

Clinical Significance of Molecular Diagnosis of Pilocytic Astrocytoma: A Case Report

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This paper reports on a case of pilocytic astrocytoma (PA), for which a diagnosis by conventional pathological diagnosis was difficult but an accurate diagnosis was possible by a new molecular diagnostic method. A 13-year-old girl whose tumor developed by a headache that gradually worsened, and a well-demarcated T₂-hyperintense lesion was found in the left cerebellum by a magnetic resonance imaging while the apparent diffusion coefficient value was also high. While the finding was a typical PA, histological features of PA were not found in the surgical specimen. An initial diagnosis was anaplastic astrocytoma (AA), and the final diagnosis through a central review was diffuse astrocytoma (DA). On the other hand, using MethylationEPIC (850 K) array, an analysis by a DNA methylation-based tumor classifier tool as reported by Capper et al. showed that this case belonged to a methylation class of PA. The copy number profile calculated from the methylation array data showed hints of BRAF/KIAA1549 fusion and no other chromosomal alterations, which also supported the molecular diagnosis. The patient was treated with local radiotherapy concomitant with temozolomide based on the initial pathological diagnosis during the consultation, but maintenance temozolomide therapy was not done according to the final molecular diagnosis. The tumor showed no recurrence for 20 months. In this case, the integrated diagnostic approach based on histological and molecular findings was clinically significant to select proper adjuvant treatment. It is crucial that the usefulness and robustness of this new molecular diagnostic method be validated further.

Keywords: pilocytic astrocytoma, copy number profile, methylation profile, tumor classifier tool

Introduction

Pilocytic astrocytomas (PA) are the most common glioma in children and adolescent, and are preferentially located in the cerebellum and cerebral midline structures. The morphological features of them are characterized as a biphasic pattern with variable proportions of compacted bipolar cells with Rosenthal fibers and loose, textured multipolar cells with microcysts and occasional granular bodies¹⁾; PA are conventionally diagnosed by microscopic examination. However, the accurate diagnosis is challenge because approximately 5% of IDH-wildtype glioma is PA diagnosed by molecular analysis, which cannot be diagnosed histologically.²⁾ While most of IDH-wildtype glioma exhibits molecular alterations and survival characteristics of IDH-wildtype glioblastoma which need aggressive multimodality therapy: surgical resection, radiotherapy combined with anti-tumoral agents,^{2–4)} PA is curable tumor by complete resection alone. The WHO diagnostic scheme is not sufficient for the clinical approach toward PA; therefore, a renewal of the diagnosis method is desired. The usefulness of a molecular diagnosis that focuses on DNA methylation profile in diagnosing PA has recently been reported,⁵⁾ but accumulation of the evidence is necessary before conclusion.

In this paper, a new molecular diagnosis was applied to a PA cases where a discrepancy was observed between the conventional pathological diagnosis and the clinical picture to investigate the role of the molecular approach.

Case Report

The patient was a 13-year-old girl with early morning headaches gradually worsened over a year. On admission, a well-demarcated T₂-hyperintense lesion with irregular enhancing effect was found in the left cerebellum by magnetic resonance imaging (MRI) (Figs. 1A and 1B). A high apparent diffusion coefficient (ADC) value was recorded at 1.95 (×10⁻³ mm²/s) (Fig. 1C). These radiological findings and the age of the patient were compatible with typical characteristics of PA. After gross total resection, light microscopic examination revealed a low to moderate proliferation of diffuse astrocytic tumor cells with mild nuclear atypia; the surgical specimen did not show typical findings for PA such as a biphasic pattern, Rosenthal fibers, and eosinophilic granular bodies (Fig. 1A). Immunohistochemical staining demonstrated an IDH1 R132H-negative, ATRX-positive, GFAP-positive, partially p53-positive, and Ki67 index of 10.5%. Pathological diagnosis was

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Received: November 15, 2018; Accepted: February 27, 2019
Online June 5, 2019

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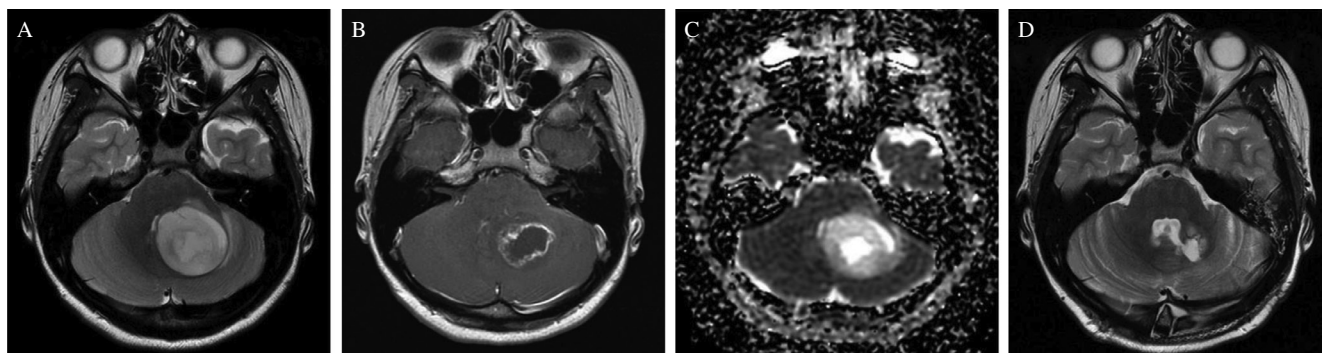


Fig. 1 MRI shows hyperintense on T₂WI (A) with irregular enhancing lesion on post-contrast T₁WI (B). ADC value of the T₂-hyperintense lesion is high (C). Post-operative MRI shows that hyperintense lesion on T₂WI is totally removed (D). MRI: magnetic resonance imaging, ADC: apparent diffusion coefficient.

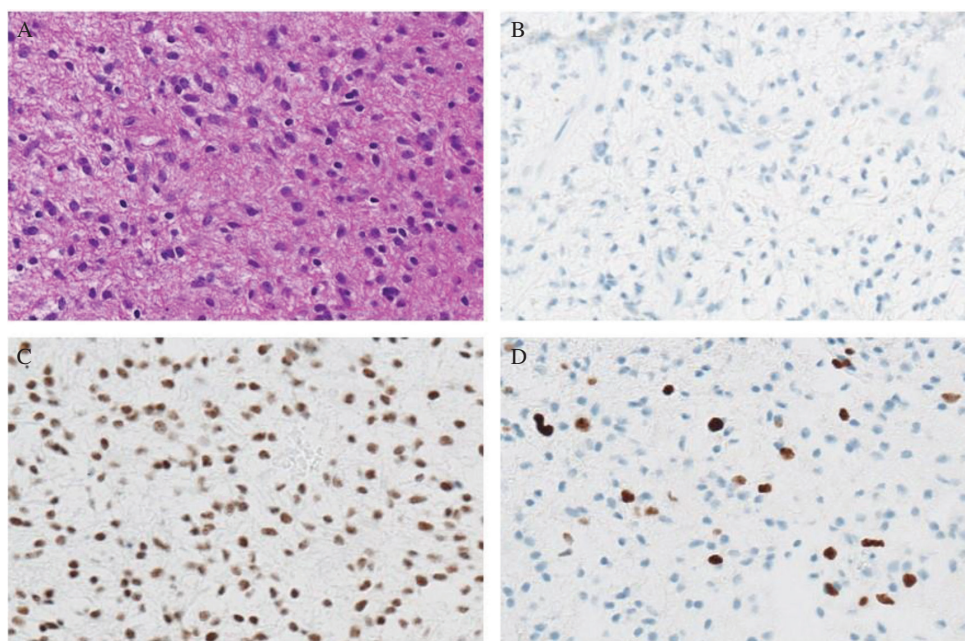
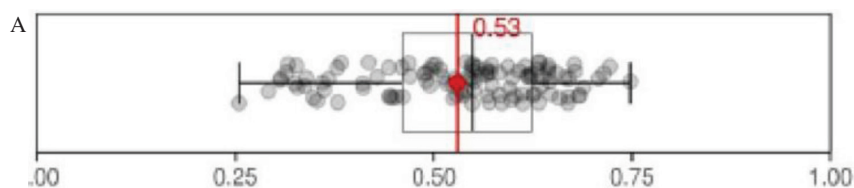


Fig. 2 Representative pathological findings. Hematoxylin and eosin staining (A) revealed low to moderate proliferation of diffuse astrocytic tumor cells. Typical biphasic pattern, Rosenthal fibers, and eosinophilic granular bodies were not seen in the section. The tumor cells were negative for IDH1-R132H (B), and positive for ATRX (C). Ki67 index at the most proliferative area was 10.5% (D).

an anaplastic astrocytoma (AA), not otherwise specified (NOS) (Figs. 2B–2D). The authors consulted the Department of Pathology at Hidaka Hospital (Professor Y. Nakazato), which made the final diagnosis of diffuse astrocytoma (DA), NOS. Because a pediatric cerebellar DA is quite rare, a request was made to Heidelberg University for a molecular diagnosis. According to the report by Capper et al.,⁵⁾ DNA methylation-based classification was performed using Infinium MethylationEPIC BeadChip (850 K) (Illumina, San Diego, CA, USA). In this method, the DNA methylation pattern of a tumor specimen is classified based on unsupervised clustering of a reference cohort (2801 reference samples and 91 methylation classes), and each cluster is well visualized using *t*-distributed stochastic neighbor embedding dimensionality reduction. The calibrated score threshold to decide methylation class family is ≥ 0.9 , and for subclasses within methylation class families, a threshold value of ≥ 0.5 is sufficient for a valid prediction. Copy number variation (CNV) profile was also calculated from the methylation array data. In this case,

methylation-based classification demonstrated a score of 1.0 for the methylation class low-grade glioma, subclass posterior fossa PA (Fig. 3A). Although not obvious, CNV profile showed hints of BRAF/KIAA1549 fusion and there are no other chromosomal alterations (Fig. 3B). Clinical and molecular characteristics except for the histological findings were compatible with PA rather than IDH-wildtype gliomas including glioblastoma, diffuse midline glioma with H3 K27M mutation, and AA with piloid features,⁶⁾ a new entity of diffuse gliomas that needs to be differentiated from PA (Table 1). Thus, we concluded that this case was a cerebellar PA without its histological features.

The patient was treated with local radiotherapy concomitant with temozolomide based on the initial pathological diagnosis (AA, NOS) during the consultation, but maintenance temozolomide therapy was not done according to the final molecular diagnosis. The tumor showed no recurrence for 20 months. The diagnosis of PA matched well with the clinical picture of this case.



Score	calibrated	quality
0.53	1.00	OK

Methylation class: low grade glioma
Subclass: posterior fossa pilocytic astrocytoma

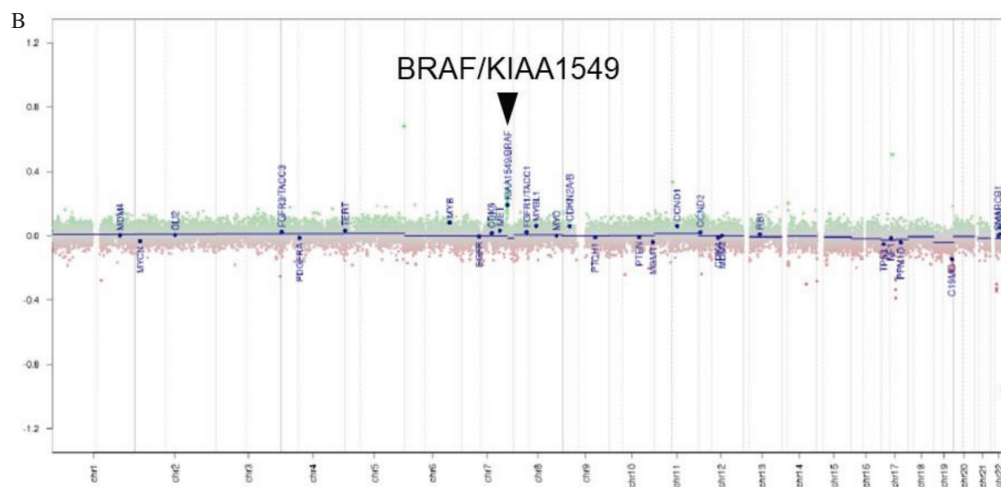


Fig. 3 In methylation-based tumor classification (A), the raw score 0.53 is equivalent to the other reference cases (gray dots) in the boxplot, and the calibrated score 1.0 demonstrates that this tumor belongs to methylation class low grade glioma, posterior fossa pilocytic astrocytoma. CNV profile (B) shows hints of BRAF/KIAA1549 fusion (arrow head) and there are no other chromosomal alterations. CNV, copy number variation.

Discussion

Pilocytic astrocytomas account for 33.2% of all gliomas and >30% of them are located in the cerebellum in the 0–14 years age group⁷; PA is the most common cerebellar glioma in children. The latest Brain Tumor Registry of Japan shows that the frequency of PA and DA in the cerebellar tumors are 42.3% and 5.5% including patients of all ages, respectively.⁸ Considering the age distribution, the frequency of pediatric cerebellar DA would be quite rare. This case was a 13-year-old girl, and the MRI findings including high ADC value of $1.95 \times 10^{-3} \text{ mm}^2/\text{s}$ and the clinically benign course were also compatible with PA,⁹ while the pathological findings suggested DA, NOS.

The most challenging and clinically most relevant differential diagnosis of PA, given the differences in prognosis and the treatment implications, is with other diffuse gliomas.¹⁰ Particularly, PA sometimes shows a predominant oligodendroglial-like or astrocytic appearance without distinctive histological features, and can be misinterpreted as high grade glioma due to the presence of nuclear pleomorphism, vascular proliferation, and necrosis.^{10–12} A previous retrospective study reported that among the 58 PA cases, 15 (25.9%) and 11 (19.0%) cases showed the monomorphous oligodendrogloma-like pattern and the monomorphous fibrous pattern, respectively.¹³ And indeed, there was a clinical trial report in which two of 30 AA cases (6.7%) were reclassified into PA

through the central review¹⁴; the current WHO classification could have the risk of the misdiagnosis. Further, a recent paper demonstrated that approximately 5% of IDH-wildtype glioma was PA diagnosed by molecular analysis, which could not be diagnosed pathologically.² More recently, mid-line PA with H3 K27M mutation^{15–17} and AA with piloid features,⁶ unclassified tumor entities which could not be diagnosed pathologically, were also reported. Diagnostic misinterpretation between PA, WHO grade I and other diffuse gliomas cause over/undertreatment of patients, and it is thought that unnecessary radiochemotherapy could be avoided in this case; therefore, a renewal of the diagnosis method is desired to distinguish them more robustly.

Focusing on the molecular point of view, the diagnostic usefulness of the tumor classifier tool based on the DNA methylation profile was reported.⁵ According to the published validation cohort data reported by Capper et al., seven of 395 IDH-wildtype gliomas are finally reclassified into PA using the classifier tool. The primary pathological diagnosis of them are DA (two cases), AA (two cases), and glioblastoma (three cases), and the tumor location are posterior fossa in one case, supratentorial in one case, supra-infratentorial in one case, and not available in four cases. The median age of the seven cases are 10 (range: 1–22). Gene fusion testing shows BRAF fusion in only four of the seven cases. These data suggest that such

Table 1 Key characteristics of PA and the differential diagnosis

Tumor entity	Age distribution	Tumor location	Histological findings		Molecular findings			Methylation class	
			Piloid features	Astrocytic features	Malignant findings	Gene fusion (%)	Gene mutation (%)		Copy number alteration (%)
PA ^{1,5,8,10}	0–14	Cerebellum Supratentorial (mainly optic pathway) Brain stem	+	+/-	-	BRAF (>70% in all, 80–90% in posterior fossa, 60% in supratentorial cases)	BRAF (5)	not applicable	PA, PF PA, SUP PA, MID
IDH-wild type GBM ^{1,5,6}	55–85	Cerebral hemisphria	-	+	+	not applicable	TERT (72–90) TP53 (28–35) PTEN (25–35) NF1 (15–18) PIC3CA (5–15)	EGFR amp (35–45) CDKN2A/B del (35–50) NFKB1A del (25) PDGFR amp (13)	GBM, RTK I GBM, RTK II GBM, RTK III GBM, G34 GBM, MES GBM, MID GBM, MYCN
DMG, H3 K27M-mutant ^{1,5,6}	5–11	Pons Thalamus Spinal cord (occasional involve the cerebellum)	-	+	+/-	not applicable	H3 K27M (100) TP53 (50) ACVR1 (20) PPM1D (15) ATRX (15)	PDGFR amp (30) CDK4/6 or CCND1–3 amp (20) MYC/PVT1 amp (15)	DMG, H3 K27M
AA with piloid features ^{5,6}	21–50	Cerebellum Cerebral hemisphria Spinal cord	+	+/-	+	BRAF (20%)	ATRX (36) (ATRX loss/mutation (45) NF1 (19) FGFR1 (17)	CDKN2A/B del (80) NF1 del (12) CDK4 amp (8)	AA with piloid features
The present case	13	Cerebellum	-	+	-	Hints of BRAF fusion	Any MAPK pathway gene alterations: 75% not done	not applicable	PA, PF

Malignant findings mean nuclear atypia, mitotic activity, microvascular proliferation, and necrosis, AA: anaplastic astrocytoma, amp: amplification, del: homozygous deletion, DMG: diffuse midline glioma, GBM: glioblastoma multiform, MES: mesenchymal, MID: midline, PA: pilocytic astrocytoma, PF: posterior fossa.

diagnostic discordance likely occur in younger patients regardless of pathological diagnosis, tumor location, and that BRAF fusion status alone is not sufficient to salvage PA from other diffuse gliomas. About BRAF fusion, the most frequent genetic abnormality in PA, while posterior fossa PA harbor BRAF fusion in up to 80–90% of cases, supratentorial PA show the fusion in only about 60% of cases.^{18,19} And, BRAF status alone is not able to distinguish PA and AA with piloid features because both tumor entities partially share the genetic characteristic of BRAF.⁶ On the other hand, the tumor classifier tool has potential to differentiate PA from IDH-wildtype gliomas including glioblastoma, AA with piloid features, and diffuse midline glioma with H3 K27M mutation.^{5,6} Also, the performance of the tool to differentiate medulloblastoma, embryonal tumor with multilayered rosettes, atypical teratoid/rhabdoid tumor, and ependymoma, all of which are major types of pediatric brain tumors, is clinically significant.⁵ In addition, CNV profile calculated from the same methylation array data (e.g. BRAF fusion, EGFR amplification, CDKN2A/B deletion, SMARCB1 deletion, C19MC amplification, etc.) is able to support the diagnosis. The tumor classifier tool is a promising approach not only to solve the problems in the diagnosis of PA, but also to classify pediatric brain tumors more robustly than the current WHO diagnostic scheme.

This case is an example of a successful diagnosis of PA by the new molecular diagnostic method, which was difficult to determine by a histological diagnosis. However, it needs to be clarified whether this diagnostic method can accurately predict clinical prognosis of the conventional PA entity, based on long-term follow-up results.

Conclusion

In this study, a new molecular diagnosis was applied in a PA cases where a discrepancy was observed between the conventional pathological diagnosis and the clinical pictures. The molecular diagnosis reflected the clinical pictures of the cases more accurately than the pathological diagnosis. However, it is necessary to validate the clinical usefulness of the new molecular diagnosis through accumulating the data before any conclusion.

Acknowledgments

The authors would like to sincerely thank Dr. Yoichi Nakazato, professor emeritus at the Department of Pathology, Gunma University Graduate School of Medicine, for conducting the central pathological diagnosis.

Molecular diagnosis in this paper was performed with a grant from the Akita University 2017 project competition (“advanced medicine” category).

Conflicts of Interest Disclosure

All authors report no conflicts of interest concerning this article.

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