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CASE REPORT

Intracranial Ewing sarcoma with whole genome study

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Abstract

Introduction Ewing sarcoma (ES) as a primary intracranial tumor is very rare. Recently, CNS embryonal tumors with ES-like genomic change have been reported. Patients and methods We report a case of intracranial Ewing sarcoma in a 13-year-old girl who complained of headache and migraine. The tumor had developed in the right middle cranial fossa with a mass effect on the brain with impending transuncal herniation.

Results Undifferentiated small round cell morphology with completely negative results for friend leukemia integration 1 transcription factor (Fli-1) and a nonspecific cytoplasmic CD99-positive staining pattern mislead the diagnosis as central nervous system (CNS) embryonal tumor, NOS. However, whole genome sequencing (WGS) revealed *Ewing sarcoma* (*EWS*)-*Fli-1* gene fusion, which was confirmed by fluorescence in situ hybridization study and the diagnosis was revised to ES.

Conclusions This case is a true intracranial but extra-axial ES confirmed by WGS. We report this case of intracranial ES to demonstrate the importance of marker gene studies using FISH or NGS.

Keywords Ewing sarcoma · CNS embryonal tumor · Genetics · EWSR1 · Next-generation sequencing · Whole genome sequencing

Introduction

Ewing sarcoma (ES)/peripheral primitive neuroectodermal tumor (pPNET) is a highly malignant small round cell tumor predominantly affecting bone and soft tissue of children and adolescents and young adults (AYA) aged 0–20 years [1–3]. Pathological diagnosis requires objective evidence-based studies such as fluorescence in situ hybridization (FISH) or genetic studies due to diagnostic difficult.

Genetically, Ewing sarcoma breakpoint region 1 (EWSR1) and various partner gene fusions are well known in this tumor. Approximately 85–90% of ES/pPNETs contain a t(11;22) chromosomal translocation, 10–15% have a t(21;22) translocation, and < 1% have other translocations such as t(2;22), t(7;22), t(17;22), or inversion of chromosome 22 [1–3].

We report a case of intracranial ES with whole genome sequencing (WGS), which revealed Ewing sarcoma (EWS)-Fli-1 fusion. Recently, central nervous system (CNS) embryonal tumor with ES-like genomic change was reported [4], but this case was a true extra-axial intracranial ES.

Case report

A 13-year-old girl was transferred to the Department of Emergency of Seoul National University Hospital (SNUH) due to an intractable headache and migraine for 1 week, and the sudden development of diplopia 1 day prior.

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She was born at gestational age (GA) of 39 weeks with a birth weight of 3.98 kg by Cesarean section due to a breech presentation. She had been healthy, but at her age of 9-year-old, the xanthogranulomatous inflammation of the pituitary stalk was surgically removed. She had received growth hormone therapy for panhypopituitarism. She showed normal growth and development without complaint of any symptoms during the follow-up period until headache and migraine developed at her age of 13-year-old.

Magnetic resonance imaging (MRI) showed a large mass on the right middle cranial fossa with a mass effect on the right temporal lobe with impending transuncal herniation. The mass showed internal heterogeneous enhancing solid portion. The radiological diagnosis was extra-axial tumor extending into the right temporal lobe, diffuse pachymeningeal enhancement, and adjacent brain parenchymal edema, suggesting a dura-origin (Fig. 1).

Emergency tumor removal was performed due to impending uncal herniation. Right temporal craniotomy was performed with the aid of neuro-navigation. Intraoperative neurophysiological monitoring was applied. The inferior-posterior site of the temporal bone showed a thinning change. After craniotomy, yellowish to pinkish large mass was revealed (Fig. 2a). The mass appeared to involve the skull and originate from the dura. We performed internal debulking of the epidural mass. It was extended to the subdural space through dural defect. Semicircular dural incision was performed and a subdural mass was removed. The mass was located extra-axially and clearly demarcated from the brain parenchyma (Fig. 2b). The mass was radically removed. The herniated uncus was suctioned with ultrasonic aspirator. The involved dura was excised and the invaded skull base bone was drilled out.

Histopathological examination revealed sheets of undifferentiated small round blue cells tumor, with extensive necrosis, which involved the skull bone. The cells have uniform rounded nuclei with fine chromatin. High mitotic rate was observed (8/10 high-power fields). The immunohistochemical test showed complete negativity for Fli-1, glial fibrillary acidic protein (GFAP), and retained expression of INI-1 and BRG1 (Fig. 3). Membranous CD99 immunostaining was focal and most cells showed nonspecific cytoplasmic staining, possibly due to the scanty cytoplasm and/or extensive necrosis (Fig. 3). Primary antibodies used and the results in this case are summarized in Table 1. The initial diagnosis of a central nervous system (CNS) embryonal tumor, NOS, was revised to ES after WGS of genomic tumor DNA extracted from fresh-frozen tissue revealed an *EWS-Fli-1* fusion of EWSR1 on chromosome 22 (position 29683929) and Fli-1 on chromosome 11 (position 128671445) (Fig. 4). This fusion was confirmed by FISH showing an EWSR1 gene (22q12) translocation (Fig. 3). No significant single nucleotide polymorphisms, insertion or deletions, copy number variations, or fusions other than the EWS-Fli-1 were observed, but an additional copy of chromosome 19q13.42 microRNA clustered region (C19MC) (chromosome 19q13.42 start, 54059940; end, 54600642) was detected.

Concurrent chemotherapy and local radiation therapy with autologous peripheral blood stem cell mobilization were administered. Radiation dose on the right temporal base was total 6120 cGy/34 fx. Four cycles of POG-VICE (carboplatin 635 mg/m^2 on day 3, etoposide 100 mg/m^2 on days 1–3, ifosfamide 2000 mg/m^2 on days 1–3, vincristine 1.5 mg/m^2 (max 2 mg)) on day 1, two cycles of KSPNO-S1101 (cisplatin 60 mg/m^2 on day 1, cyclophosphamide 1000 mg/m^2 on days 1

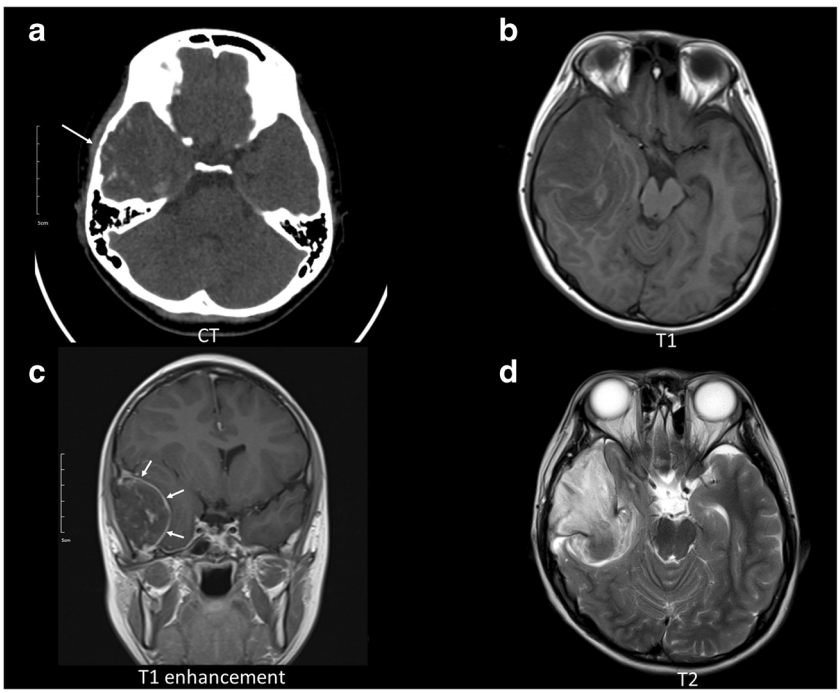

Fig. 1 Magnetic resonance images. **a** CT scan shows a dura-based mass with calcification and hemorrhage in the right middle cranial fossa. Thinning of the right temporal skull (arrow) with permeable bone destruction is noted. **b** T1-weighted MR image shows low-signal intensity mass with mass effect on the right temporal lobe with impending transuncal herniation. **c** T1 enhancement MR image shows focal heterogeneous enhancement with diffuse pachymeningeal enhancement (arrows). **d** T2-weighted MR image shows an about 5.5 × 7.5-cm size dural based mass in the right middle cranial fossa, with hemorrhage

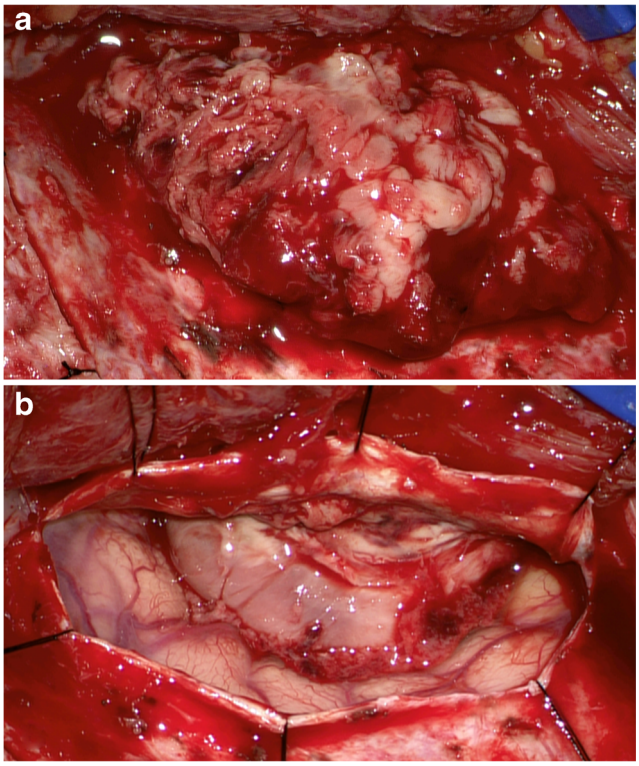

Fig. 2 **a** After craniotomy, the exposed tumor shows yellowish pink solid and hemorrhagic appearance, which extended to the subdural space through dural defect. **b** After debulking of epidural tumor with attached dura matter, the brain looks depressed but the pial surface of cortex is free from tumor without surgical defect because of well demarcation of mass from the brain parenchyma

and 2, etoposide 100 mg/m^2 on days 1–3, vincristine 1.5 mg/m^2 (max 2 mg)) on days 1 and 8, and tandem high-dose chemotherapy with autologous stem cell rescue were done. Busulfan (120 mg/m^2) was administered as a starter dose on day 8 and once daily thereafter. The dose of busulfan was analyzed for the therapeutic drug monitoring (TDM). The total target area under the curve (AUC) of busulfan was set at 74,000 to 76,000 μg × h/L and melphalan 70 mg/m^2 was given on days 2 and 3. Second melphalan 140 mg/m^2 was given on day 7 and 70 mg/m^2 on day 6, etoposide 200 mg/m^2 on days 5–8 (total 4 days), and carboplatin 350 mg/m^2 on days 5–8 (total 4 days).

Pachymeningeal dissemination of the right middle cranial fossa found on the initial MRI was not shown in the postoperative MRI.

The patient has been relatively well with a second peripheral blood stem cell transfusion at 15 months after surgery. There was no tumor recurrence, but there was retinal hemorrhage and decreased hearing ability of the right ear. Her panhypopituitarism was controlled by minirin (0.0125T bid).

Discussion

ES/pPNETs are rare tumors in children and AYA, with a peak incidence at age 15 [5]. It occurs in 1–3 individuals per million annually in the Western Hemisphere and has a slight male predominance (M:F ratio, 1.4:1). ES/pPNETs usually occur in the long bones [5]. Extraosseous intracranial ES/pPNET is very rare, so far, fewer than 50 cases have reported in the English literature and half of which with genetic studies [5–13].

Recently, Cherif El Asri et al. comprehensively reviewed the previously reported primary intracranial ES/pPNET and Mobley et al. summarized intradural extramedullary spinal ES/pPNET [10, 13]. They tended to develop in children and

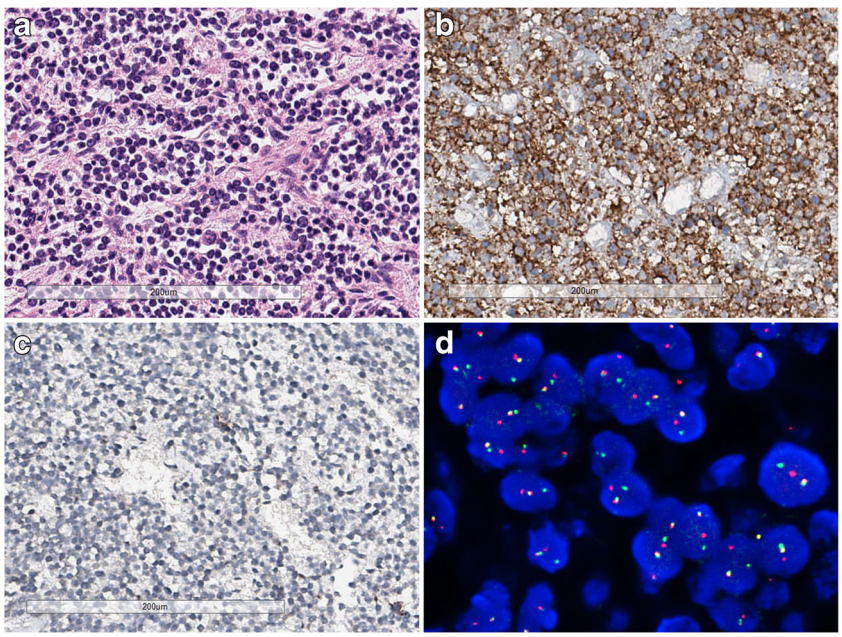

Fig. 3 Pathological findings. **a** Viable area of the tumor shows a sheet of small round cells with uniform round nuclei. **b** CD99 immunostaining in viable area shows membranous staining pattern. **c** Fli-1 is completely negative in the tumor cells despite of repeat stainings. **d** EWSR1 FISH study exhibited 1 yellow or fusion signal, 1 red, and 1 green (break-apart) signal

Table 1 Antibodies used in this case (alphabetical order)

Antibody	The result in our cases	Dilution	Source
Brahma-related gene-1 (BRG1)	+ (retained)	1:1000	Millipore, Temecula, USA
CD34	–	1:200	Dako, Glostrup, Denmark
CD99	+ (actually membranous)	1:50	Novocastra (Leica), Muchen, Germany
Fli-1	–	1:30	Becton Dickinson, Franklin Lakes, USA
GFAP	–	1:200	Dako, Glostrup, Denmark
Histone 3 K27M mutant (H3 K27M)	–	1:1000	Millipore, Temecula, USA
Isocitrate dehydrogenase 1 (IDH1)	–	1:100	Dainova, Hamburg, Germany
Integrase interactor 1 (INI-1)	+ (retained)	1:100	Cell Marque, Rocklin, USA
Ki-67	14.4% in the hot spot	1:100	Dako, Glostrup, Denmark
Lin-28 homolog A (Lin28A)	–	1:100	Cell signaling
Olig2	–	1:500	Cell Marque, Rocklin, USA
p53	+	1:1000	Dako, Glostrup, Denmark
pHH3	15/10HPF	1:5000	Cell Marque, Rocklin, USA
Synaptophysin	Focal +	1:200	Novocastra, Newcastle, UK
S-100 protein	Focal +	1:3000	Dako, Glostrup, Denmark
Vimentin	Diffuse +	1:500	Dako, Glostrup, Denmark

+, positive; −, negative

AYA (median age, 15 years old; age range, 1~67 years old). About 80% were under 20 years old. Spinal ES/pPNET occurred in little bit older patients than intracranial tumors (median, 31 years old; range, 11~52 years old). They occurred in the coverings of the brain (40% in convexity, 15% in the anterior and middle skull base, 11% in the tentorium, 10% in the para-falcian, 15% in the posterior fossa, and 8% in the cerebellopontine angle) as well as the dura of the spinal cord. Patients usually presented with symptoms and signs of increased intracranial pressure (56%), such as headache, nausea

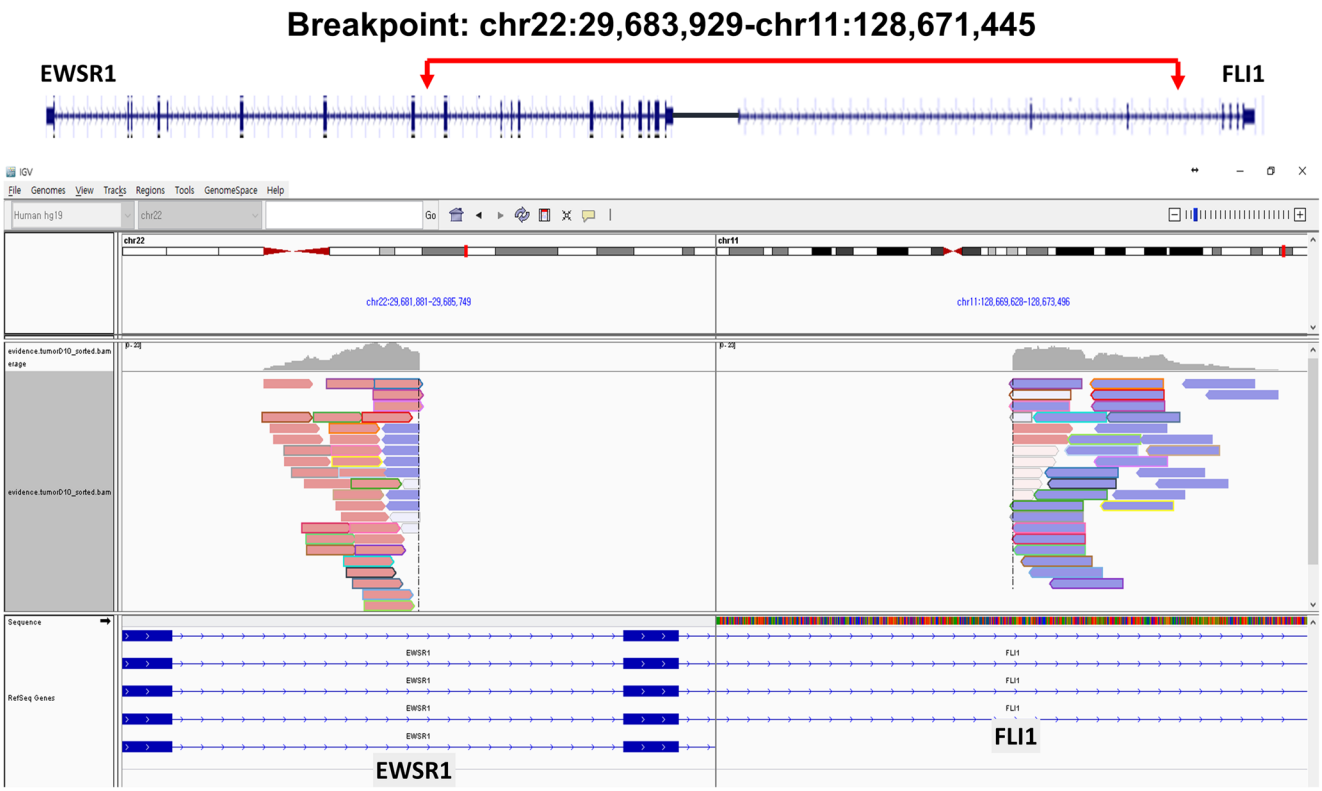

Fig. 4 Whole genome sequencing. Integrative genomic view (IGV) shows spanning fragment of EWSR1 and Fli-1 fusion

and vomiting and neurologic deficit (21%) or seizures (about 8%), or masses of the scalp swelling (about 10%) while spinal tumors presented with low-back pain, numbness, or urinary incontinence, which showed lumbar predominance (lumbar, 63%; cervical, 25%; and thoracic, 13%).

In imaging study, the T1-low or isointense and T2-high intense (70% of the tumor) extra-axial enhancing tumors often pressurized the brain and spinal cord making a remarkable peri-tumoral edema or brain shift. However, the remained 30% can show T2-low or iso-signal intensity. Cystic component and bone involvement were found in 27.5% of and 28.5% of the patients; however, calcifications were very rare, found in only one case (2%).

Combining maximal surgical resection, chemotherapy, and radiation therapy is the main strategy of treatment. Chemotherapy was alternated with vincristine, cyclophosphamide, doxorubicin, ifosfamide, etoposide, or actinomycin D. Most patients received topical radiation therapy, but exceptionally, in rare cases, craniospinal radiation was given.

Of the 44 patients, 73% had no tumors during the 12-month follow-up (up to 20 years) and 27% died or recurred within the first few years after diagnosis. Cherif El Asri et al. recommended radiation and chemotherapy whenever tumors were incompletely removed [13].

While the origin of extraskeletal ES/pPNET has not been clearly elucidated, the presumptive precursor cells are probably neural crest or mesenchymal stem cell origin [11, 14].

ES/pPNET characteristically expresses a fusion of the EWSR1 gene (22q12) with a member of the ETS gene family, including Fli-1 (11q24), ERG (21q22), ETV1 (7p22), ETV4 (E1AF, 17q12), and FEV (2q36) [5].

CNS embryonal tumor, atypical teratoid/rhabdoid tumor (ATRT), and ES/pPNET were considered for differential diagnosis in our patient because CD99-positive pattern was not clearly membranous, except a small area, most-likely due to diffuse necrosis. Fli-1 remained negative even in repeat tests. WGS revealed a breakpoint in chromosomes 22 (EWSR1) and 11 (Fli-1), which was confirmed by FISH study.

The differential diagnosis of intracranial ES from CNS embryonal tumor, NOS, is important because intracranial ES does not require craniospinal irradiation (CSI), whereas this CSI should be a standard treatment for CNS embryonal tumor. ES often metastasizes by hematogenous spread to the bones and lungs compared to craniospinal metastasis in CNS embryonal tumors. Our case involved the skull bone but not the underling brain and a recent review has shown that infratentorial location or skull bone involvement in intracranial ES is associated with poor prognosis such as tumor recurrence or short survival [13].

We report this case of intracranial ES to demonstrate the importance of marker gene studies using FISH or NGS.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Informed consent The authors got the informed consent from the patients.

References

1. Maire G, Brown CW, Bayani J, Pereira C, Gravel DH, Bell JC, Zielenska M, Squire JA (2008) Complex rearrangement of chromosomes 19, 21, and 22 in Ewing sarcoma involving a novel reciprocal inversion-insertion mechanism of EWS-ERG fusion gene formation: a case analysis and literature review. Cancer Genet Cytogenet 181:81–92
2. Charville GW, Wang WL, Ingram DR, Roy A, Thomas D, Patel RM, Hornick JL, van de Rijn M, Lazar AJ (2017) EWSR1 fusion proteins mediate PAX7 expression in Ewing sarcoma. Mod Pathol 30:1312–1320
3. Smith SC, Palanisamy N, Martin E, Almenara J, McHugh JB, Choi EK, Lucas DR, Betz BL, Thomas D, Patel RM (2017) The utility of ETV1, ETV4 and ETV5 RNA in-situ hybridization in the diagnosis of CIC-DUX sarcomas. Histopathology 70:657–663
4. Sturm D, Orr BA, Toprak UH, Hovestadt V, Jones DTW, Capper D, Sill M, Buchhalter I, Northcott PA, Leis I, Ryzhova M, Koelsche C, Pfaff E, Allen SJ, Balasubramanian G, Worst BC, Pajtler KW, Brabetz S, Johann PD, Sahm F, Reimand J, Mackay A, Carvalho DM, Remke M, Phillips JJ, Perry A, Cowdrey C, Drissi R, Fouladi M, Giangaspero F, Lastowska M, Grajkowska W, Scheurlen W, Pietsch T, Hagel C, Gojo J, Lotsch D, Berger W, Slavc I, Haberler C, Jouvet A, Holm S, Hofer S, Prinz M, Keohane C, Fried I, Mawrin C, Scheie D, Mobley BC, Schniederjan MJ, Santi M, Buccoliero AM, Dahiya S, Kramm CM, von Bueren AO, von Hoff K, Rutkowski S, Herold-Mende C, Fruhwald MC, Milde T, Hasselblatt M, Wesseling P, Rossler J, Schuller U, Ebinger M, Schittenhelm J, Frank S, Grobholz R, Vajtai I, Hans V, Schneppenheim R, Zitterbart K, Collins VP, Aronica E, Varlet P, Puget S, Dufour C, Grill J, Figarella-Branger D, Wolter M, Schuhmann MU, Shalaby T, Grotzer M, van Meter T, Monoranu CM, Felsberg J, Reifenberger G, Snuderl M, Forrester LA, Koster J, Versteeg R, Volckmann R, van Sluis P, Wolf S, Mikkelsen T, Gajjar A, Aldape K, Moore AS, Taylor MD, Jones C, Jabado N, Karajannis MA, Eils R, Schlesner M, Lichter P, von Deimling A, Pfister SM, Ellison DW, Korshunov A, Kool M (2016) New brain tumor entities emerge from molecular classification of CNS-PNETs. Cell 164:1060–1072
5. Tanboon J, Sitthinamsuwan B, Paruang T, Marrano P, Thorner PS (2012) Primary intracranial Ewing sarcoma with an unusually aggressive course: a case report and review of the literature. Neuropathology 32:293–300
6. Chebib I, Jo VY (2016) Round cell sarcoma with CIC-DUX4 gene fusion: discussion of the distinctive cytomorphologic, immunohistochemical, and molecular features in the differential diagnosis of round cell tumors. Cancer Cytopathol 124:350–361
7. Picarsic J, Reyes-Mugica M (2015) Phenotype and immunophenotype of the most common pediatric tumors. Appl Immunohistochem Mol Morphol 23:313–326
8. Antonelli M, Caltabiano R, Chiappetta C, Oliva MA, Giangaspero F, Lanzafame S (2011) Primary peripheral PNET/Ewing's sarcoma arising in the meninges, confirmed by the presence of the rare translocation t(21;22) (q22;q12). Neuropathology 31:549–555

9. Kleinschmidt-DeMasters BK, Lovell MA, Donson AM, Wilkinson CC, Madden JR, Addo-Yobo SO, Lillehei KO, Foreman NK (2007) Molecular array analyses of 51 pediatric tumors shows overlap between malignant intracranial ectomesenchymoma and MPNST but not medulloblastoma or atypical teratoid rhabdoid tumor. Acta Neuropathol 113:695–703
10. Mobley BC, Roulston D, Shah GV, Bijwaard KE, McKeever PE (2006) Peripheral primitive neuroectodermal tumor/Ewing's sarcoma of the craniospinal vault: case reports and review. Hum Pathol 37:845–853
11. Mazur MA, Gururangan S, Bridge JA, Cummings TJ, Mukundan S, Fuchs H, Larrier N, Halperin EC (2005) Intracranial Ewing sarcoma. Pediatr Blood Cancer 45:850–856
12. Tison V, Cerasoli S, Morigi F, Ladanyi M, Gerald WL, Rosai J (1996) Intracranial desmoplastic small-cell tumor. Report of a case. Am J Surg Pathol 20:112–117
13. Cherif El Asri A, Benzagmout M, Chakour K, Chaoui MF, Laaguili J, Chahdi H, Gazzaz M, El Mostarchid B (2018) Primary intracranial pPNET/Ewing sarcoma: diagnosis, management, and prognostic factors dilemma-a systematic review of the literature. World Neurosurg 115:346–356
14. Torchia EC, Jaishankar S, Baker SJ (2003) Ewing tumor fusion proteins block the differentiation of pluripotent marrow stromal cells. Cancer Res 63:3464–3468