstem pilocytic astrocytoma (PA) harboring the H3 K27M mutation. The tumor presented typical clinical, pathological, and DNA ploidy phenotypes of PA.

Case Report

History and Presentation

A mass lesion in the midbrain tectum was incidentally diagnosed on CT scans obtained after a minor head injury in a 53-year-old man. On MRI, the tumor showed low intensity on T1-weighted images (Fig. 1A), mixed high intensity on T2-weighted images (Fig. 1B), and partial enhancement by gadolinium uptake (Fig. 1C). Computed

ABBREVIATIONS H3 = histone 3; MET-PET = methionine-PET; PA = pilocytic astrocytoma.

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FIG. 1. Radiological findings. **A–D:** Axial MRI and MET-PET studies obtained at onset. The tumor was located in the tectum and exhibited low intensity on T1-weighted images (A), mixed high intensity on T2-weighted images (B), and partial enhancement after gadolinium administration (C). An MET-PET study showed moderate uptake (tumor/normal ratio: 2.73) in the enhanced lesion (D). **E–H:** Axial MRI and MET-PET studies obtained before resection. The MRI revealed an increase in uptake in the enhanced lesion (G), although the size of the entire tumor was unchanged (E and F). The signal intensity in the MET-PET study was unchanged (H). **I–K:** Axial MRI studies obtained after resection showing almost no residual tumor. The labels T1, T2, and gadolinium apply to all 3 panels in the column above them.

tomography showed partial calcification in the tumor and mild ventricular dilation. A methionine-PET (MET-PET) study showed moderate uptake in the enhanced lesion (tumor/normal ratio: 2.73; Fig. 1D). The patient exhibited no neurological deficit, and he was consequently followed up with MRI every 6 months. Three years later, MRI showed increased tumor size and enhancement of the lesion (Fig. 1E–G), whereas MET-PET examination showed no significant upregulation of the signal intensity in the enhanced lesion (Fig. 1H). The patient showed mild ataxia due to hydrocephalus.

Operation and Postoperative Course

First, endoscopic third ventriculostomy was performed for hydrocephalus, after which the ventricle size was reduced. A week later, gross-total removal of the tumor via the occipital transtentorial approach was performed. The boundary between the tumor and normal tissue was clear, and necrotic changes were observed in the enhanced lesion. The extent of resection of the tumor was estimated to be > 95%, as calculated using MRI before and after the tumor resection (Fig. 1I–K). The patient showed mild ataxia and blurred vision after the surgery, although the symptoms fully resolved in 1 month. Postoperatively, he did not receive any adjuvant therapy, based on the original

WHO classification (Grade I), rather than on the revised classification (Grade IV). At the latest follow-up, 8 months after the surgery, the patient was free from recurrence.

Pathological Findings

The surgically resected specimens were composed of several small pieces, measuring between 0.5 × 1.0 cm and 1.0 × 2.0 cm. All specimens were embedded into 2 blocks and showed essentially identical histological features. The majority of the lesions consisted of densely fibrillary or fascicular areas with long spindle cells. The nuclei were oval with faint chromatin, although small numbers of cells with large nuclei and increased chromatin were present. The overall cellularity was low, but the background was densely gliotic. Numerous Rosenthal fibers were diffusely scattered, whereas only a few scattered eosinophilic granular bodies were observed. Narrow, loose, or microcystic areas with small round cells interposed between the dense cellular areas were noted, giving a vague biphasic appearance (Fig. 2A).

The tumor tissue was sharply demarcated from the adjacent cerebellar cortex, and there was a thick layer of gliosis at the boundary (Fig. 2B). Dilated or “wickerwork” vessels were conspicuous, a few of which were glomeruloid (Fig. 2C). Aggregate hyalinized vessels were occa-

FIG. 2. Light microscopic features of the resected specimen. **A:** A representative area showing predominately dense fibrillary components with narrow loose components, giving a vague biphasic appearance. Rosenthal fibers are abundant in the dense component. Dilated vessels are conspicuous in the background. **B:** The tumor (lower left corner) appeared sharply demarcated from the adjacent altered cerebellar cortex. Note the abundant Rosenthal fibers in the tumor tissue. **C:** Microvascular hyperplasia, some of which is slightly glomeruloid, can be seen. **D:** A focus of coagulation necrosis in the tumor tissue associated with hyalinized vessels. **E:** Nestin immunohistochemical study highlighting the thin piloid features of the tumor cells. **F:** Scattered Ki-67-positive cells were seen. **G:** Results of the isocitrate dehydrogenase R132H immunohistochemical analysis were negative. **H:** The histone H3 K27M immunohistochemical staining was diffusely and strongly positive. H & E (A–D), immunohistochemical staining (E–H). Bar = 200 μ m (A) and 100 μ m (B–H).

sionally associated with coagulative necrosis in the loose or collagenous background (Fig. 2D). Microcalcification was present, and mitosis was rare. On immunohistochemical investigation, the tumor cells were diffusely positive for glial fibrillary acidic protein, vimentin, and nestin (Fig. 2E), whereas the loose areas were focally positive for only oligodendrocyte transcription factor 2. The Ki-67 index was low (average of 10 independent fields, 3.32%; maximum, 7.4%; Fig. 2F), and phospho-histone 3-positive mitoses were rare. The cells were negative for isocitrate

FIG. 3. Genetic profiling of the tumor specimen in the present case. **A:** The histone H3 K27M mutation was detected by Sanger sequencing. **B:** The *BRAF* V600E mutation was negative. **C:** Break-apart fluorescence in situ hybridization did not indicate the presence of *KIAA1549-BRAF* fusions. **D:** Flow cytometric analysis. Single-cell suspension of the tumor specimen was stained with propidium iodide, and the DNA content was analyzed, revealing mainly diploid DNA contents (2N). CV = coefficient of variation; MI = malignancy index.

dehydrogenase R132H (Fig. 2G), whereas they were diffusely and strongly positive for H3 K27M (Fig. 2H). The p53 protein was only focally positive (< 5% of the tumor cells), and the alpha-thalassemia/mental retardation syndrome X-linked protein expression was retained. According to the revised WHO classification, this tumor corresponded to a diffuse, midline, H3 K27M-mutant glioma, Grade IV.

Genetic Analysis

The tumor samples were subjected to molecular genetics screening to ensure diagnostic accuracy. Consistent with the immunohistochemical findings, the H3 K27M

mutation was confirmed by Sanger sequencing (Fig. 3A). Next, we examined the *BRAF* gene status; despite the presence of the H3 K27M mutation, the tumor displayed the typical clinical, radiological, and histological characteristics of PA, which is genetically characterized by frequent oncogenic fusions of the *BRAF* and *KIAA1549* genes and, in very rare cases, *BRAF V600E* mutations, leading to aberrant activation of the mitogen-activated protein kinase pathway. Interestingly, neither *BRAF V600E* mutation (Fig. 3B) nor *KIAA1549-BRAF* fusion (Fig. 3C) was detected by Sanger sequencing and break-apart fluorescence in situ hybridization, respectively—instead supporting the integrated diagnosis of the present case as diffuse, midline, H3 K27M–mutant glioma. Moreover, the DNA content of the cells showed no significant proliferating or aneuploidy population, suggesting a lower-grade tumor (Fig. 3D),¹⁴ although the new 2016 WHO classification assigns Grade IV to diffuse, midline, H3 K27M–mutant glioma.

Discussion

The present case showed a relatively long clinical course of > 3 years and was accompanied by only mild neurological symptoms. The radiological, surgical, and pathological findings all supported the diagnosis of a tumor with less invasive characteristics. The pathological findings from the resected specimen, which contained a sufficient amount of material to evaluate the whole lesion, exhibited typical features of PA and showed a very low Ki-67 index. Flow cytometric analysis showed no significant aneuploid population. Furthermore, the necrotic changes suspected on MRI and observed intraoperatively were in fact found to be vascular coagulation necrosis, which is a characteristic feature of PA. All pathological and clinical findings corresponded to PA with WHO Grade I according to the integrated diagnosis by the Haarlem consensus guideline, which is the basis of the updated WHO classification.⁸ Moreover, the tectum is one of the most common sites of PA, and this location supports this diagnosis. On the other hand, the H3 K27M mutation was detected in the tumor by both immunohistochemical analysis and Sanger sequencing, and such tumors are classified as diffuse, midline, H3 K27M–mutant glioma, in the updated 2016 WHO classification.⁹ Thus, we concluded that the current case was a brainstem PA with the H3 K27M mutation rather than a clinically benign subtype of diffuse midline glioma with the H3 K27M mutation.

Pilocytic astrocytoma is the most common brain tumor in children and is, as mentioned above, classified as a WHO Grade I tumor.² The incidence of the H3 mutation in PA is currently unknown. Two cases of pediatric PA with the H3 K27M mutation have been previously reported.^{5,11} The first case involved a 7-year-old girl in whom the tumor arose in the cervical spinal cord and showed no significant chromosomal aberrations on comparative genome hybridization analysis at the initial diagnosis.⁵ However, 10 years after the initial diagnosis, recurrence occurred, and p53 mutation and drastic chromosomal aberrations were detected in addition to the H3 K27M mutation. The second case was a thalamic PA that exhibited the H3 K27M mutation. This case rapidly recurred in 14 months.¹¹ Both

cases lacked the *BRAF V600E* mutation and *BRAF* fusion protein.^{5,11} Hence, the H3 K27M mutation may play a role in the malignant transformation of PA. In addition, the significance of the H3 K27M mutations in the tumorigenesis and biological behavior of PA is largely unclear. There have been some reports that adult PA shows a more aggressive clinical course compared with pediatric PA,⁷ and that malignant transformation is more frequent in adult PA.^{3,12,13} Thus, the H3 K27M mutation may contribute to the malignant biological features of these tumors. However, because it is not unusual for PAs to grow rapidly, the change in the behavior of the tumor in this case does not necessarily indicate malignant progression. Accordingly, because the follow-up period in our case is still relatively short (< 4 years), we will need to carefully observe this patient to determine whether this tumor will continue to have a benign course in the long term.

The H3 K27M alteration is known to play a causal role in gliomagenesis as a driver mutation,^{1,10} but it may not directly determine the biological behavior of the tumor. Furthermore, it may be present in a spectrum of gliomas with less aggressive clinical behaviors and more favorable prognoses. In fact, this mutation has been found in other glioma subtypes such as ependymoma.⁴ Therefore, diffuse midline gliomas with the H3 K27M mutation may behave differently depending on the site of occurrence or the patient's age at onset. The present case may prove to be an exception to the general rule that diffuse midline gliomas with the H3 K27M mutation behave in an aggressive manner. Considering that this is a newly defined entity, the frequency of such exceptional cases is unclear, and the accumulation of additional cases is needed. Thus, the treatment strategy should be determined not only by detecting the H3 K27M mutation but also by considering the clinical, pathological, and genetic information of each individual patient.

Conclusions

The present case suggests that the H3 K27M mutation may not always indicate infiltrating astrocytoma morphology or Grade IV malignancy. Additional studies are required to elucidate the prognostic impact of the H3 mutation in PA, as well as to establish proper criteria for the diagnosis of midline gliomas.

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Disclosures

The authors report no conflict of interest concerning the materials or methods used in this study or the findings specified in this paper.

Author Contributions

Conception and design: Nitta. Acquisition of data: Nitta, Masui, Maruyama, Ichimura, Nakano, Sawada, Koriyama, Tsuzuki, Yasuda, Hashimoto, Niwa. Analysis and interpretation of data: Nitta, Komori, Masui, Ichimura. Drafting the article: Nitta, Morita. Critically revising the article: Nitta, Muragaki, Komori, Ichimura. Reviewed submitted version of manuscript: Nitta, Muragaki, Komori. Approved the final version of the manuscript on behalf of all authors: Nitta. Administrative/technical/material support: Kawamata. Study supervision: Muragaki, Komori, Kawamata.

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