Adult Diffuse Astrocytoma in the Medulla Oblongata: Molecular Biological Analyses Including H3F3A Mutation of Histone H3.3

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Unlike in children, brain stem gliomas in adult are rare and still poorly understood. In addition, most adult brain stem gliomas result predominantly in the pons and are less often found in the medulla oblongata. Here, we report a case of an adult glioma in the medulla oblongata and its molecular biological features. A 46-year-old male presented with gait disturbance, paresthesia, and dysphagia. Magnetic resonance imaging (MRI) showed a diffuse hyper-intensive lesion in the medulla oblongata on a T1-weighted image without gadolinium contrast enhancement. We performed an open biopsy and the lesion was pathologically diagnosed as a diffuse astrocytoma. Molecular biological analyses revealed the absence of histone H3.3 mutation (H3F3A K27M), and presence of methylation of O-6-methylguanine-DNA methyltransferase (MGMT) promoter and a mutation in isocitrate dehydrogenase 1 (IDH-1). The patient received local radiotherapy and temozolomide chemotherapy. The patient’s symptoms were ameliorated, and MRI showed no tumor growth at 6 months after the initial treatment. Biopsy for brain stem lesions is generally thought to have risk of complications, but if performed minimally, it is useful to diagnose and determine treatment strategy. Obtaining patient characteristics and molecular biological features will provide insight towards therapeutic treatment for adult brain stem gliomas.

Keywords: diffuse astrocytoma, medulla oblongata, histone H3.3 mutation (H3F3A K27M), O-6-methylguanine-DNA methyltransferase promoter, adult

Introduction

Brain stem gliomas constitute 10% of brain tumors in children and less than 2% of brain tumors in adults.1) Adult brain stem gliomas are mostly histologically low grade,1-3) and result predominantly in the pons and less often in the medulla oblongata and midbrain.4) Ueoka et al. examined 86 patients with brain stem gliomas, including 24 (27.9%) adult patients. Lesions were located in the pons in 75.6% of patients, the midbrain in 15.1% of patients and the medulla in 9.3% of patients.5) Although midbrain and cervicomedullary gliomas are reported to demonstrate better survival than pontine gliomas,6) medullary gliomas appear to be associated with high morbidity and mortality, because the medulla oblongata is an essential autonomic center in the brain.

Moreover, adult patients occasionally present a rapidly growing tumor similar to pediatric cases.2,4) Recently, recurrent H3F3A mutations of histone H3.3 have been identified as a frequent event in pediatric diffuse intrinsic brain stem gliomas (DIPGs) and thalamic glioblastomas (GBMs), associated with prognosis and survival.6-9) However, due to its rarity, the relationship between the clinical course and molecular biological features in adult brain stem gliomas have still been poorly understood.

Here, we present a case of an adult glioma in the medulla oblongata and demonstrate its histopathological features and genetic analyses.

Case Report

I. History and examination

A 46-year-old male presented with a 2-month history of dizziness followed by headache, paralysis, and paresthesia of the limbs, dysphoria, and dysphagia. One month later, gait disturbance and loss of appetite were deteriorated. Magnetic resonance imaging (MRI) demonstrated a diffuse hyper-intensive lesion on a T2-weighted image (T2-WI) in the medulla oblongata (Fig. 1a, b) without contrast enhancement on T1-weighted gadolinium (Gd) in the lesion (Fig. 1c). Positron emission tomography (PET) showed a negative accumulation of 11C-methionine and 18F-fluorodeoxyglucose (FDG) at the lesion (data not shown). The patient was admitted to our hospital. Upon admission, neurological examinations revealed hoarseness, difficulty of swallowing, loss of gag reflex, vocal cord palsy, and paralysis and paresthesia of the limbs. Karnofsky performance status (KPS) scale was 40 on admission. To pathologically diagnose and determine the therapeutic strategy, we performed an open biopsy. Via a suboccipital craniectomy with C1 laminectomy, the medulla oblongata was exposed (Fig. 2a). The medulla oblongata was swollen. A medullary incision was performed upon the median sulcus of the fourth ventricular floor and minimal amount of tissue sample was obtained. Postoperative computed tomography (CT) (Fig. 2b) and T2-weighted MRI (Fig. 2c) showed minimal extent of craniectomy and biopsy. The patient showed no worsening of his symptoms after the surgery.

II. Pathological findings and genetic analyses

Informed consent was obtained from the patient and his family. Molecular analysis was approved by the Research Ethics Committee of the Institutional Review Board of Kumamoto University Hospital.

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Immunohistochemistry was performed with validation of positive and negative controls as previously described. Briefly, formalin-fixed, paraffin-embedded (FFPE) tissue samples were obtained and 4 μm thick serial sections were stained. The following primary antibodies were used: anti-human glial fibrillary acidic protein (GFAP) antibodies (Z0334, rabbit, 1:2000, Dako, Glostrup, Denmark), anti-Olig2 antibodies (18953, rabbit, 1:100; IBL, Gunma), anti-human Ki-67 antibodies (M7240, mouse, clone MIB-1, 1:100, Dako), anti-trimethyl-histone H3 (Lys27) antibodies (#07-449, rabbit, EMD Millipore Corporation, Temecula, California, USA), anti-IDH-1-R132H antibodies (DIA-H09, mouse, 1:50; Dianova, Hamburg, Germany), anti-p53 (Ab-2) antibodies (#OP09, mouse, 1:100; Oncogene Research Products, La Jolla, California, USA), anti-alpha thalassemia/mental retardation syndrome X-linked (ATRX) antibodies (HPA001906, rabbit, 1:200, Sigma, St. Louis, Missouri, USA), and anti-BRAF V600E antibodies (# E19290, mouse, 1:50, Spring Bioscience Corporation, Pleasanton, California, USA).

Hematoxylin and eosin (H&E) staining showed moderately increased cellularity and weak nuclear atypia without mitotic cells, necrosis, and microvascular proliferation (Fig. 3a). Immunohistochemical analysis showed positive staining for GFAP (Fig. 3b) and Olig2 (Fig. 3c). The MIB-1 staining index was 2–3% (Fig. 3d). Histological analysis identified the tumor as World Health Organization (WHO) grade II, diffuse astrocytoma. Immunohistochemical analyses showed positive staining for trimethyl-histone H3 (Lys27) (Fig. 3e). Positive staining for IDH-1 (Fig. 3f), negative staining for mutated p53 (Fig. 3g), and positive staining for ATRX (Fig. 3h), are shown in the respective figures.

The methylation-specific polymerase chain reaction (MS-PCR) assay was performed to evaluate the methylation status of the MGMT promoter in the tumor tissue as previously described. Briefly, genomic DNA was obtained from each sample (2 μg) and treated with sodium bisulfite using the Epitect Bisulfite Kit (QIAGEN Inc., Valencia, California, USA). Human glioma-initiating cell lines were established in our institution as previously described and were used as methylated and unmethylated controls. In our patient, the methylated MGMT promoter was included (Fig. 4).

Pyrosequencing analysis was performed to evaluate the histone H3.3 mutation H3F3A K27 and G34 mutational status in the tumor tissue. Briefly, genomic DNA was obtained from each sample (2 μg) and treated with sodium bisulfite using the Epitect Bisulfite Kit (QIAGEN Inc., Valencia, California, USA). Human glioma-initiating cell lines were established in our institution as previously described and were used as methylated and unmethylated controls. In our patient, the methylated MGMT promoter was included (Fig. 4).

Pyrosequencing was performed to evaluate the histone H3.3 mutation H3F3A K27 and G34 mutational status in the tumor tissue. Briefly, genomic DNA was extracted from a frozen tumor tissue sample. The mutation hotspot codons at K27 and G34 of the H3.3 genes (AAG → ATG; GGG → AGG; GGG → GTG) were screened by pyrosequencing according to a previous report. Briefly, pyrosequencing was...
performed using PyroGold reagents (QIAGEN GmbH, Hilden, Germany) on the PSQ 96MA instrument (QIAGEN GmbH), according to the manufacturer’s instructions. Control oligonucleotides (QIAGEN GmbH) were used to detect the background signal. Pyrogram outputs were analyzed by the aqueous assay of a PyroMark Q96 (version 2.5.8) on a PSQ 96MA pyrosequencer (QIAGEN GmbH) to determine the percentage of mutant versus wild-type alleles according to percentage relative peak height as per the manufacturer’s recommendation. A 240 bp fragment of exon 2 of H3.3 containing the coding region was amplified in a polymerase chain reaction (PCR) using the primer set H3.3 fwd 5’-TGTTTGGTAGTTGCATATGGT-3’ and H3.3 rev 5’-biotin-TACAAGAGACTTTTGTCC-3’ and 20 ng genomic DNA as template. PCR amplicons of H3.3 flanking both mutation hotspots (codon 27 and 34) were analyzed on 2% agarose gels and subjected to pyrosequencing reactions using the pyrosequencing primer H3.3-Py-5’-CAAAAAGCGC- GCTCGCA-3’ with the following nucleotide dispensation order: GATGAGTGCGCTCTACTCGAGCGTGTGA. In this patient’s sample, wild-type sequence patterns for H3F3A position 27 and 34 are shown in Fig. 5.

III. Treatment and clinical course

Radiation therapy (total 50 Gy/25 days) and concomitant temozolomide (TMZ) chemotherapy at 75 mg/m²/day (140 mg daily) was performed in combination with corticosteroid therapy. After discharge, he attended our hospital and continued to receive the maintenance of adjuvant TMZ chemotherapy (150 mg/m² for 5 days/28 days at first time, from the second time 200 mg/m² for 5 days/28 days). During chemoradiotherapy, the patient went through rehabilitation. Gait disturbance and dysphagia were partially ameliorated and the KPS was increased by 80. Follow-up MRI after 6 months from the initial treatment revealed little change in the findings and slightly enhanced area in the lesion (Fig. 1d–f). However, the tumor volume analysis compared using the Ellipsoidal method showed that there was no difference between the tumor volume before therapy and that after 6 months.

Discussion

We presented a case of an adult glioma in the medulla oblongata, and a biopsy was performed on the patient. The patient was then treated with radiotherapy and TMZ chemotherapy. In our case, the molecular biological features showed (1) absence of the histone H3.3 mutation (H3F3A K27M), (2) presence of a mutation in IDH-1 and absence of a mutation in p53 and ATRX, and (3) presence of methylation of the MGMT promoter.
Adult brain stem gliomas may resemble supratentorial gliomas in adults and a different behavior compared to childhood tumors, known as pediatric DIPG.1–3 Although a biopsy is rarely performed in intrinsic diffuse gliomas in adults, if it is performed, a low-grade histology (grade II gliomas) is found in up to 80% of cases, while in children a grade IV, GBM is the most frequent phenotype reported in 50–60% of cases.5 The adult form probably represents better prognosis, whereas adult patients occasionally present a rapidly growing tumor similar to the DIPG found in the pediatric population.5–7 A recent study identified recurrent histone H3.3 H3F3A mutations affecting two critical amino acids (K27 and G34) as a frequent event in pediatric DIPG and thalamic GBMs.8 H3F3A mutation is important to define epigenetic subgroup of GBM with a distinct global methylation pattern, together with IDH-1 mutations.7 A report demonstrated that 78% of DIPGs and 22% of non-brainstem pediatric GBM contained a mutation in H3F3A.8 Other report revealed that Lys27Met alteration in histone H3.3 (H3F3A K27M) occurred in 71%, p53 mutations in 77%, and ATRX mutations in 9% of DIPGs.8 Moreover, H3F3A K27M is commonly associated with short survival in DIPG, while patients with wild-type for H3.3 show improved survival.8 In our case, immunohistochemical analyses and pyrosequencing indicated the absence of H3F3A K27M, which demonstrated that the case had different characteristics from pediatric DIPG and would present better prognosis. More new knowledge of the profile of H3F3A mutation will help to classify and predict tumor behavior in adult and pediatric brain stem gliomas.

GBM can be divided into two subtypes, primary GBM or secondary GBM, with different genetic pathways. The IDH-1 mutation is common in secondary GBM, but is rare in primary GBM, which helps to distinguish secondary from primary GBM.13 The IDH-1 mutation has also been reported to be a favorable prognostic factor in GBM patients.13 Moreover, p53 mutations and alterations in ATRX are frequent in adult diffuse gliomas and are specific to astrocytic tumors carrying IDH1/2.14 A recent study reported that, in adult cerebral gliomas, ATRX aberrations were detected in 33% of grade II and 46% of grade III gliomas, as well as in 80% of secondary and 7% of primary GBMs. Thus, combined alterations of these genes, including p53 and ATRX, may drive neoplastic growth in a major subset of diffuse astrocytomas in adults.14 Oka et al. reported 25 brain stem gliomas, and 4 tumors were located in the medulla oblongata.15 In the four medullary gliomas, three cases were diagnosed with histopathological analyses; two cases were grade II astrocytomas and one case was grade IV, GBM.15 In all the three cases, IDH-1 mutation was negative.15 Yoshikawa et al. also reported an extremely short clinical course of adult GBM in the medulla oblongata, in which the case had no IDH-1 mutation.14 In our case, immunohistochemical analyses showed positive staining for IDH-1, which indicated the presence of IDH-1 mutation, and negative staining for mutated p53 was shown as the wild-type pattern, and ATRX mutations (loss of ATRX expression) were not detected. Taken together, these findings indicated that the patient exhibited similar features to cerebral low-grade gliomas and the potential to progress to secondary GBM.

Hypermethylation of the MGMT promoter is associated with an improved outcome in GBM and is a predictive marker of sensitivity to alkylating agents, such as TMZ and radiotherapy.16 Although MGMT promoter methylation ranges from 35% to 73% in GBM,17 its frequency in brain stem lesions has not yet been reported. Oka et al. reported that, in four medullary gliomas, two cases of grade II astrocytoma showed unmethylated MGMT promoter and one case of grade VI GBM showed methylated MGMT promoter.15 Yoshikawa et al. reported the case of adult GBM in the medulla oblongata with an unmethylated MGMT promoter, and the patient had an extremely short clinical course.4 In our case, MS-PCR showed the methylation of the MGMT promoter, which indicated that TMZ chemotheraphy and radiotherapy would be effective.

Finally, the medulla oblongata is the most critical area in the brain stem and surgical intervention in this area poses a risk of serious morbidity or mortality. While, a previous report presented two cases of gross total surgical removal of malignant glioma from the medulla oblongata, which were unilateral and the margin appeared to be relatively clear on MRI.18 By contrast, in diffuse intrinsic gliomas, gross total removal would be impossible and even an indication of biopsy is controversial. Because biopsy was rarely performed, the distinct molecular biological patterns of diffuse brainstem gliomas remain almost unknown. In our case, open biopsy was safely performed, and we propose that biopsy is useful for the diagnosis and determination of treatment strategy. Usually the regimen of chemotherapy is determined by the histology of the tumor. In our institute, cisplatin plus vincristine is used for pilocytic astrocytomas, ACNU (nimustine hydrochloride), or TMZ for grade III gliomas and TMZ for GBM, respectively. In the brain stem glioma, the treatment strategy should be different from that in supratentorial glioma. At first, there is no surgical indication except for the biopsy in the diffuse intrinsic brain stem glioma. Therefore, radiotherapy is a most promising treatment in the low-grade brain stem gliomas. Salmaggi et al. demonstrated clinical improvement in 60% of cases and radiological responses in 25% of cases of adult brain stem gliomas. TMZ is effective for high-grade gliomas but its efficacy in low-grade brainstem gliomas is unknown. There is no randomized study evaluating the efficacy of TMZ in low-grade brain stem gliomas. However, Taal et al.20 have reported about the first-line TMZ therapy in progressive low-grade gliomas after radiotherapy. They concluded that MGMT status and IDH1 mutations did not seem to predict the progression-free survival to TMZ. While there is a report that TMZ is a potentially useful treatment for recurrent diffuse infiltrating low-grade brainstem gliomas relapsing after radiation therapy.21 Now, the issue about TMZ therapy for low-grade brainstem gliomas is controversial. In our case, we have an impression that TMZ is an effective agent because the tumor size is stable 6 months after initial therapy. In conclusion, we presented a rare case of adult diffuse astrocytoma in the medulla oblongata and its histopathological and molecular
biological features. Biopsy appears to be useful to diagnose and determine treatment. Because there is no effective therapy to date, further studies examining patient characteristics and the molecular biological features, including histone H3.3 mutation, will provide further insight towards predicting tumor behavior and establishing an effective therapy for adult brain stem gliomas.

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Conflicts of Interest Disclosure

The authors declare that they have no conflict of interest. All authors who are the members of The Japan Neurosurgical Society (JNS) have registered online Self-reported COI Disclosure Statement Forms through the website for JNS members.

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