Detailed molecular and pathological analyses of primary intracranial embryonal rhabdomyosarcoma with a **BRAF** mutation: illustrative case

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**BACKGROUND** The etiological significance of the RAS and PI3K pathways has been reported in systemic embryonal rhabdomyosarcoma (ERMS) but not in primary intracranial ERMS (PIERMS). Herein, the authors present a unique case of PIERMS with a **BRAF** mutation.

**OBSERVATIONS** A 12-year-old girl with progressive headache and nausea was diagnosed with a tumor in the right parietal lobe. Semi-emergency surgery revealed an intra-axial lesion that was histopathologically identical to an ERMS. Next-generation sequencing indicated a **BRAF** mutation as a pathogenic variation, but the RAS and PI3K pathways showed no alteration. Although there is no established reference class for PIERMS, the DNA methylation prediction was closest to that of ERMS, indicating the possibility of PIERMS. The final diagnosis was PIERMS. The patient underwent local radiotherapy (50.4 Gy) and multiagent chemotherapy, with no recurrence for 12 months after surgery.

**LESSONS** This may be the first case demonstrating the molecular features of PIERMS, especially the intra-axial type. The results showed a mutation in **BRAF** but not in the RAS and PI3K pathways, which is different from the existing ERMS features. This molecular difference may cause differences in DNA methylation profiles. Accumulation of the molecular features of PIERMS is necessary before any conclusions can be drawn.

https://thejns.org/doi/abs/10.3171/CASE23207

**KEYWORDS** rhabdomyosarcoma; next-generation sequencing; **BRAF**; methylation profile; intra-axial tumor

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Rhabdomyosarcoma (RMS) is the most common malignant soft tissue sarcoma in pediatric patients and is categorized into embryonic, alveolar, pleomorphic, and spindle cell types. In systemic cases of embryonic RMS (ERMS), genomic studies have identified the etiological significance of genes involved in the RAS pathway, those encoding effectors of the PI3K pathway, and those that control the cell cycle.1–3 However, there are few studies on the histopathological and molecular features of primary intracranial ERMS (PIERMS).

Here, we present a case of intra-axial PIERMS with unique molecular features. In the present case, a **BRAF** mutation, which has not yet been reported as a driver of ERMS, was identified as the pathogenic variant. The DNA methylation profile was similar to that of existing ERMS clusters; however, there was no established reference class. These findings may represent novel molecular features of previously uncharacterized PIERMS.

**Illustrative Case**

**History and Examination**

A 12-year-old girl had a headache accompanied by nausea that had gradually worsened over the previous 3 weeks. She had no notable medical, familial, or psychosocial history. Magnetic resonance imaging (MRI) performed at a local hospital revealed a right parietal tumor with intratumoral hemorrhage and signs of intracranial hypertension.

She presented with left hemianopia and constructional apraxia and was immediately admitted to the Akita University Hospital. Soon after, she began to present with consciousness disturbance...
and Cheyne-Stokes respiration, and semi-emergency surgery was decided upon. Preoperative MRI showed a relatively well-demarcated multilocular tumor in the right parietal lobe (Fig. 1A). The solid lesion was homogeneously enhanced (Fig. 1B and C) and hyperintense on diffusion-weighted imaging (Fig. 1D).

In addition to glioblastoma, pleomorphic xanthoastrocytoma, astroblastoma, and embryonal tumors were considered as preoperative diagnoses. We planned to perform maximum resection followed by an applicable after-treatment based on the resulting diagnosis.

Surgery was performed using the right parietal approach with the patient prone. The right parietal lobe was swollen (Fig. 2A), and a grayish intra-axial tumor was observed after corticotomy. The boundary with the normal cerebrum was relatively well demarcated compared with typical intra-axial tumors. The tumor was removed en bloc with some margins, and the solid lesion of the resected sample was elastic and hard (Fig. 2B), differing from typical intra-axial tumors.

**Histopathological Findings**

Hematoxylin-and-eosin staining revealed marked proliferation of monomorphic tumor cells with a high nuclear-to-cytoplasm ratio. The tumor contained elongated strap cells with eosinophilic cytoplasm (Fig. 3A). Geographic necrosis, microvascular proliferation, and brisk mitotic figures were observed. Immunohistochemical staining was positive for cytokeratin, vimentin, myogenin, desmin, BRAF V600E, and p53 (Fig. 3B and C) and negative for GFAP, Olig2, ATRX, synaptophysin, SMA, and MYOD1. These findings indicated a diagnosis of ERMS, rather than gliomas or other sarcomas. The Ki-67 index was 81.5% (Fig. 3D), suggesting high tumor malignancy.

**Molecular Findings**

We consulted Heidelberg University (Heidelberg, Germany) to perform molecular analyses using a next-generation sequencing (NGS) panel (Table 1) and a methylation-based classification. NGS demonstrated mutations in **BRAF** (V600E), **ESR1**, **POLD1**, **RB1**, and **TP53**. Among these, mutations in **BRAF**, **RB1**, and **TP53** were found to be relevant to tumorigenesis, and the allele frequency of these 3 genes exceeded 0.9. In contrast, mutations in the RAS family and the PI3K pathway, which are common mutations in ERMS, were not observed.

In the methylation-based classification, although the highest score for the methylation class was ERMS (score: 0.78; calibrated score threshold to decide methylation class family is ≥ 0.9), there was no established reference class. Two-dimensional t-distributed stochastic neighbor embedding (t-SNE) analysis of the RMS groups demonstrated that the present case was localized to the ERMS methylation class (Fig. 4A). However, the robustness of this result was limited by the small number of reference groups. Although the
copy number variation profile revealed several chromosomal gains and losses, including the loss of chromosome 7p, segmental loss of chromosome 7q, and loss of chromosomes 10 and 22q (Fig. 4B), these chromosomal alterations were not specific.

**Postoperative Course**

Postoperative MRI demonstrated that the enhancing lesion was completely removed. The symptoms of intracranial hypertension improved within a few days after surgery. General examination using contrast-enhanced computed tomography and $^{18}$F-fluorodeoxyglucose–positron emission tomography revealed no evidence of the systemic lesion. Based on the histopathological features, we concluded that the present case was PIERMS, and the clinical and molecular findings were consistent with it. The patient underwent local radiotherapy (50.4 Gy, 28 fractions) and multiagent chemotherapy by vincristine, inototecan, doxorubicin, cyclophosphamide, ifosfamide, and actinomycin, according to the standard treatment protocol for RMS.7 Twelve months after surgery, MRI showed no evidence of tumor recurrence.

**Discussion**

Here, we report a case of intra-axial PIERMS with unique molecular features. Although primary intracranial RMS is rare, a number of cases have already been reviewed. However, the histological subtype was not described in approximately half of the reported cases, and the tumors originating from intra- and extra-axial tissues were mixed. The histopathological and molecular features of PIERMS, as well as the differences between intra- and extra-axial cases, remain unclear. Thus, the current World Health Organization (WHO) diagnostic criteria define this tumor entity only histologically, and the present case was diagnosed accordingly.

NGS results indicated that mutations in BRAF, RB1, and TP53 were associated with tumorigenesis in the present case. A previous report described genetic abnormalities of the RB pathway and TP53 pathway as significant alterations associated with the cell cycle in ERMS. In that report, while 15% of the cases showed TP53 mutation, none of the cases showed RB1 mutation. Although direct mutation in RB1 seems to be rare, it is not uncommon as an abnormality of the entire RB pathway (~30%). It seems that such cell cycle activation is a molecular feature of all ERMS, and not only in intra-axial cases.

**BRAF** mutation was presumed to be the most significant variation in tumorigenesis in the present case. Genetic alterations in the RAS family, which regulates both the MAPK and PI3K pathways, are recognized as an etiological factor for tumorigenesis in ERMS.1-3 BRAF is located upstream of the MAPK pathway and is implicated in cell proliferation and differentiation via MEK/ERK activation. It was suggested that alteration of the MAPK pathway via

**TABLE 1. One hundred twenty-two genes included in the next-generation sequencing panel**

<table>
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<tr>
<th>ABL1</th>
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<th>AKT1</th>
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<td>ATR</td>
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the BRAF mutation contributed to tumorigenesis in the present case. However, tumorigenesis in ERMS driven by BRAF mutations alone has not been previously reported. Further studies are needed before conclusions can be drawn.

Focusing on the PI3K pathway, there was no evidence of an abnormality in the present case. The PI3K pathway is one of the representative oncogenic pathways that regulates essential cellular functions, including growth, metabolism, survival, and proliferation. In addition, some previous reports have indicated that the PI3K pathway could mediate DNMT and TET expression and promote transcriptional competence via regulation of DNA methylation status. There is a possibility that the differences in PI3K status in the present case and the existing ERMS cases could explain the difference in DNA methylation profiles between them.

However, careful attention should be paid to the possibility of a rare tumor not fitting into the current WHO classification and not being represented in the reference set of the classifiers. Although the methylation profile was supportive in the present case, this alone could not clearly indicate a diagnosis of PIERMS; therefore, the diagnosis of other sarcomas, not classified elsewhere, cannot be altered. It is necessary to construct a more robust methylation class through additional accumulation of intracranial sarcoma cases, especially sarcomas with rhabdomyoid morphology and no known extracranial site of origin.

**Observations**

Integrating the comprehensive information presented herein, the final diagnosis of the present case was intra-axial PIERMS. The characteristics of the present case were as follows: 1) BRAF mutation as a pathogenic variant, 2) normal PI3K pathway status, and 3) a differential DNA methylation profile compared with existing ERMS. To the best of our knowledge, the present case is the first of an intra-axial PIERMS with a BRAF mutation. Since there are few studies on PIERMS, it is still unclear whether the above-described molecular features are also observed in other intra-axial cases or whether the present case is a previously unestablished sarcoma. The molecular characteristics of PIERMS need to be comprehensively elucidated through the accumulation of additional cases.
Lessons

Here, we present a case of intra-axial PIERMS with unique molecular features. Tumorigenesis in the present case was presumed to be driven by activation of the MAPK pathway via a BRAF mutation. There was no evidence of a PI3K pathway abnormality, which could explain the difference in DNA methylation profiles between the present case and the existing ERMS cluster. It is possible that these findings are previously undescribed molecular features of intra-axial PIERMS or intracranial sarcoma, as in the present case, and form a previously unestablished sarcoma class. Therefore, informing molecular diagnostics is extremely important for future case reports, case series, and rigorous clinical investigations.

Acknowledgments

Takahiro Ono has received support from the Ministry of Education, Culture, Sports, Science and Technology (MEXT), Japan Society for the Promotion of Science (JSPS, KAKENHI, no. JP19K16823).

References


Disclosures

Dr. von Deimling reported a patent for a DNA-methylation–based method for classifying tumor species EP16710700 issued.

Author Contributions

Conception and design: Ono, Abe, M Takahashi. Acquisition of data: Ono, Abe, M Takahashi, Kodama, von Deimling. Analysis and interpretation of data: Ono, Abe, Hinz, M Takahashi, Hiroshima, Nanjo, von Deimling, Shimizu. Drafting of the article: Ono, Abe, Hinz, Yano, Shimizu. Critically revising the article: Ono, Abe, Hinz, Yano, von Deimling, Shimizu. Reviewed submitted version of the manuscript: Ono, Yano, von Deimling, Shimizu. Approved the final version of the manuscript on behalf of all authors: Ono. Study supervision: T Takahashi, Shimizu.

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