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CASE REPORT

Pediatric thalamic glioma with *H3F3A* K27M mutation, which was detected before and after malignant transformation: a case report

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Abstract
Purpose Histone H3.3 (*H3F3A*) mutation in the codon for lysine 27 (K27M) has been found as driver mutations in pediatric glioblastoma and has been suggested to play critical roles in the pathogenesis of thalamic gliomas and diffuse intrinsic pontine gliomas. We report a case of thalamic glioma with *H3F3A* K27M mutation, which was detected in both the primary tumor diagnosed as diffuse astrocytoma obtained during the first surgery and also in the tumor diagnosed as anaplastic astrocytoma obtained at the second surgery.
Case presentation A 14-year-old girl presented with mild headache. Magnetic resonance imaging (MRI) showed a small intraaxial lesion in the left thalamus, which increased in size. Stereotactic tumor biopsy was performed 2 years after the initial diagnosis, and a pathological diagnosis of diffuse astrocytoma (WHO grade 2) was made. The tumor grew further and showed contrast enhancement on MRI despite 16 months of chemotherapy. Surgical removal via the transcallosal approach was then performed, and postoperative pathological diagnosis was anaplastic astrocytoma (WHO grade 3), indicating malignant transformation of the tumor. Molecular diagnosis of tumor tissue obtained at first and second surgeries revealed *H3F3A* K27M mutation in both primary and secondary specimens.
Conclusion This report demonstrates minute neuroradiological and pathological features of malignant transformation from thalamic low grade glioma with *H3F3A* K27M mutation. It is noteworthy that this mutation was found in this case when the tumor was still a low-grade glioma. Tissue sampling for genetic analysis is useful in patients with thalamic gliomas to predict the clinical course and efficacy of treatments.

Keywords *H3F3A* K27M mutation · Malignant transformation · Thalamic gliomas

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Introduction

Approximately 1 – 5 % of pediatric brain tumors occur in the thalamus [6, 23], and half are high-grade astrocytomas [2, 23]. Thalamic gliomas usually occur in deep areas of the brain and are close to many critical structures. Therefore, the clinical prognosis has been considered to be poor because surgical resection is difficult or impossible [13]. A number of studies of treatment strategies for thalamic gliomas have provided evidence of the efficacy of chemotherapy and radiation

therapy [22]. Recently, histone H3.3 (H3F3A) mutations in the codon for lysine 27 (K27M) and glycine 34 (G34R/V) at two critical positions within the histone tail have been found as driver mutations in pediatric glioblastoma multiforme (GBM) [25] and have been suggested to play critical roles in the pathogenesis of thalamic gliomas and diffuse intrinsic pontine gliomas (DIPG) and midline high-grade gliomas (mHGG) [1, 4, 8, 11, 25, 26]. Here, we report a 14-year-old girl with thalamic glioma that was initially diagnosed as low-grade astrocytoma and showed malignant transformation over 3-year follow-up. Molecular genetic diagnosis indicated that this tumor tissue had *H3F3A* mutation both at the time of initial diagnosis and after malignant transformation. We discuss the molecular biological role of this mutation in the pathogenesis of thalamic glioma.

Case presentation

A 14-year-old girl visited our hospital due to mild headache that had persisted for 1 month. Neurological examination revealed no neurological deficits. Magnetic resonance imaging (MRI) showed an intramedullary tumor in the left thalamus. The tumor was not enhanced by gadolinium and showed no mass effect (Fig. 1a). The patient was followed up conservatively by MRI every 6 months. The tumor had obviously increased in size on MRI 2 years after the initial diagnosis (Fig. 1b–d).

On stereotactic biopsy, a diagnosis of diffuse astrocytoma (WHO grade 2) was made (Fig. 1e, f). In addition, the validity of the sampling site was assured by postoperative MRI (Fig. 1g). Under a diagnosis of thalamic low-grade astrocytoma, the patient underwent chemotherapy (8 kur of carboplatin + vincristine followed by 3 kur of temozolomide + interferon-beta), but the tumor showed gradual enlargement (Fig. 2a–d), and the patient suffered severe headache and consciousness disturbance for 16 months after stereotactic biopsy. Surgical excision via the transcallosal approach and simultaneous ventriculoperitoneal shunting was then carried out (Fig. 2e). The pathological diagnosis showed anaplastic astrocytoma, WHO grade 3 (Fig. 3a–f). Subsequently, the patient underwent intensity modulated radiation therapy and chemotherapy with bevacizumab and temozolomide. However, the patient's consciousness deteriorated because of tumor dissemination to the subarachnoid space 12 months after second surgery (Fig. 2f). The total follow-up period of this patient was 4 years and 11 months.

Pathological findings

Microscopic examination of the tissue obtained in the first surgery showed the characteristic features of diffuse astrocytoma with a slight increase in the astrocyte population; the nuclei of which had a short spindle shape. Almost no nuclear division or vascular proliferation was observed (Fig. 1e). MIB-1 labeling index was 2.2 % (Fig. 1f). Microscopic examination of the material obtained in the second surgery showed evidence of anaplastic astrocytoma, marked increase in cellularity, prominent disparity of nuclear size and nuclear division, occasional intratumoral hemorrhage, and proliferation of microvessels, without necrosis (Fig. 3a, b). On immunohistochemical examination, a few p53 cells (Fig. 3d), no $IDH1^{R132H}$-positive cells (Fig. 3e), and O^6-methylguanin-

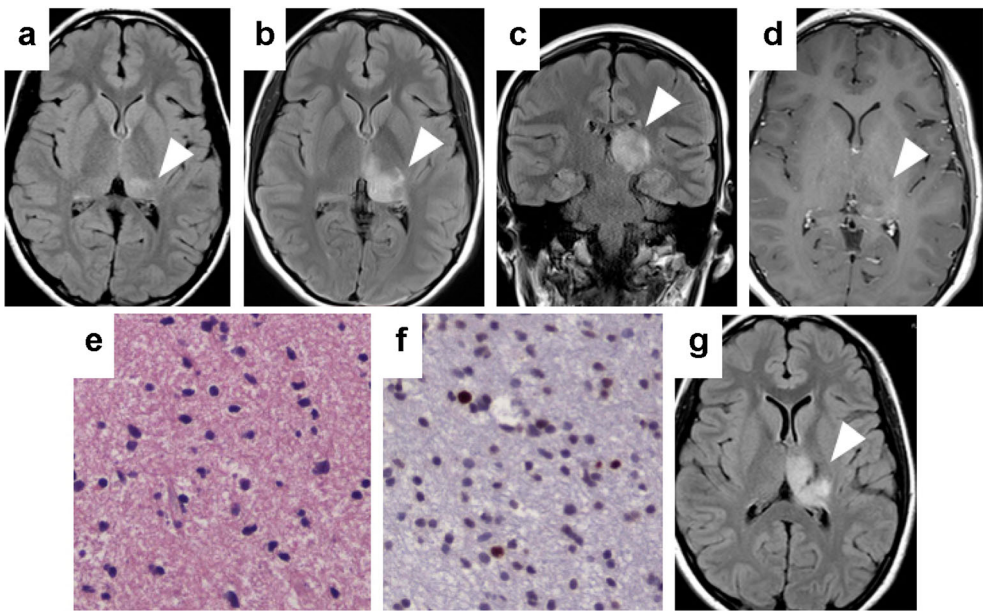

Fig. 1 RI at initial diagnosis (**a**, FLAIR) and 2 years later (**b**, **c**: FLAIR, **d**: Gd), showing the tumor infiltrating the left thalamus (*arrowheads*). Stereotactic biopsy indicated pathological diagnosis of diffuse astrocytoma with 2.2 % MIB-1 labeling index (HE: **e**, MIB-1: **f**). Postoperative MRI indicated that the sampling site was appropriate (**g**, FLAIR)

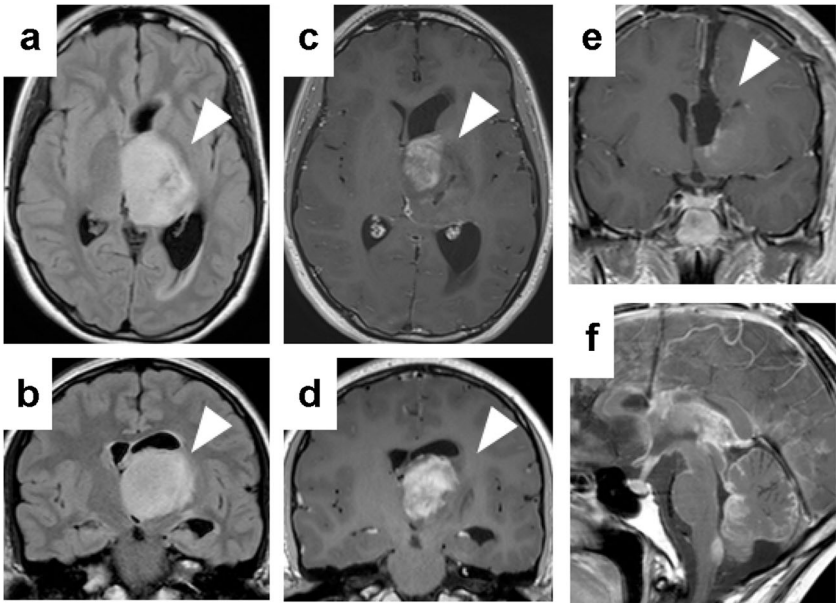

Fig. 2 MRI at 1 year after stereotactic biopsy (**a**, **b**: FLAIR, **c**, **d**: Gd) showing marked enlargement and contrast enhancement effect of the tumor (*arrowheads*). Transcallosal tumor resection was carried out (**e**: Gd), and tumor dissemination to the subarachnoid space occurred 12 months after second surgery (**f**: Gd)

DNA-methyltransferase (MGMT)-positive cells were seen scattered throughout the tumor (Fig. 3f), and MIB-1 labeling index was 1.4 % (Fig. 3c). These findings indicated that the tumor followed a course of malignant transformation.

Genetic findings

Fresh tumor tissue specimen (obtained at second surgery) and formalin-fixed paraffin-embedded (FFPE) tissue sections (obtained at first surgery) were obtained with written informed consent, and used for genomic DNA sample extraction. We examined the gene mutation status at histone *H3F3A* (first coding exon), *HIST1H3B*, *p53* (exon 2-exon 11), *IDH1* (exon 4 containing codon 132), and *IDH2* (exon 4 containing codon 172) by direct DNA sequencing [19]. MGMT promoter methylation status was also determined by quantitative methylation-specific PCR, as described previously [19].

H3F3A K27M mutation was equally verified in the tissue obtained at both first and second surgeries (Fig. 4). No mutation was recognized in *HIST1H3B* (Fig. 4). MGMT methylation status was 0.12 % ± 0.07 % (at first surgery) and 0.46 % ± 0.06 % (at second surgery), and was considered to represent an unmethylated pattern. Both *IDH1/2* and *p53* genes were wild-type in both first and second surgery samples (data not shown).

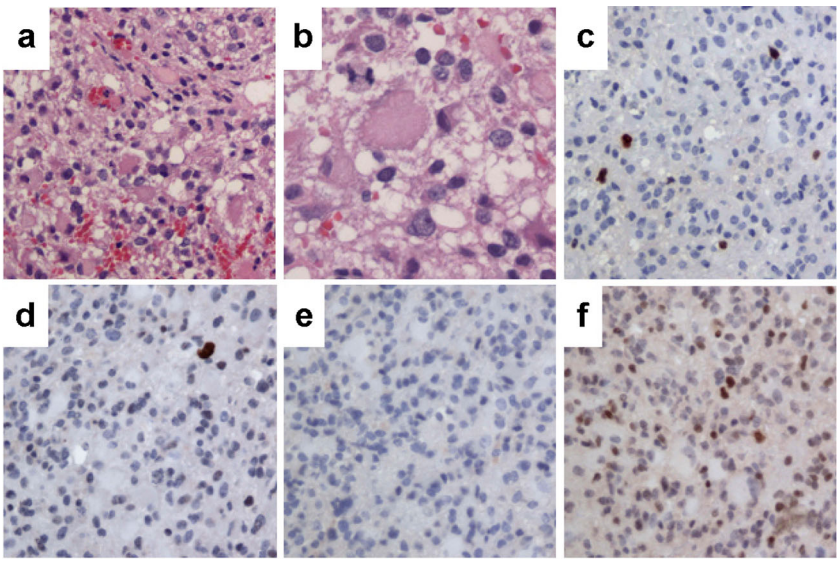

Fig. 3 Pathological micrographs from the surgical specimen obtained at the second surgery. H & E-stained section of the resected tumor (**a** × 100, **b** × 400). Microscopic examination (**a**, **b**) showed evidence of anaplastic astrocytoma, marked increase of cellularity, prominent disparity of nuclear size and nuclear division, occasional intratumoral hemorrhage, proliferation of microvessels, and no evidence of necrosis. Immunohistochemical examination showed a few p53 cells (**d**), no IDH-1-positive cells (**e**), and MGMT-positive cells scattered throughout the tumor (**f**). MIB-1 labeling index was 1.4 % (**c**)

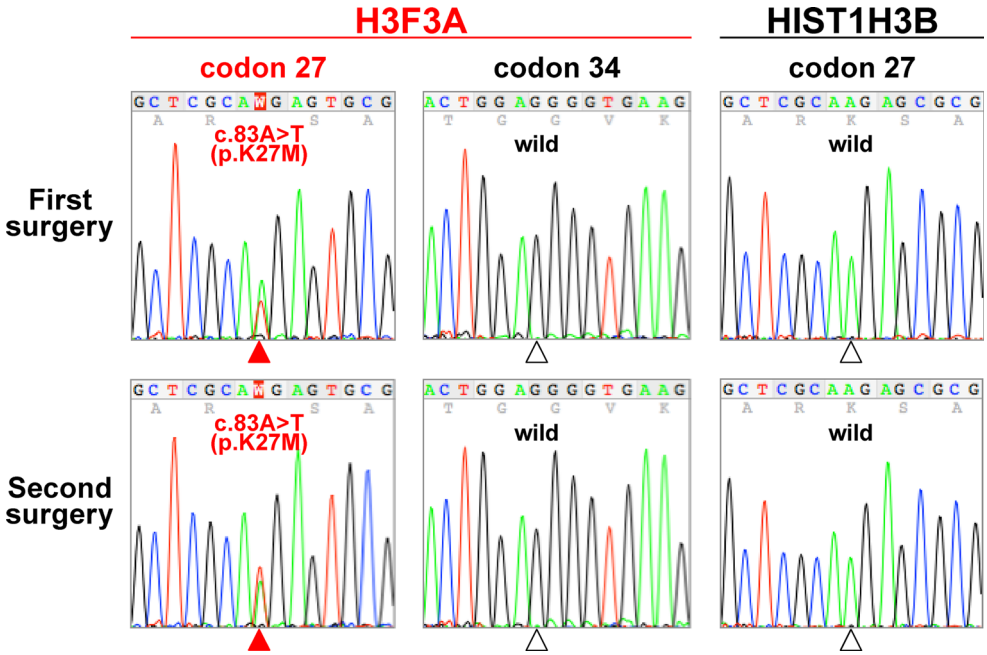

Fig. 4 On DNA sequence analysis of tumor tissue obtained at the first surgery (stereotactic biopsy) and second surgery (transcallosal approach), *H3F3A* K27M mutations were equally identified in both samples. No mutation was recognized at the R34 site of *H3F3A* and *HIST1H3B*

Diagnosis

According to histopathological features and DNA-sequencing results, we diagnosed the tumor as thalamic glioma with *H3F3A* K27M mutation, which followed a course of malignant transformation.

Discussion

Thalamic glioma, a relatively rare tumor in pediatric and young adult patients, is a challenging disease for which effective chemotherapy has not been reported [20], and the efficacy of irradiation was also reported to be temporary or restricted [7, 21]. Many of these patients were treated palliatively [13, 14]. A few reports mentioned that radical resection is favorable for prognosis [3, 24], but in some cases of thalamic glioma, the tumors seemed quite unresectable and were treated conservatively by only imaging diagnosis without tumor biopsy [3, 7, 12, 22].

Although the detailed molecular mechanism of malignant transformation of glioma has not been clarified, *H3F3A* and *HIST1H3B* K27M mutation may play an important role in tumorigenesis of mHGG and DIPG [5]. *H3F3A* K27M mutation is a missense mutation of histone H3.3, which was recently identified [25], leading to tumorigenesis of pediatric GBM, DIPG, and thalamic high-grade glioma. In addition, this mutation determines the critical properties of the tumor and strongly affects patient prognosis [5, 26]. *H3F3A* K27M mutation causes the development of glioma via demethylation at the K27 site [4, 18] by inhibition of PRC2 activity [15]. Recent studies reported the spatial homogeneity of this prognostically relevant somatic mutation in mHGG and DIPGs, and possible utilization for therapeutic target [11, 17]. Considering these observations, it is clear that gene mutation analysis as part of current diagnostic pathology in brain tumor management is extremely important.

Detailed natural and clinical courses of tumors with *H3F3A* K27M mutation have not been reported. To our knowledge, this is the first case report demonstrating minute neuroradiological and pathological features of malignant transformation from thalamic low grade glioma with *H3F3A* K27M mutation. The present case was first diagnosed as low-grade astrocytoma in combination with standard histopathology and MRI findings, and treated according to the results of diagnosis. There was little possibility of misdiagnosis from sampling error at the time of stereotactic biopsy because postoperative MRI indicated an appropriate sampling site. Recently, histopathological heterogeneity ranging from WHO grade II to IV astrocytoma was reported in DIPGs and mHGG with *HIST1H3B* K27M mutation [17]. These findings will suggest more precise diagnosis, and better estimation of prognosis and treatment response can only be obtained with gene mutation analysis together with usual pathological diagnosis. Furthermore, such analysis will allow the establishment of molecular targeting treatment for pathologies caused by gene mutations. A recent study suggested the potential efficacy of an antitumor agent, such as panobinostat or GSK-J4 [9, 16], that negatively affects a glioma cell line with *H3F3A* K27M mutation by normalizing aberrant demethylation at the K27 site [10].

Conclusion

Based on these findings, the present case suggested that thalamic glioma in younger patients may have potential malignant properties with *H3F3A* mutations, even if the clinical features are not malignant at initial presentation. Tumor tissue sampling and detailed assessment of gene mutations should be considered because molecular biological information may improve the clinical prognosis of thalamic glioma patients.

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Conflict of interest Kanemura Y received research funding from Kaneka Corp. The authors declare no conflicts of interest associated with this manuscript.

Ethical approval Genetic testing was approved by the Ethics Committees of both Osaka City General Hospital and Osaka National Hospital and was carried out at Osaka National Hospital in accordance with the principles of the Declaration of Helsinki.

Informed consent We obtained written informed consent from the patient for publication of this case report and any accompanying images.

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