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Case report

# Spindle cell rhabdomyosarcoma in a lumbar vertebra with *FUS-TFCP2* fusion

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ABSTRACT

A 70-year-old woman developed severe buttock pain that progressed to a walking disturbance. Radiographs and computed tomography scans revealed an osteolytic lesion with osteosclerosis extending from the body to the arch of the fifth lumbar vertebra. Magnetic resonance imaging showed multinodular masses in the fifth lumbar vertebral body extending into the spinous processes and right transverse process. The masses were hypointense to isointense on T1-weighted images and hypointense to hyperintense on T2-weighted images. Histologic examination of biopsy specimens showed destruction of the trabecula of the vertebral bone by a fascicular and solid proliferation of spindle tumor cells and scattered rhabdomyoblasts, in a fibrotic background. The tumor cells were immunoreactive for keratins, vimentin, desmin, MyoD1, myogenin, and anaplastic lymphoma kinase. Fluorescence *in situ* hybridization detected split signals for *FUS* and *TFCP2* in 80% and 64% of the tumor cells, respectively, suggesting *FUS-TFCP2* fusion. Reverse transcription-polymerase chain reaction revealed a *FUS-TFCP2* fusion. The final diagnosis was spindle cell rhabdomyosarcoma of a lumbar vertebra with a *FUS-TFCP2* fusion. A spindle cell rhabdomyosarcoma with a *FUS-TFCP2* fusion in a vertebral bone is rare and should be differentiated from metastatic carcinoma, particularly in the elderly.

## 1. Introduction

The current World Health Organization classification divides rhabdomyosarcoma (RMS) into four subtypes: embryonal, alveolar, pleomorphic, and spindle cell/sclerosing. Spindle cell/sclerosing RMS is an uncommon variant of RMS, accounting for up to 5%–10% of all cases of RMS and mainly affecting children and young adults with a male predominance. Spindle cell/sclerosing RMS arise in the paratesticular region in the pediatric population and tend to arise in the deep soft tissue of the head and neck in adults. Primary RMS of bone is extremely rare.

Very recently, a unique type of RMS showing epithelioid to spindle morphology with specific chimeric fusions of *FUS-TFCP2* or *EWSR1-TFCP2* has been reported [1,2]. Such cases have arisen in the pelvic bone, chest wall, sphenoid bone, and jaw; those that arose in the first three of these sites were demonstrated to be “a new epithelioid rhabdomyosarcoma entity characterized by *EWSR1/FUS-TFCP2* fusion” and to occur mainly in young women [1]. These tumors consisted of small sheets or fascicular proliferations of epithelioid tumor cells with high-grade features and monotonous round nuclei with prominent nucleoli. They were positive for desmin, MyoD1, myogenin, and anaplastic lymphoma kinase (ALK) on immunohistochemistry (IHC). All three cases were very aggressive and the patients died within 5 months. On the other hand, Dashti et al have reported a case of spindle cell RMS of the jaw in a 72-year-old man [2]. The tumor was a relatively monomorphic spindle cell neoplasm that consisted of a short fascicular proliferation of primitive-appearing, hyperchromatic spindled to somewhat epithelioid tumor cells with scant eosinophilic cytoplasm. The tumor cells in this patient were also positive for desmin, MyoD1, and ALK on IHC and variable aberrant keratin expression was observed. RNA sequencing detected a specific *FUS-TFCP2* fusion in the tumor. The common findings in RMS with *FUS/EWSR1-TFCP2* fusion include not only a spindled to epithelioid morphology, but also characteristic expression of MyoD1, keratin, and ALK. These features are very different from those associated with spindle cell/sclerosing RMS.

Here we report a further rare case of spindle cell RMS in a lumbar vertebra with a *FUS-TFCP2* fusion detected by fluorescence *in situ*

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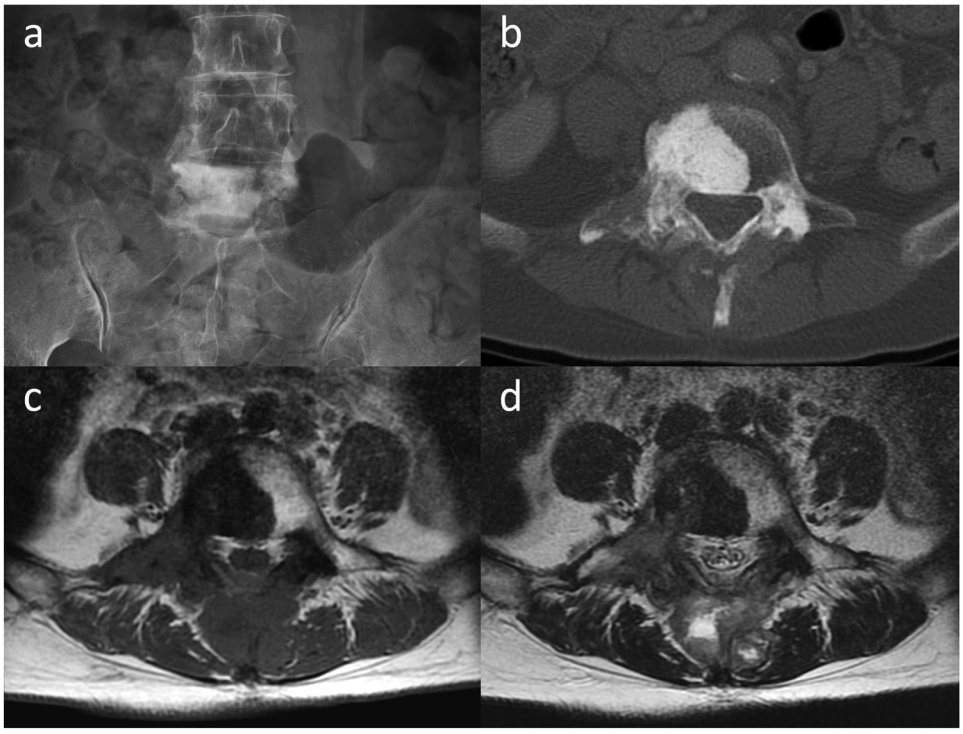


**Fig. 1.** Radiologic findings in a patient with spindle cell rhabdomyosarcoma in a lumbar vertebra.
a. Anteroposterior radiograph showing an osteolytic lesion with osteosclerosis in the body of the fifth lumbar vertebra.
b. Axial computed tomography showing a mixed osteolytic and osteosclerotic lesion extending from the body to the arch and spinous process of the fifth lumbar vertebra.
c. Axial T1-weighted magnetic resonance image showing hypointense to isointense multinodular lesions in the fifth lumbar vertebral body extending into the transverse processes and spinous process.
d. Axial T1-weighted magnetic resonance image showing low to high-intensity multinodular lesions.

hybridization (FISH) and reverse transcription-polymerase chain reaction (RT-PCR). The tumor expressed myogenic markers, including MyoD1, and showed strong keratin and ALK expression on IHC. This tumor needed to be differentiated from metastatic carcinoma and from carcinosarcoma with rhabdomyosarcomatous components.

## 2. Materials and methods

### 2.1. Case presentation

A 70-year-old woman developed severe buttock pain that progressed to a walking disturbance. Radiographs and computed tomography (CT) scans revealed an osteolytic lesion with osteosclerosis in the fifth lumbar vertebra extending from the body to the arch (Fig. 1a, 1b). Magnetic resonance imaging (MRI) showed multinodular masses in the body of the fifth lumbar vertebra that extended into the spinous processes and right transverse process. The masses were hypointense to isointense on T1-weighted images and hypointense to hyperintense on T2-weighted images (Fig. 1c, d). CT, MRI, and FDG-positron emission tomography (PET) detected no other lesion suggestive of a primary tumor except in the vertebra. The final diagnosis of spindle cell RMS of the lumbar vertebra with a *FUS-TFCP2* fusion was made by pathologic examination, which included morphology, IHC, FISH, and RT-PCR. The tumor was not completely resectable, so the patient underwent chemotherapy with docetaxel and radiotherapy (30 Gy/10 fr + boost 21 Gy/7 fr). The patient subsequently received 7 courses of adriamycin as chemotherapy and was still alive with disease 6 months after the open biopsy.

### 2.2. Immunohistochemistry

We selected representative sections from formalin-fixed and paraffin-embedded tissue sliced into 3-μm-thick sections and examined them using an automated IHC system at Sapporo Medical University Hospital. All slides were loaded into a PT Link module (Agilent Technologies, Santa Clara, CA) and subjected to a heat-induced and/or enzyme-induced antigen-retrieval protocol with EnVision FLEX Target Retrieval Solution (Agilent) before being transferred to an Autostainer Link 48 (Agilent) and Dako Omnis (Agilent). We used antibodies against the following antigens: keratin AE1/AE3 (AE1/AE3, Agilent), CK7 (OV-TL12/30, Agilent), CK20 (Ks20.8, Agilent), epithelial membrane antigen (E29, Agilent), vimentin (V9, Agilent), α-smooth muscle actin (1A4, Agilent), desmin (D33, Agilent), muscle-specific actin (HHF35, Agilent), myogenin (F50, Agilent), MyoD1 (5.8 A), S-100 protein (polyclonal, Agilent), CD99 (12E7, Agilent), INI1 (25/BAF47, Biosciences, San Jose, CA), SOX-9 (3C10, Agilent), leukocyte common antigen (2B11, Agilent), CD3 (Polyclonal, Agilent), CD20 (L26, Agilent), and ALK (5A4, Nichirei, Tokyo, Japan).

### 2.3. Fluorescence in situ hybridization

FISH for *FUS*, *EWSR1*, *TFCP2*, *FOXO1*, *PAX3*, and *PAX7* rearrangements was performed using 4-μm sliced formalin-fixed, paraffin-embedded tissue sections with the Vysis Paraffin Pretreatment Reagent Kit (Abbott, Abbott Park, IL). Commercially available dual-color and split-signal probes were used according to the manufacturer's instructions. The Vysis EWSR1 Break Apart FISH Probe Kit (Abbott) was used for *EWSR1* rearrangement, the Vysis FUS Break Apart FISH Probe Kit (Abbott) for *FUS* rearrangement, the Vysis FOXO1 Break Apart FISH Probe Kit (Abbott) for *FOXO1* rearrangement, the PAX3 Break Apart FISH Probe Kit (CytoTest Inc., Rockville, MD) for *PAX3* rearrangement, the PAX7 Breakapart kit (Cytocell, Cambridge, UK) for *PAX7* rearrangement, and the TFCP2 Break Apart FISH Probe (Empire Genomics, Buffalo, NY) for *TFCP2* rearrangement. We counted 50 tumor cell nuclei under fluorescence microscopy, and positivity for rearrangement was defined if split signals were observed in more than 10% of tumor cells [3]. We did not evaluate any truncated or overlapping cells in the FISH analysis.

### 2.4. Reverse transcription-polymerase chain reaction

RT-PCR analysis was performed to detect *FUS-TFCP2* fusions. Total RNA was extracted from formalin-fixed, paraffin-embedded tissue using NucleoSpin FFPE RNA (Macherey-Nagel GmbH & Co. KG, Düren, Germany) according to the manufacturer's instructions. Reverse transcription was carried out with the RevertAid Reverse Transcription Kit

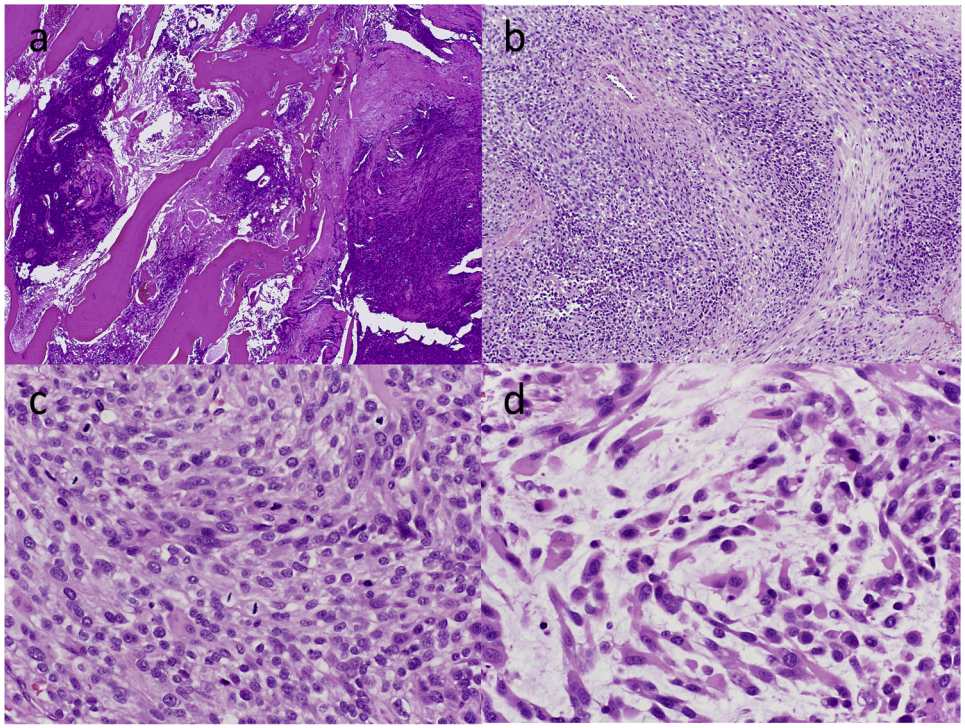


**Fig. 2.** Histologic findings in a patient with spindle cell rhabdomyosarcoma in a lumbar vertebra.
a. Tumor destroying the trabecula in the vertebral body.
b. Tumor composed of a fascicular or small sheet-like proliferation of spindle to round cells and a fibrous stroma.
c. Tumor cells with round to oval nuclei and mild nuclear pleomorphism and frequent mitotic activity.
d. Rhabdomyoblasts with abundant eosinophilic cytoplasm are observed.

(Thermo-Fisher Scientific, Waltham, MA). To detect *FUS-TFCP2*, we used a primer set of *FUS* exon 6- (forward) 5′-TGGCTATGAACCCAGA GGTC′ and *TFCP2* exon 2 (reverse) 5′GGAGGCAAACTCGACTCTTCT -3′.

## 3. Results

### 3.1. Pathologic findings

Grossly, the tumor was whitish and solid on cut sections. Histologically, the tumor had destroyed the trabecula of the vertebral body (Fig. 2a). The tumor consisted of a multinodular proliferation with a fascicular or small sheet-like proliferation of spindle to round cells (Fig. 2b). The spindle cell component occupied about 50% of the tumor. Foci of collagen-rich stroma were found in the tumor. The tumor cells had round to oval nuclei with mild nuclear pleomorphism. Mitotic figures (12/10 high-power fields) were frequently observed (Fig. 2c). Foci of necrosis were found within the tumor. Rhabdomyoblasts with abundant eosinophilic cytoplasm were occasionally observed (Fig. 2d). On IHC, the tumor cells were diffusely positive for keratin AE1/AE3 (Fig. 3a), vimentin, MyoD1 (Fig. 3b), CD99, muscle-specific actin, CK7, SOX-9, and INI1. They were focally positive for desmin (Fig. 3c) and myogenin and showed strong ALK expression (Fig. 3d). Among the myogenic markers, the ratios of cells immunopositive for myogenin, desmin, and myoD1 were 10%, 30%, and 90%, respectively. They were slighly positive for epithelial membrane antigen but were negative for leukocyte common antigen, CD3, CD20, CD34, α-smooth muscle actin, and S-100 protein.

### 3.2. Fluorescence in situ hybridization

FISH detected split signals for *FUS* and *TFCP2* in 80% and 64% of tumor cells, respectively (Fig. 3e, f), suggesting a *FUS-TFCP2* fusion in the tumor. FISH also revealed split signals for *EWSR1*, *FOXO1*, *PAX3*, and *PAX7* in 8%, 0%, 0%, and 2% of cells, respectively.

### 3.3. Reverse transcription-polymerase chain reaction

Using RT-PCR analysis, we detected the expected 105 bp *FUS* (exon 6)-*TFCP2* (exon 2) PCR products by electrophoresis (Fig. 4). The final diagnosis was spindle cell rhabdomyosarcoma of a lumbar vertebra with a *FUS-TFCP2* fusion.

## 4. Discussion

Specific chimeric fusions of *FUS-TFCP2* or *EWSR1-TFCP2* were recently detected in a small group of unusual RMS [1,2]. Histologically, these RMS tended to show epithelioid and spindle cell morphology. Interestingly, these tumors were positive for keratin and ALK as well as myogenic markers, including MyoD1, on IHC. Of the five RMS reported to date, including our present case, 4 cases were primary osseous RMS affecting the sphenoid bone, jaw, pelvis, and lumbar vertebra (Table 1). We have now reported a fourth case of RMS that had a specific fusion of *FUS-TFCP2* arising in bone. RMS of bone is extremely rare, although there have been previous reports of sporadic cases [4,5]. While many cases of osseous RMS are conventional RMS, including embryonal, alveolar, and pleomorphic RMS, a few cases have shown a spindle cell morphology similar to fibrosarcoma or leiomyosarcoma [4,6,7]. Although specific fusion of *FUS/EWSR1-TFCP2* was not tested in those cases, it is possible that the tumors corresponded to this unique RMS in view of their spindle cell morphology.

Differential diagnosis was difficult in the present case. Given that the tumor strongly expressed keratin, we considered the possibility of metastatic carcinoma because of the patient's advanced age and the tumor site arising in the vertebra. Metastatic leiomyosarcoma was also one of the most important differential diagnoses because the patient was female and the tumor showed a spindle cell morphology with expression of myogenic markers. We initially thought the patient had a metastatic tumor, specifically metastatic carcinosarcoma, because the tumor cells strongly expressed cytokeratin and exhibited rhabdomyoblastic differentiation on IHC in addition to spindle cell morphology. However, whole-body CT, MRI, and FDG-PET scans did not identify a primary site elsewhere in this patient. Other differential diagnoses considered were spindle cell sarcoma, including malignant peripheral nerve sheath tumor, synovial sarcoma, desmoplastic small round cell tumor (DSRCT), and Ewing's sarcoma. However, the tumor was not reactive for S-100 protein and was positive for myogenic markers,

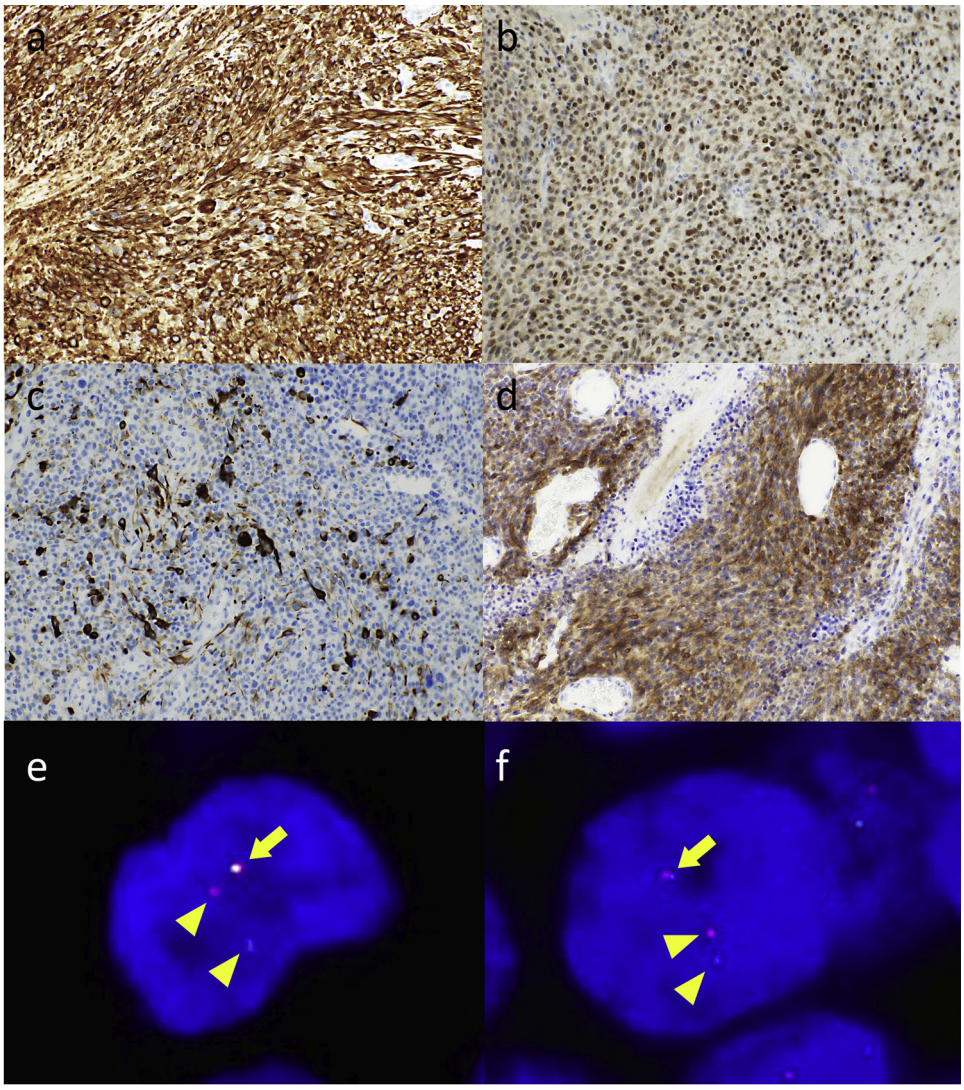


**Fig. 3.** Immunohistochemistry and fluorescence *in situ* hybridization analysis of the tumor.
a. Tumor cells positive for keratin AE1/AE3
b. Tumor cells positive for MyoD1
c. Tumor cells focally positive for desmin
d. Tumor cells strongly express ALK
e. FISH analysis of *FUS*. Tumor cells showing a split signal for *FUS* with a pair of fused (arrow) and split (arrow head) patterns of red and green signals.
f. FISH analysis of *TFCP2*. Tumor cells showing a split signal for *TFCP2* with a pair of fused (arrow) and split (arrow head) patterns of red and green signals.
ALK, anaplastic lymphoma kinase; FISH, fluorescence *in situ* hybridization; IHC, immunohistochemistry

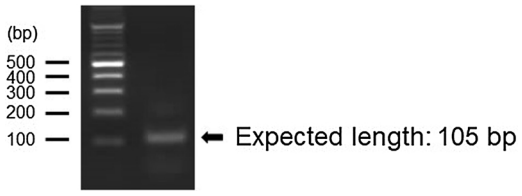


**Fig. 4.** Results of reverse transcription-polymerase chain reaction analysis of the tumor.
RT-PCR detected the expected 105 bp *FUS* (exon 6)-*TFCP2* (exon 2) PCR products by electrophoresis.
RT-PCR, reverse transcription-polymerase chain reaction

including MyoD1 and myogenin. DSRCT may reveal a polyphenotypic profile including expression of keratin, EMA, vimentin, and desmin although diffuse expression of MyoD1 is not usually observed in DSRCT. Some Ewing's sarcomas have been reported to show myogenic differentiation [8], although our case showed diffuse keratin and ALK expression in addition to myogenic markers but *EWSR1* rearrangement was not detected. Furthermore, it is necessary to differentiate this case from the recently recognized spindle and round cell sarcoma with *EWSR1-PATZ1* gene fusion [9]. *EWSR1-PATZ1* sarcoma consisted of solid proliferation of spindle and round tumor cells with dense intratumoral fibrocollagenous stroma similar to DSRCT. The present case showed fibrous stroma but lacked dense desmoplastic fibrous stroma and had no areas of pseudoalveolar architecture with focal microcystic

**Table 1**
Clinicopathological summary of previously reported cases of *EWSR1/FUS-TFCP2* fusion rhabdomyosarcoma.

| Case | Age (years)/Sex | Site | Positivity for myogenic and epithelial markers, and ALK on IHC | Fusion gene | Reference |
|---|---|---|---|---|---|
| 1 | 38/female | Chest wall | MYOD1, desmin, myogenin, ALK | *EWSR1-TFCP2* | Watson et al. |
| 2 | 26/female | Pelvic bone | MYOD1, desmin, myogenin | *FUS-TFCP2* | Watson et al. |
| 3 | 16/female | Sphenoid bone | MYOD1, desmin, myogenin | *FUS-TFCP2* | Watson et al. |
| 4 | 72/male | Mandible | MYOD1, desmin, myogenin, keratin, ALK | *FUS-TFCP2* | Dashti et al. |
| 5 | 70/female | Lumbar vertebra | MYOD1, desmin, myogenin, keratin, ALK | *FUS-TFCP2* | Present case |

ALK, anaplastic lymphoma kinase; IHC, immunohistochemistry.

and macrocystic changes seen in *EWSR1-PATZ1* sarcoma. On IHC, *EWSR1-PATZ1* sarcoma showed expression of CD99, desmin, myogenin, MyoD1, S-100 protein, SOX10, and CD34 but was negative for keratins. In our case, there was strong expression of keratins.

The College of American Pathologists (CAP) protocol classification and the current WHO classification have defined spindle cell/sclerosing RMS. The spindle cell RMS is composed almost exclusively (minimum 80% of tumor) of elongated spindle cells in 1 of 2 recognizable patterns. The collagen-poor pattern has a whorled, fascicular growth of spindle cells without significant collagen and resembles a smooth muscle tumor both grossly and microscopically. The collagen-rich form shows spindle cells with variable myogenic differentiation in a dense collagenous stroma [10]. Our case had a spindle cell component that occupied about 50% of the tumor in addition to foci of collagen-rich stroma. The histological findings were not identical to those of spindle cell RMS. Moreover, sclerosing RMS may show only a very limited expression of desmin and myogenin, whereas it is often strongly positive for MyoD1 [10]. Although our case had diffuse expression of MyoD1, myogenin and desmin expression was not limited. The expression pattern of myogenic markers in our case was different from sclerosing RMS. We finally detected specific rearrangements of *FUS* and *TFCP2* by FISH and *FUS-TFCP2* fusion using RT-PCR, thus distinguishing the present case from the previously known spindle cell/sclerosing RMS. We confirmed a diagnosis of spindle cell RMS of the lumbar vertebra with a *FUS-TFCP2* fusion.

Another intriguing finding for spindle cell RMS with this specific fusion is the presence of strong ALK expression in the tumor cells. ALK is a transmembrane tyrosine kinase protein that functions as an oncogene in various tumors, including non-small cell lung carcinoma, malignant lymphoma, renal carcinoma, soft tissue tumors, neuroblastoma, and thyroid carcinoma. These tumors have a chimeric fusion gene involving *ALK* or a mutation of *ALK*. As a result, activation of *ALK* is associated the oncogenesis of these tumors, and such tumors have been called “ALKoma” [11]. Patients with ALKoma can be successfully treated with ALK inhibitors. Approximately 3%–7% of lung adenocarcinomas have an *EML4-ALK* fusion, and patients with this type of lung carcinoma can be treated successfully with ALK inhibitors. In the present case, there was no fusion gene involving *ALK*, although the tumor showed strong ALK expression on IHC. The anti-ALK antibody (5A4) recognized the ALK protein itself independent of the partners of *ALK* fusion genes, ALK positivity on IHC without the fusion gene would then suggest ALK protein overexpression. Although the mechanism of high ALK expression in sarcoma without *ALK* fusion gene has not been fully elucidated, it is possible that high ALK expression would be observed if there is *ALK* gene amplification. Also, transcriptional *ALK* gene overexpression by aberrant signal transduction would also result in ALK protein expression. Considering the mechanism of action of ALK inhibitors, we can expect successful treatment of such tumors with these agents. Therefore, this patient may have had an ALKoma amenable to treatment with ALK inhibitors.

In summary, we have encountered a patient with spindle cell RMS of a lumbar vertebra with a specific *FUS-TFCP2* fusion detected by FISH and RT-PCR. This tumor showed strong expression of cytokeratin, MyoD1, and ALK, so metastatic carcinoma and carcinosarcoma was included in the differential diagnosis. This unusual type of spindle cell RMS should be included in the differential diagnosis if a spindle cell tumor of bone that expresses both myogenic markers and keratins is encountered. The clinicopathologic features of spindle cell RMS with a *FUS-TFCP2* fusion are not well recognized on account of the extreme rarity of these tumors. Further case reports are needed to clarify the pathologic nature and clinical significance of this subtype of rhabdomyosarcoma.

## Conflicts of interest

None.

## Funding

None.

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