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ONCOLOGY: BRIEF REPORT

Malignant brain tumor in an infant showing histopathological features of yolk sac tumor but genetic and epigenetic features of AT/RT

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Abstract

SMARCA4 pathogenic variants are rarely detected in pediatric brain tumors other than atypical teratoid rhabdoid tumors (AT/RTs) without INI1 deficiency or in some cases of medulloblastoma. Here, we report an atypical intracranial immature teratoma that recurred as a yolk sac tumor with metastatic spinal and lung lesions. Sequencing of the tumor revealed two *SMARCA4* variants, including a splice-site variant and a non-synonymous variant of uncertain significance. Additionally, the methylation signature of the tumor was close to that of AT/RTs. Our case might be a yet-unrecognized subtype of pediatric tumors in which inactivation of *SMARCA4* contributes to the pathogenesis.

KEYWORDS
atypical teratoid rhabdoid tumor, intracranial germ cell tumor, methylation analysis, *SMARCA4*

1 | INTRODUCTION

Comprehensive molecular analyses of pediatric brain tumors have identified various tumor-specific pathogenic variants and have revealed new entities, which has advanced our understanding of tumor biology and therapeutic approach.[1–3] BRG1 and INI1, encoded by *SMARCA4* and *SMARCB1*, respectively, are components of the SWI/SNF complex, and atypical teratoid rhabdoid tumors (AT/RT) are characterized by loss of function of either of these two genes. Additionally, *SMARCA4* variation has been detected in some cases of medulloblastoma, but it is rarely observed in other pediatric brain tumors.[4–6] Here, we report a case with an atypical germ cell tumor

Abbreviations: AFP, alpha-fetoprotein; AT/RT, atypical teratoid rhabdoid tumors; SCCOHT, small-cell carcinoma of the ovary of the hypercalcemic type; *t*-SNE, *t*-distributed stochastic neighbor embedding.

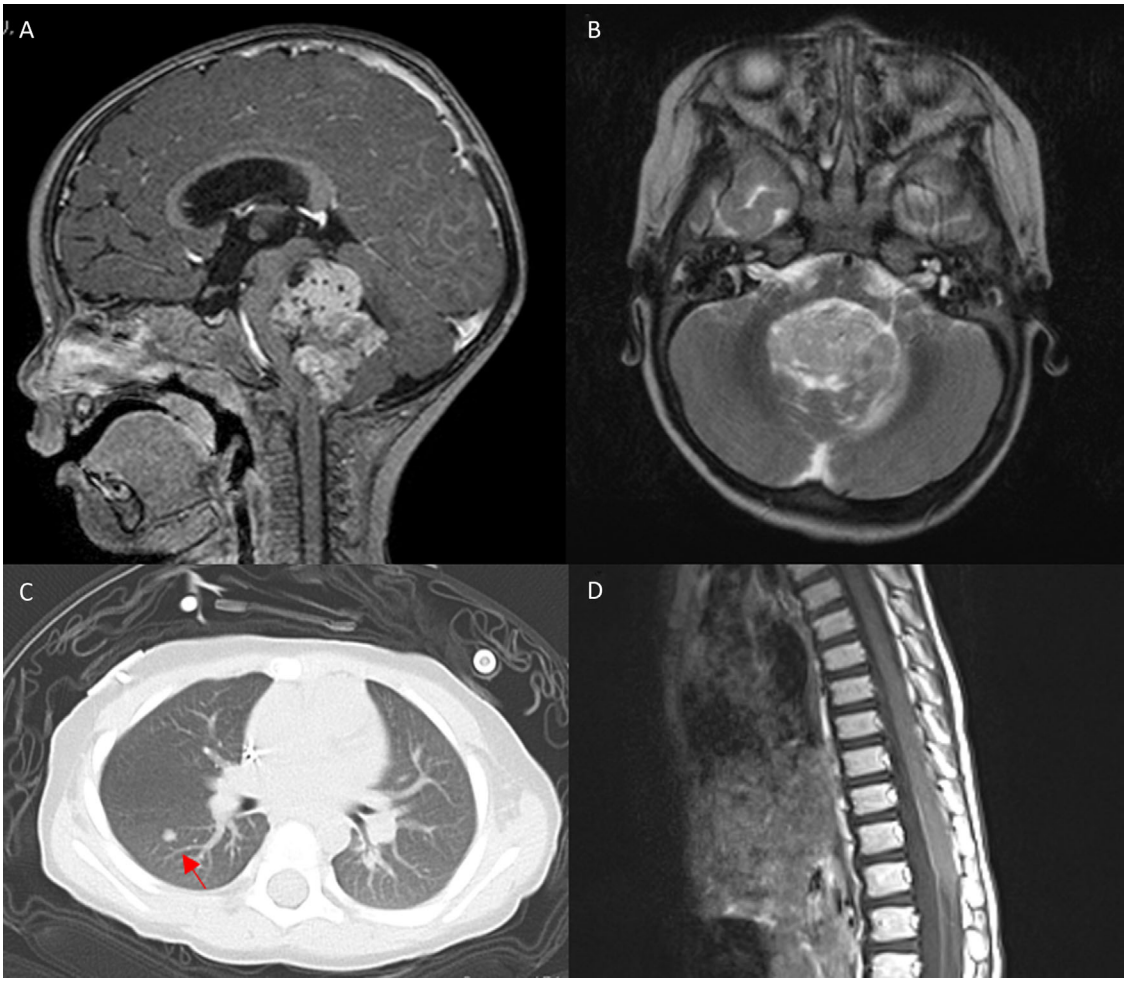

FIGURE 1 (A and B) Magnetic resonance imaging (MRI) findings of the primary lesion. An enhanced tumor was observed in the fourth ventricle. (C) Lung lesions were detected 10 months after the initial diagnosis. Representative slice of computed tomography (CT) imaging of the multiple lung lesions. The arrow indicates one of the nodular lesions. (D) A metastatic spinal lesion was detected on MRI 12 months after the initial diagnosis

harboring abnormal sequences of *SMARCA4* in which the methylation signature was close to AT/RT.

2 | RESULTS

2.1 | Clinical summary

A 1-year-old boy, who presented with persistent vomiting, was diagnosed with brain tumor of the fourth ventricle and underwent partial tumor resection (Figure 1A,B). The serum alpha-fetoprotein (AFP) level was 40.9 ng/ml (within the normal range for his age) and human chorionic gonadotropin (hCG) was negative. He was diagnosed with an immature teratoma and received chemotherapy, radiotherapy, and repeat surgery. Although the primary lesion was refractory to carboplatin, etoposide, and ifosfamide (CE/IE/ICE), it responded to gemcitabine, paclitaxel, and oxaliplatin (GemPOx) along with radiotherapy. However, 10 months after the initial diagnosis, several progressive lung nodules were observed, and 2 months later a metastatic spinal lesion was detected (Figure 1C,D, Figure S1). The patient died of the disease 15 months after the initial diagnosis.

2.2 | Pathological findings

The tumor sample obtained at initial surgery was composed of variable components. Pathological examination showed immature neuroectodermal cells with a high nuclear–cytoplasmic ratio and dense proliferation along the neural rosettes (Figure 2A). Stratified squamous epithelium, columnar epithelium, and cartilaginous tissue were observed (Figure 2B). A yolk sac tumor-like area consisting of reticular and trabecular structures was seen in a small part of the tissue (Figure 2C). Immunohistochemically, the tumor cells were positive for INI1 and negative for AFP. The MIB-1 labeling index was as high as 80% in the immature components. The tumor was pathologically diagnosed as an immature teratoma with focal embryoid components by both institutional and central pathologists. A sample obtained from the local recurrent lesion after chemotherapy, 8 months after the initial diagnosis,

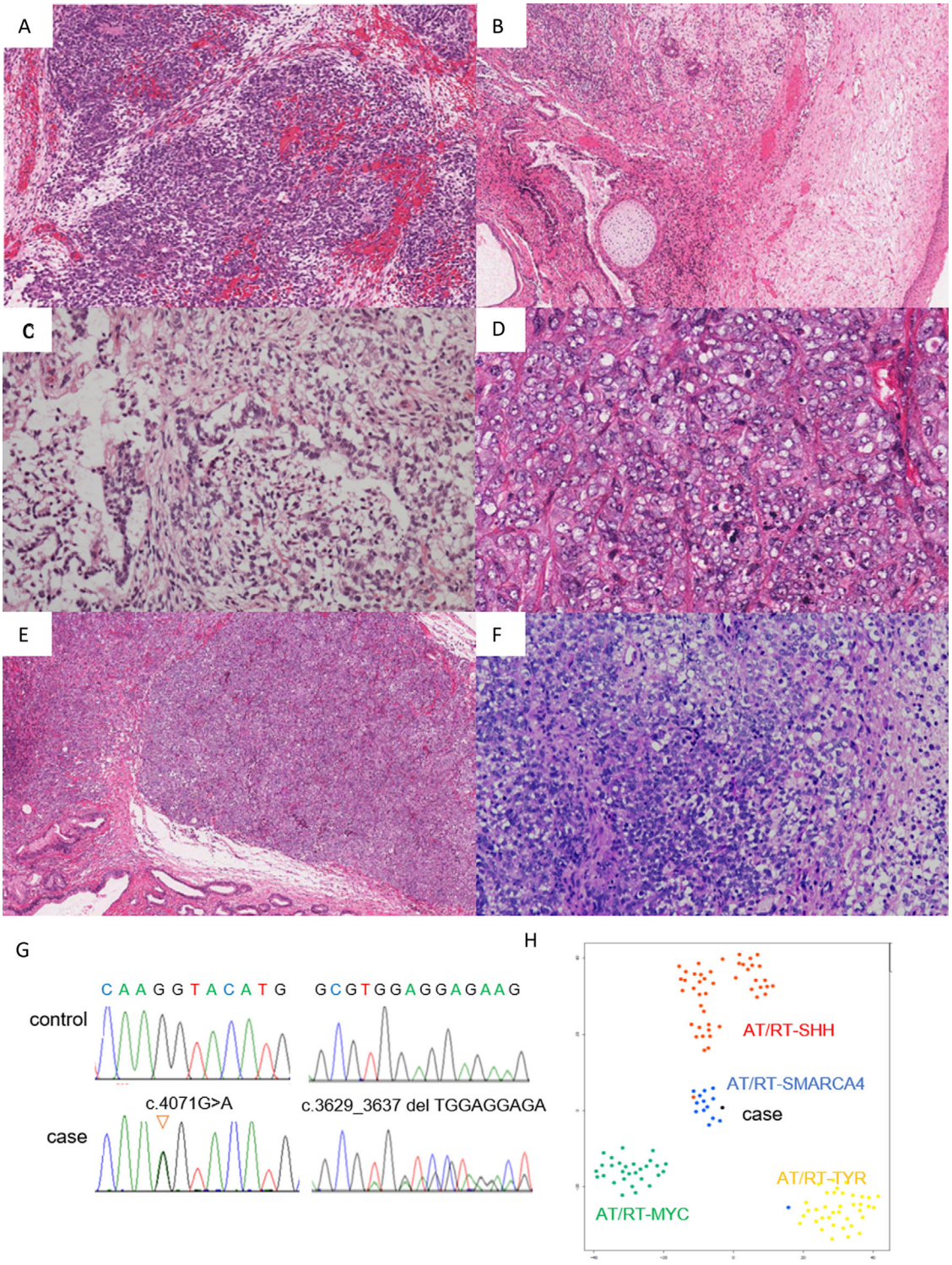

FIGURE 2 Pathological (A–F) and molecular (G and H) analysis of the tumor. Microscopic appearance of the tissue sample obtained from the primary lesion at initial surgery (A–C), repeat surgery (D and E), and the metastatic spinal lesion (E). The tumor showed variable features including densely proliferating cells with neuronal rosettes (A), stratified squamous epithelium, columnar epithelium, and cartilaginous tissue (B). In a small part of the sample, embryonal components were also observed (C). The tumor was mainly composed of the yolk component (D), while columnar epithelium was observed in part (E). The spinal metastatic lesion was compatible with a solid pattern yolk sac tumor (F). *SMARCA4* splice-site mutation and non-synonymous mutation were validated by Sanger sequencing. (H) *t*-Distributed stochastic neighbor embedding (*t*-SNE) plot showing clustering of the present case (black) in relation to 112 cases of teratoid rhabdoid tumors (AT/RT), which were reported as AT/RT-SHH, AT/RT-TYR, or AT/RT-MYC by dkfz classifier and 14 SMARCA4-deficient AT/RT (AT/RT-SMARCA4). The present case was plotted close to AT/RT-SMARCA4

mainly demonstrated yolk sac-like tumor cells with swollen nuclei and clear cytoplasm, proliferating in sheets (Figures 2D,E). These cells were positive for SALL4 and Glypican3 but negative for AFP or AE1/AE3 cytokeratin. Columnar epithelium was partially observed. Tumor samples from the metastatic spinal lesion demonstrated distinct sheet-like proliferation of cells harboring a vesicular nucleus and clear cytoplasm (Figure 2F). These cells were positive for SALL4, Glypican3, and SMARCA4/BRG1. They were negative for EMA, synaptophysin, GFAP, and neurofilament, which are commonly positive in AT/RT (Figure S3). The metastatic lesion was pathologically diagnosed as a yolk sac tumor.

2.3 | Molecular analysis

DNA was extracted from the metastatic spinal tumor sample. All coding regions and 25 bp exon–intron boundaries of the 147 genes listed in Table S1 were sequenced by the sequence capture method (Agilent Technologies, CA, USA), using NextSeq500 (Illumina, CA, USA). Consequently, the splice-site variant c.4071G>A at the exon27–intron27 boundaries of *SMRCA4* (NM_001128845) and 10 non-synonymous variants of uncertain significance, including *SMARCA4*, p.Val1210_Lys1213delinsGlu (c.3629_3637del TGGAGGAGA) (NM_001128845) exon25 were identified (Table S2). These two *SMARCA4* variants were confirmed by Sanger sequencing (Figure 2G). DNA methylation-based classification and copy number analysis were performed using an Infinium Human MethylationEPIC BeadChip array (Illumina, San Diego, CA, USA) and an online DNA methylation-based classifier developed by the DKFZ (https://www.molecularneuropathology.org/mnp). The tumor was classified as a methylation class family AT/RT, with a low calibrated score of 0.76; subclass SHH 0.5 and subclass MYC 0.21. Partial deletions of chromosome 4q and chromosome 8p were detected (Figure S2). Raw IDAT file of the tumor was processed using the minfi package (version 1.34.0) in R statistical environment (version 4.0.2), following which *t*-distributed stochastic neighbor embedding (*t*-SNE) cluster analysis was performed with 126 AT/RT samples, including SMARCA4-deficient cases. The *t*-SNE showed that the present case plotted close to the SMARCA4-deficient AT/RTs (Figure 2H). Unprocessed IDAT files of 112AT/RT samples (SHH, TYR, and MYC) and 14 SMARCA4-deficient cases were downloaded from the NCBI Gene Expression Omnibus (GEO) with accession numbers GSE90496 and GSE161692, respectively.[3,7]

3 | DISCUSSION

We have described a case with an atypical germ cell tumor harboring *SMARCA4* alternations. Analysis of the recurrent tumor revealed that it was mainly composed of yolk sac tumor component, and demonstrated two variants of *SMARCA4*: splice-site variant (c.4071G>A) and non-synonymous variant (c.3629_3637del TGGAGGAGA). We initially considered the pathogenic significance of these variants to be indefinite. However, subsequent methylation analysis revealed that the tumor was clustered very close to AT/RT, which are characterized by the loss of expression of either SMACRB1/INI1 or SMARCA4/BRG1. Moreover, it has been reported that the methylation signature of small-cell carcinoma of the ovary of the hypercalcemic type (SCCOHT), which is characterized by a loss of expression of SMARCA4/BRG1, is close to AT/RT, and splice-site variants in *SMARCA4* are reported in some cases of SCCOHT.[8,9] Accordingly, we speculated that both or at least one of the detected *SMARCA4* variants may be associated with the pathogenesis in this case.

However, SMARCA4/BRG1 was positive in the nuclei of the tumor cells in the present case, and a loss of expression was not validated immunohistochemically. This might be because the antibody corresponding to the residue 214–279 (exon 4–5) might not be suitable to detect the abnormalities. Furthermore, it has been reported that SMARCA4/BRG1 staining is not as sensitive as SMARCB1/INI1 staining; hence, some cases of AT/RT with pathogenic *SMARCA4* variants may be positive for SMARCA4/BRG1.[10,11] Therefore, positive SMARCA4/BRG1 staining does not necessarily exclude the possibility of its loss of function of SMARCA4/BRG1.

Although the results of molecular findings and clinical presentation of the present case seemed to be compatible with AT/RT, the pathological findings including the results of immunohistochemical staining that was positive for INI1 or BRG1, indicated germ cell tumor and were unusual for AT/RT. Interestingly, there is a case report of a pediatric patient with an INI1-deficient inguinal tumor with lung metastasis, which was pathologically diagnosed as a yolk sac tumor.[12] There is also a report of infantile pulmonary teratoid tumors with biallelic *SMARCA4* variants, one was germline and the other somatic.[13] These and our case suggest the existence of a yolk sac-like or teratoid tumor, which is driven by the loss of function of the SWI/SNF complex. Furthermore, several cases of SMARCA4-deficient SCCOHT accompanying immature teratomas and yolk sac tumors have been reported, and possibility of existence of shared tumor cell origin has been discussed.[14] In the present case, a possibility exists that the patient had a *SMARCA4* germline pathogenic variant and the lung lesion was a second tumor (not metastatic). However, this cannot be confirmed as the samples of the germline and lung lesion were unavailable, which is a limitation of this study. Recognizing and performing further analysis of similar cases may be important for selecting therapeutic options based on tumor biology, such as, EZH2 inhibitors.[15,16] Furthermore, performing surveillance according to the result of germline genetic testing would potentially benefit the patients.

In summary, we have reported a case, pathologically diagnosed as germ cell tumor, in which genetic and epigenetic findings indicated *SMARCA4*-deficient AT/RT. Our case emphasizes the importance of molecular analysis and suggested the existence of a rare brain tumor entity, which has pathological feature of yolk sac tumor and molecular feature of AT/RT.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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