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CASE STUDY

Rhabdomyosarcoma of the skull with *EWSR1* fusion and ALK and cytokeratin expression: a case report

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Rhabdomyosarcoma (RMS) comprises of heterogeneous group of neoplasms that occasionally express epithelial markers on immunohistochemistry (IHC). We herein report the case of a patient who developed RMS of the skull with *EWSR1* fusion and anaplastic lymphoma kinase (ALK) and cytokeratin expression as cytomorphologic features. A 40-year-old man presented with a mass in his forehead. Surgical resection was performed, during which intraoperative frozen specimens were obtained. Squash cytology showed scattered or clustered spindle and epithelioid cells. IHC revealed that the resected tumor cells were positive for desmin, MyoD1, cytokeratin AE1/AE3, and ALK. Although *EWSR1* rearrangement was identified on fluorescence in situ hybridization, *ALK*, and *TFCP2* rearrangement were not noted. Despite providing adjuvant chemoradiation therapy, the patient died of tumor progression 10 months after diagnosis. We emphasize that a subset of RMS can express cytokeratin and show characteristic histomorphology, implying the need for specific molecular examination.

Key Words: Rhabdomyosarcoma; Skull; *EWSR1* protein; Keratins; Cytology

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Rhabdomyosarcoma (RMS) is a heterogeneous group of malignant neoplasms that share common embryonic skeletal muscle features. It is one of the most common pediatric sarcomas and rarely presents in adults. Adults diagnosed with RMS have a poorer prognosis than do pediatric patients, with 5-year survival rates of 27% compared with 61%, respectively [1]. Currently, RMS is classified into embryonal, alveolar, pleomorphic, and spindle cell/sclerosing types and subdivided through molecular studies. For example, spindle cell/sclerosing RMS has a subset of genetic groups that include *MYOD1* mutation [2], *NCOA2* fusions [3], and *VGLL2* rearrangement [4].

Considering that all RMSs exhibit skeletal muscle differentiation, the diagnosis of this neoplasm has been primarily based on confirmation of the immunophenotype. Interestingly, RMSs occasionally exhibit aberrant cytokeratin expression [5]. Some of these multi-phenotypic RMSs have characteristic clinical, histologic, and molecular properties [6]. For example, one study recently described RMS with *EWSR1/FUS-TFCP2* fusion as a distinct entity of epithelioid and spindle cell RMS, characterized by a predilection for the craniofacial bone and the expression of cytokeratin AE1/AE3 and anaplastic lymphoma kinase (ALK) on immunohistochemistry (IHC) [7].

We herein report a case of RMS arising in the skull with *EWSR1* fusion and ALK and cytokeratin expression with squash smear cytology and surgical specimens.

CASE REPORT

A 40-year-old previously healthy man presented with a palpable, hard, unmovable mass in his right forehead that appeared 3 months prior. Magnetic resonance imaging detected a 2.7-cm-sized dumbbell-shaped enhancing mass in the right frontal bone involving the scalp and meninges (Fig. 1A). The mass was suspected to be a malignant bone tumor, such as Langerhans cell histiocytosis or metastasis.

Surgical resection was performed without biopsy, and sam-

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ples from the scalp were obtained for intraoperative frozen section diagnosis. Squash cytology revealed small to large loosely cohesive clusters with smear artifact (Fig. 2A). The background was clear with few-to-no inflammatory cells and no necrosis. The clusters consisted of mixed epithelioid and spindle cells showing round-to-oval nuclei, prominent nucleoli, and granular chromatin with eosinophilic cytoplasm (Fig. 2B). Marked nuclear pleomorphism and scant mitotic activity were observed (Fig. 2C). Frozen section diagnosis confirmed positivity for malignancy.

During gross examination, we observed an ill-demarcated, homogenous, whitish, solid, hard mass in the skull measuring 2.5×2.2×1.1 cm and penetrating through the dura mater (Fig. 1B). The resected tumor consisted of spindle and epithelioid cells with primarily a fascicular or whirling arrangement and few portions of solid growth (Fig. 2D, E). The neoplastic cells had round, vesicular nuclei, prominent one or two macronucleoli and moderate-to-abundant eosinophilic cytoplasm. Brisk mitosis and necrosis were noted.

The tumor cells were diffusely positive for desmin (clone D33, 1:200, Dako, Santa Clara, CA, USA), MyoD1 (EP212, 1:50, Cell Marque, Rocklin, CA, USA), and cytokeratin AE1/AE3 (1:400, Novocastra, San Jose, CA, USA) and focally positive for ALK (5A4, 1:200, Novocastra), smooth muscle actin (M0851, 1:500, Dako), myogenin (F5D, 1:100, Cell Marque), epithelial membrane antigen (EMA; E29, 1:100, Dako), p53 (DO-7, 1:1,000, Dako), S100 (4C4.9, 1:400, Cell Marque), nestin (10C2, 1:1,000, Cell Marque), SATB2 (polyclonal, 1:200, Cell Marque) on IHC (Fig. 2F–I). Conversely, the tumor cells tested negative for myoglobin (Z001, 1:1,000, Thermo Fisher Scientific, Waltham, MA, USA), signal transducer and activator of transcription 6 (polyclonal, 1:1,000, Abcam, Cambridge, UK), cyclin-dependent kinase 4 (DCS-31, 1:100, Santa Cruz Biotechnology, Santa Cruz, CA, USA), MDM-2 (SPM14, 1:50, Zeta, Sierra Madre, CA, USA), CD34 (1:400, Cell Marque), ERG (EP111, 1:400, Cell Marque), and HMB45 (1:50, Dako). *EWSR1*, *ALK*, and *TFCP2* break-apart fluorescence in situ hybridization (FISH) revealed *EWSR1* rearrangement (Fig. 3A) but no *ALK* or *TFCP2* rearrangement (Fig. 3B, C). A molecular study using next-generation sequencing was attempted, but DNA quality was too degraded by decalcification.

Although our patient received one cycle of adjuvant chemotherapy (vincristine, doxorubicin, and cyclophosphamide according to the IRS-III protocol), the size of the suspicious mass continued to increase for 2 months. No gross tumor remained at the time of surgery. Intensity-modulated radiation therapy (4680 cGY/26fx), three cycles of second-line chemotherapy (ifosfamide, carboplatin, and etoposide), and one cycle of third-line chemotherapy (gemcitabine and doxorubicin) were provided consecutively for residual/recurrent tumor. Unfortunately, the patient's condition deteriorated with multiple metastases to the dura mater, skin, lung, rib, and abdominal cavity. The patient eventually died of tumor progression 10 months after diagnosis.

DISCUSSION

RMSs occurring in the head and neck, including the parame-

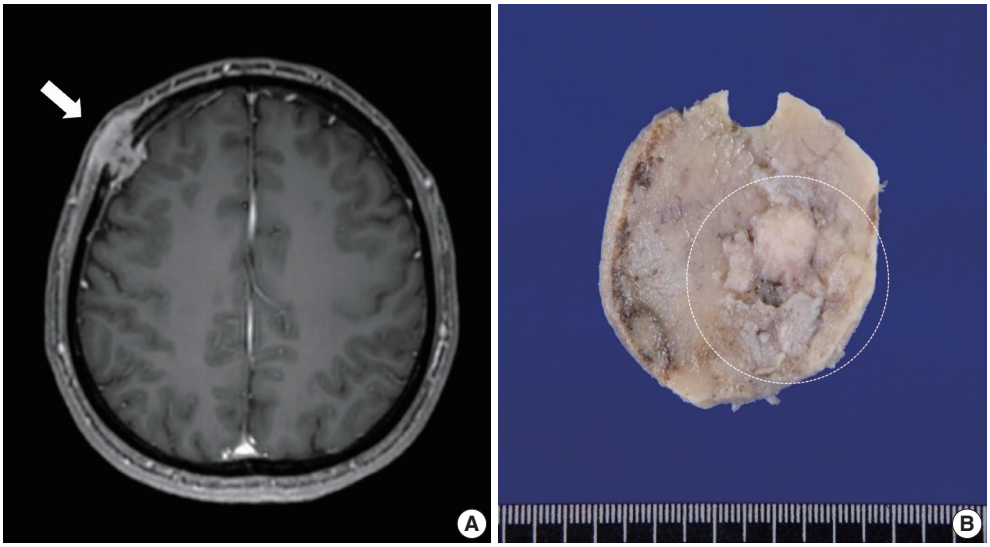

Fig. 1. Radiologic and gross findings. (A) Ga-enhanced T1-weighted magnetic resonance imaging shows a transdiploic mass in the frontal scalp involving the dura mater (arrow). (B) Grossly, an ill-circumscribed, whitish solid mass infiltrates the skull (circle).

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ningeal or orbital area, account for one-third of all cases of RMS in children and adolescents. Among adults, RMSs are most frequently observed in the extremities, followed by the chest/abdominal/pelvic, genitourinary, and head or neck regions [1]. However, RMSs of the skull are quite uncommon, with most cases originating from the skull base or temporal bone [8]. Primary tumors detected in the vault of the skull should be preferentially considered osteoma, osteosarcoma, Ewing sarcoma, Langerhans cell histiocytosis, plasmacytoma, or chondrosarcoma [9]. Moreover, for transdiploic lesions in the skull vault, radio-

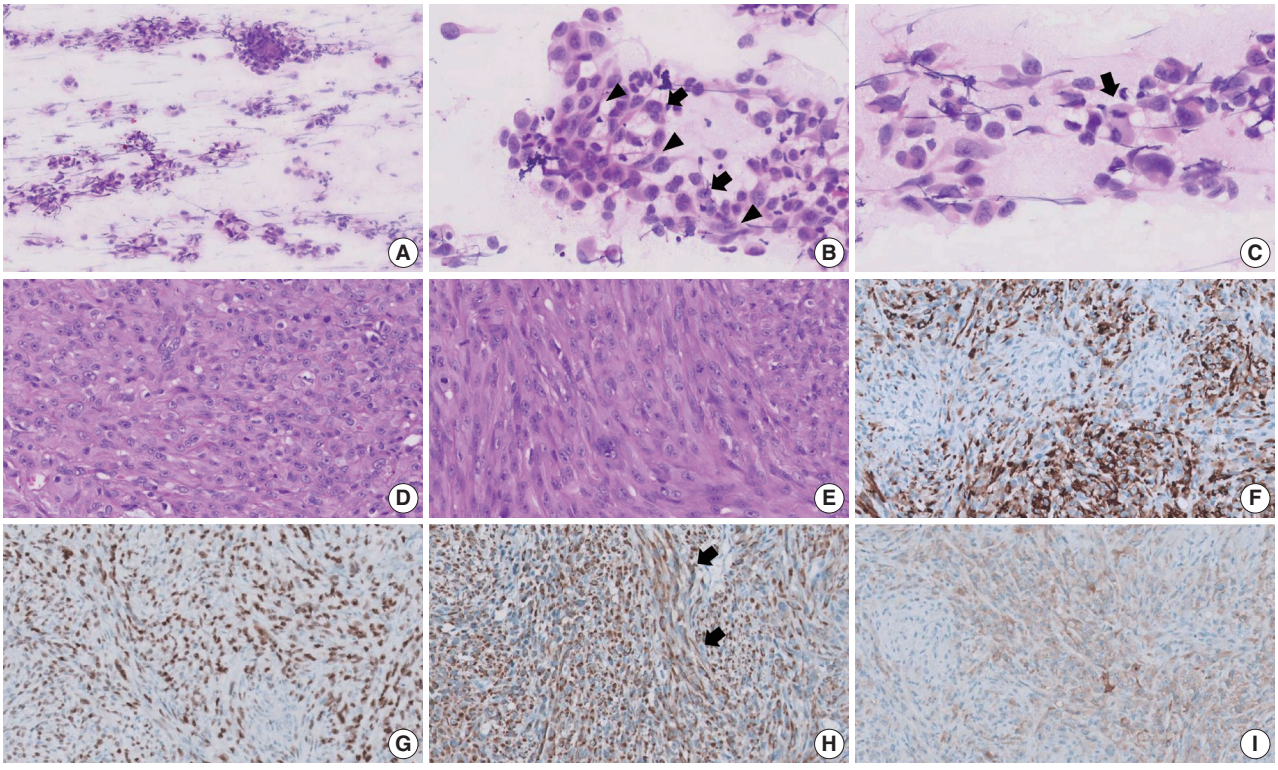

Fig. 2. Representative images of squash cytology, histologic, and immunohistochemical findings. (A) On frozen section, tumor clusters with smear artifacts are scattered in a clear background. (B) Epithelioid cells with abundant cytoplasm and prominent nucleoli are identified. A few spindle-shaped cells (arrowheads) and binucleation (arrows) are noted. (C) Marked nuclear pleomorphism and mitosis (arrow) are observed. (D) In permanent section, the epithelioid cell component shows glassy cytoplasm with a solid pattern. (E) Spindle cell component shows a fascicular pattern. Tumor cells express immuno-positivity for desmin (F), MyoD1 (G), cytokeratin AE1/AE3 (H), and anaplastic lymphoma kinase (I). Cytokeratin is expressed in not only epithelioid cells but also spindle cells (arrows, H).

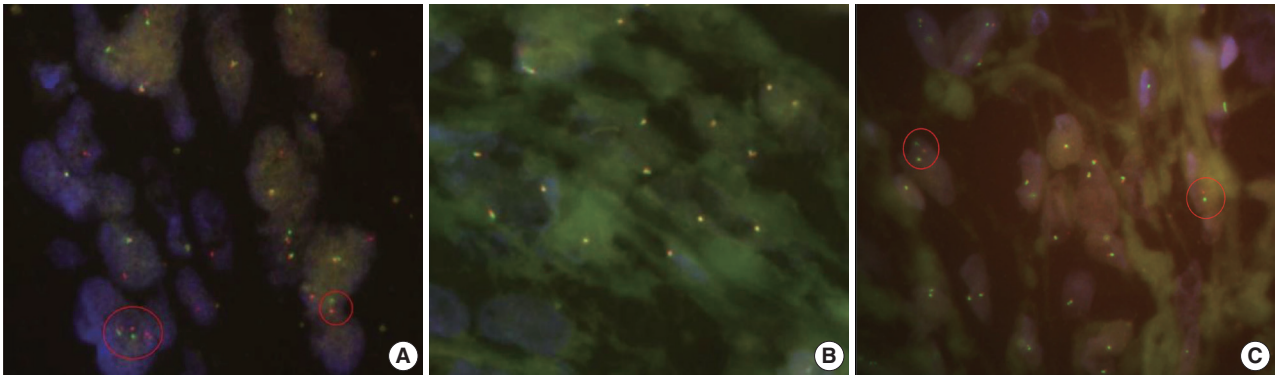

Fig. 3. Results of break-apart fluorescence in situ hybridization. (A) *EWSR1* rearrangement is identified (circles). (B) Anaplastic lymphoma kinase copy number change or translocation is not observed. (C) TFCP2 break-apart signals are detected in a few tumor cells (circles). Seven of 100 cells present a splitting signal on counts, indicating a negative result.

logic differential diagnoses include meningioma, solitary fibrous tumor, lymphoma, plasmacytoma, and metastasis [10]. As the present case was initially suspected to be Langerhans cell histiocytosis or metastasis, establishing a correct diagnosis of RMS of the skull vault was challenging given its rarity in this location.

The *EWSR1* gene, which stands for Ewing sarcoma breakpoint region 1, is ubiquitously involved in various cellular processes, and its rearrangements with diverse partner genes have been associated with the development of multiple types of tumors [11]. For example, *ESWR1* rearrangements have been frequently detected and serve as a key diagnostic finding in certain soft tissue tumors, such as Ewing sarcoma, round cell sarcomas with *EWSR1–non-ETS* fusions, desmoplastic small round cell tumor, myxoid liposarcoma, tumors with *EWSR1/FUS* fused to the *CREB*-family, sclerosing epithelioid fibrosarcoma, extraskeletal myxoid chondrosarcoma, and RMS with *EWSR1/FUS-TFCP2* fusion. Considering the presence of *EWSR1* fusion in various sarcomas, *EWSR1*-rearranged tumors necessitate further examination for the detection of the partner gene, especially in cases showing unusual clinicopathologic features. Despite having identified *EWSR1* break-apart on FISH, we failed to confirm the counterpart gene to which the *EWSR1* gene fused.

The findings of the present report raise questions about a series of differential diagnoses, due to the present case's unique and complex characteristics. *EWSR1-PATZ1* and *EWSR1-NFATC2* sarcomas are classified as round cell sarcomas with *EWSR1–non-ETS* fusions according to World Health Organization tumor classification and can show diverse histopathologic and immunophenotypic features [12]. *EWSR1-PATZ1* sarcomas usually originate from the deep soft tissue of the chest wall and abdomen; however, they can develop in the head and neck as previously reported [13]. They express myogenic and neurogenic markers (S100P, SOX10, and GFAP) but rarely exhibit epithelial markers. Furthermore, ALK overexpression has not yet been reported in this tumor. Although *EWSR1-NFATC2* sarcomas can show mixed epithelioid and spindle cell histology and dot-like positivity for cytokeratin, they are usually accompanied by abundant myxohyalinized stroma and do not express skeletal muscle differentiation [14]. A subset of tumors with *EWSR1/FUS* fused to the *CREB*-family has shown a predilection for intracranial location and exhibited both EMA and desmin [15]. These tumors consisted of ovoid or round cells in a myxoid background. Other *EWSR1-CREB* fusion neoplasms demonstrated a hybrid epithelioid and spindle morphology with cytokeratin and desmin positivity, and one expressed diffuse ALK positivity [16]. However, they commonly arise in mesothelial-lined cavities.

RMSs with *EWSR1/FUS-TFCP2* fusion are a newly emerging sub-classification characterized by a predilection for craniofacial bone, specific immunoprofiles, and molecular alterations [7,17]. This tumor comprises hybrid epithelioid and spindle cells. The latter are arranged in fascicular growth and have nuclei that are ovoid and fusiform with prominent nucleoli and mild pleomorphism, whereas the former contain an abundance of eosinophilic, often glassy cytoplasm arranged in solid sheets [18,19]. Several cases contain a portion of small round or rhabdoid cytologic features. Almost all cases have high mitotic rates and tumor necrosis. The tumor displayed positivity for myogenic markers, such as MyoD1, myogenin, and desmin. Interestingly, tumors in most cases show positivity for cytokeratin AE1/AE3 and ALK on IHC, and molecular studies often reveal ALK overexpression. One study showed that *EWSR1/FUS-TFCP2* can activate *ALK* upregulation [20]. However, *ALK* rearrangement has not been reported previously [18]. Although we could not directly confirm *TFCP2* rearrangement or fusion, we suspected that the tumor in the present case might have been RMS with *EWSR1-TFCP2* fusion based on the tumor's location in the craniofacial bone, the presence of mixed epithelioid and spindle cells, and the tumor's immunopositivity for myogenic markers, cytokeratin, ALK, and *EWSR1* rearrangement. According to a report on spindle cell RMS, aberrant keratin expression was a unique feature of RMS with *EWSR1-TFCP2* fusion, which differed from spindle cell RMS harboring other fusions [6]. Additionally, a literature review revealed that *EWSR1* rearrangement in RMS is exceedingly rare, and most of these cases involve RMS with *EWSR1-TFCP2* fusion. However, some round cell sarcomas with *EWSR1* fusion express skeletal muscle markers, which necessities differential diagnosis [21]. Meanwhile, we interpreted the results of *TFCP2* FISH in our case as negative despite the identification of a few break-apart signals. Although Bin Xu et al. counted 200 nuclei and considered split signal of over 20% to be positive [18], currently no definite criteria for interpreting *TFCP2* FISH results have been established.

The prognosis of RMS with *EWSR1/FUS-TFCP2* is incredibly poor, with a median survival time of less than 21 months (Table 1) [6,7,18,19]. Over half of patients develop local recurrence, regional lymph node metastasis, or distant metastasis to the bones and lungs [18]. Treatment options involve surgical resection, chemotherapy, and radiotherapy, all of which have limited efficacy. Although *ALK* inhibitors have been proposed to be a potential target therapy, their effectiveness remains unclear [20,22,23].

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Table 1. Summary of major published cases of rhabdomyosarcoma with *TFCP2* fusion

Study	No. of cases	Age (yr)	Sex (M:F)	Locations	Outcomes
Le Loarer et al. (2020) [7]	14	11–86 (mean, 31)	3:4	Craniofacial (8/14), other bone (4/14), soft tissue (2/12)	Median survival: 8 months
Chrisinger et al. (2020) [19]	23[a]	11–86	1:2.7	Craniofacial (12/23), other bone (9/23), soft tissue (2/23)	Median survival: 15 months
Xu et al. (2021) [18]	27[a]	11–74 (mean, 25)	1.25:1	Craniofacial (18/27), other bone (8/27), soft tissue (1/27)	1- and 2-year disease-specific survival rate: 74% and 35%, respectively
Dehner et al. (2023) [6]	56[a]	8–86 (mean, 34)	5:9	Craniofacial (37/56), other bone (13/56), soft tissue (6/56)	Median survival: 21 months

[a]This number includes data from previously reported cases through a literature review.

In summary, we report an unusual case of RMS of the skull with *EWSR1* fusion and ALK and cytokeratin expression, highlighting the importance of precise histopathologic examination along with comprehensive immunohistochemical and molecular evaluation for diagnosis and management.

Ethics Statement

This study was approved by the Institutional Review Board, and the need for informed consent was waived (Asan Medical Center IRB No. 2023-1548).

Availability of Data and Material

The datasets generated or analyzed during the study are available from the corresponding author on reasonable request.

Code Availability

Not applicable.

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Conflicts of Interest

J.S.S., a contributing editor of the *Journal of Pathology and Translational Medicine*, was not involved in the editorial evaluation or decision to publish this article. All remaining authors have declared no conflicts of interest.

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