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Case Report

Atypical ossifying fibromyxoid tumor unusually located in the mediastinum: report of a case showing mosaic loss of INI-1 expression

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Abstract: Ossifying fibromyxoid tumor (OFMT) is a rare soft tissue tumor. OFMT mostly arises in subcutaneous tissue or skeletal muscle of the extremities and is extremely unusual in the mediastinum. OFMT is classified as typical, atypical, or malignant as tumor aggressiveness increases. Herein, we presented a case of atypical OFMT that developed in the mediastinum of a 43-year-old woman. Because of its predominant hypercellular area and some tumor cells with high nuclear grade, it was not a typical OFMT. However, it did not have a sufficient number of mitotic figures to be classified as malignant. Hence, we classified it as atypical OFMT with some apparent characteristic features of OFMT, such as the presence of spicules of bone at the periphery of the tumor. Upon immunohistochemistry, it was positive for vimentin, S-100 protein, and CD10, which was consistent with a diagnosis of OFMT. Particularly noteworthy was the mosaic loss of INI-1 expression. Some OFMT and other exceptionally rare tumors have been reported to exhibit mosaic INI-1 loss. Inactivation of *INI-1* gene and deregulation of *PHF1* gene are thought to be involved in tumorigenesis of OFMT. Therefore, we speculated that the mosaic loss of INI-1 observed in the present case might also be related to a kind of abnormality of *INI-1* as was reported previously.

Keywords: Ossifying fibromyxoid tumor, atypical, mediastinum, ini-1, immunohistochemistry

Introduction

Ossifying fibromyxoid tumor (OFMT) in soft parts, described by Enzinger et al. in 1989, is a rare soft tissue tumor [1] of uncertain origin and composed of relatively uniform spindled to ovoid cells, often arranged in a corded or trabecular pattern and embedded in a fibromyxoid matrix [1, 2]. In addition, spicules of metaplastic bone are commonly seen at the periphery of the tumor [1, 2]. Initially, it was described as a benign to low-grade malignant tumor [1]. Subsequently, several reports have documented both histopathologically and clinically atypical and malignant OFMTs [3-5].

OFMT affects males to females at a ratio of approximately 1.5:1. The overall median age is about 50 years [2]. OFMT mostly arises in subcutaneous tissue or skeletal muscle of the extremities, although it has been occasionally reported at other sites such as the trunk, and head and neck [1, 2, 4, 6]. To date, only one case located in the mediastinum was reported in the English literature [7].

Recently, a mosaic pattern of INI-1 protein loss, as seen by immunohistochemistry (IHC), was shown by Graham et al. to be common in typical, atypical, and malignant OFMTs [8]. However, Gebre-Medhin et al. reported that a reduction or absence of immunostaining for INI-1 was not observed [9].

Herein, we present the second reported case of OFMT in the mediastinum. It was classified as an atypical OFMT in which mosaic loss of INI-1 expression was observed.

Clinical summary

A 43-year-old woman was referred from another hospital. She presented mainly with numbness and weakness of the right arm, related to the abnormal mass in the upper mediastinum

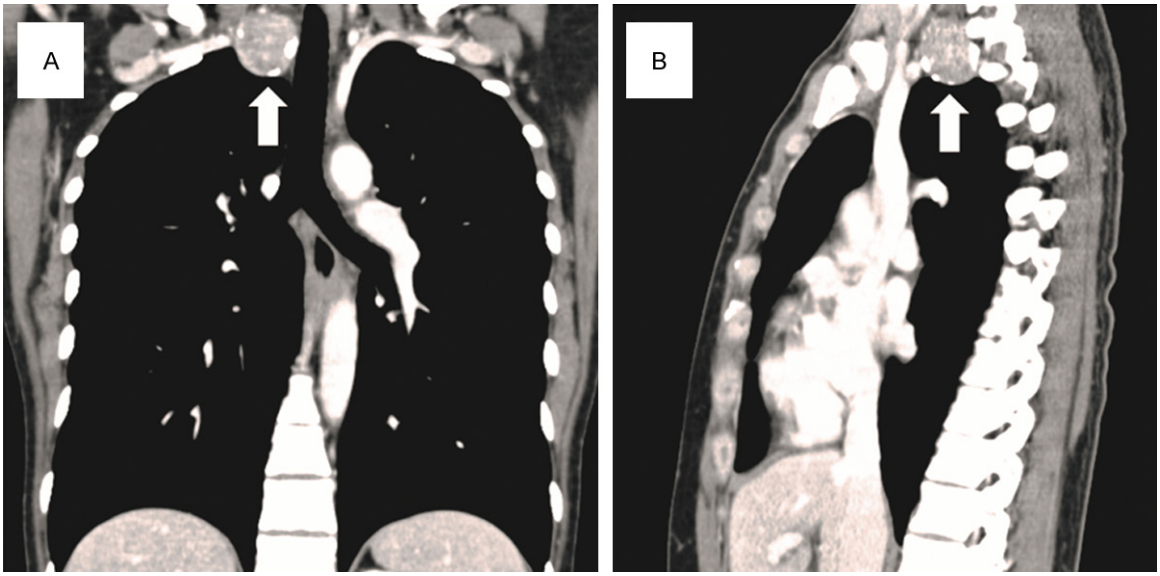

Figure 1. Radiological findings. Coronal (A) and sagittal sections (B) of contrast-enhanced computed tomographic images. The mass (arrow) was located in the upper mediastinum and was 3.2 × 3 × 2.8 cm. Moderate enhancement with well-demarcated borders and peripheral calcifications were observed.

seen on a chest radiograph. The mass was initially detected 4 years prior to the examination and enlarged over time. Laboratory tests revealed no abnormalities. On contrast-enhanced computed tomography, the mass was located in the upper mediastinum, measured 3.2 × 3 × 2.8 cm, was moderately enhanced with defined circumscription, and showed peripheral calcification (**Figure 1A**, **1B**). Surgery was the only available treatment option, and transcervical resection of the mass was performed. Intraoperative examination revealed that the mass originated from the sympathetic trunk. The patient's postoperative course was uneventful, and she has been recurrence-free for 6 years.

Pathological findings

The surgically resected specimen was fixed in 10% buffered formalin for 24 h, cut into 5-mm-thick tissue slices, and embedded in paraffin. Sections were cut for hematoxylin and eosin staining (2.5 μm) and IHC (4 μm). IHC was performed by using an automated slide stainer (Bench-Mark GX; Ventana Medical Systems, Tucson, AZ, USA).

The mass was artificially hemorrhagic because of physical pressure applied during surgical resection. The mass measured approximately 3.2 × 3 × 2.8 cm and was mainly grayish white in areas without hemorrhage. Hard substances were present at the periphery of the mass.

Histopathological examination revealed a largely hypercellular tumor with a focal hypocellular area within a myxoid background, which was well demarcated. At the periphery of the tumor were spicules of metaplastic woven or lamellar bones scattered throughout without entire circumscription (**Figure 2A**). In the hypocellular and myxoid areas, spindled tumor cells were arranged in cords with intermediate nuclear atypia (**Figure 2B**). In the hypercellular area, almost no matrix was intervening with the ovoid tumor cells, resulting in a sheet-like growth pattern. Nuclear atypia was intermediate to high in these cells, with some nuclei exhibiting an irregular nuclear membrane, obvious nucleoli, and several mitotic figures (1/50 high-power fields [HPFs]; **Figure 2C**). Necrosis was absent, and infiltration into the surrounding tissue was not detected. The surgical margin was free of the tumor.

Diffuse and strong expression of vimentin (V9, prediluted; Ventana) was observed in the sections using IHC (**Figure 3A**). The sections were also extensively positive for S-100 protein (polyclonal, prediluted; Ventana) with a range of intensities (**Figure 3B**) and erratically positive for CD10 (56C6, prediluted; Ventana; **Figure 3C**). Weak immunoreactivity was observed only

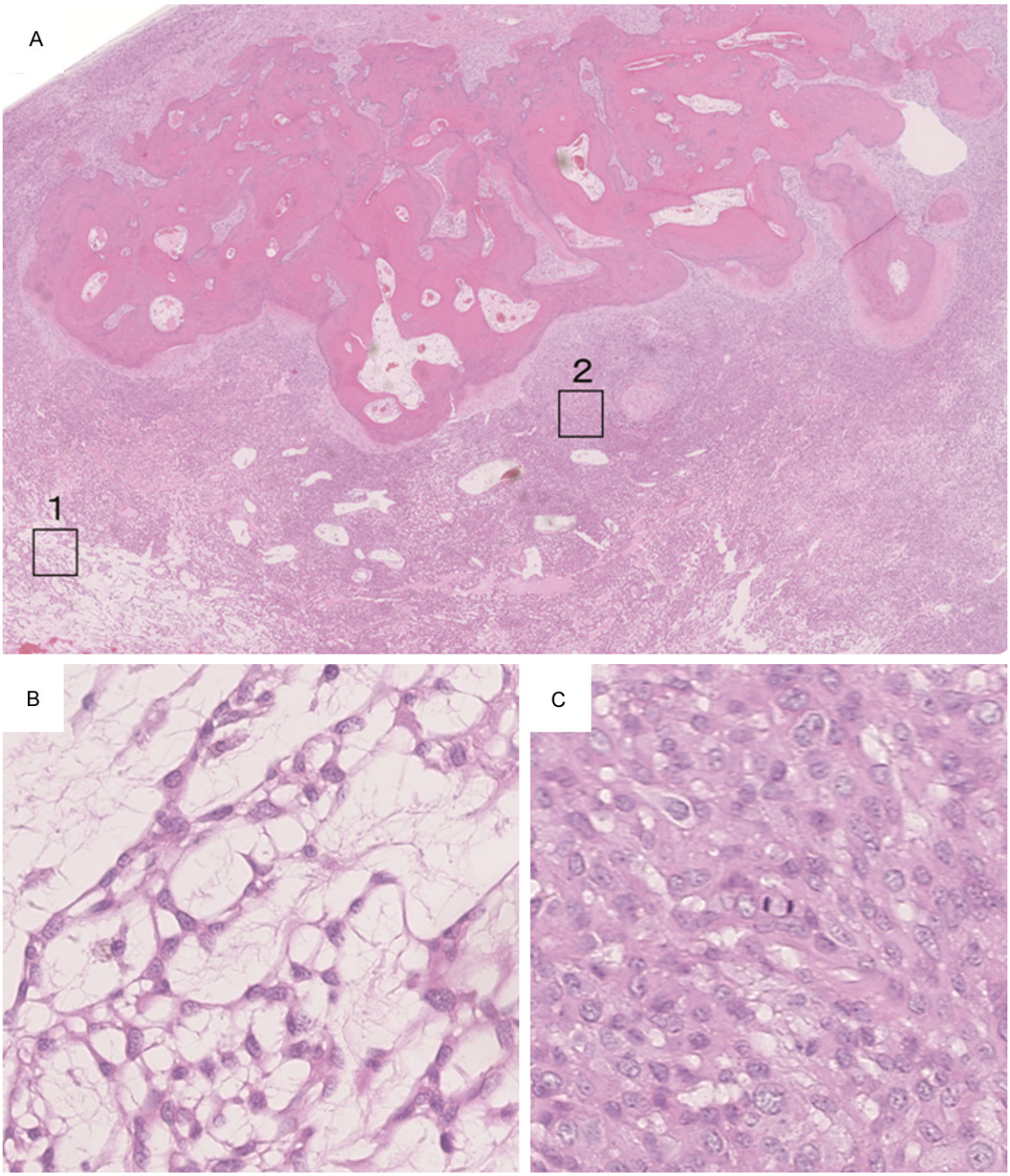

Figure 2. Microscopic findings. (A) The well-demarcated tumor was predominantly hypercellular with a focal hypocellular area within a myxoid background. At the periphery of the tumor were spicules of metaplastic woven or lamellar bones with patchy distribution (× 20); (B). Magnified view of the boxed area 1 in (A) corresponding to the hypocellular and myxoid areas. Spindled tumor cells with intermediate nuclear atypia were arranged in cords (× 400); (C) Magnified view of the boxed area 2 in (A) corresponding to the hypercellular area, in which a sheet-like growth pattern was observed. Nuclear atypia was intermediate to high, with some nuclei showing irregular nuclear membranes and obvious nucleoli. Note the mitotic figure in the center of the field (× 400).

focally for αSMA (1A4, prediluted; Ventana; **Figure 3D**). A characteristic mosaic loss of expression of INI-1 (25/BAF47, 1:200; BD Transduction Laboratories, Franklin Lakes, NJ) was observed (**Figure 3E**). The samples were negative for cytokeratin (AE1/AE3, prediluted; Ventana), GFAP (polyclonal, prediluted; Ventana), and desmin (DE-R-11, prediluted;

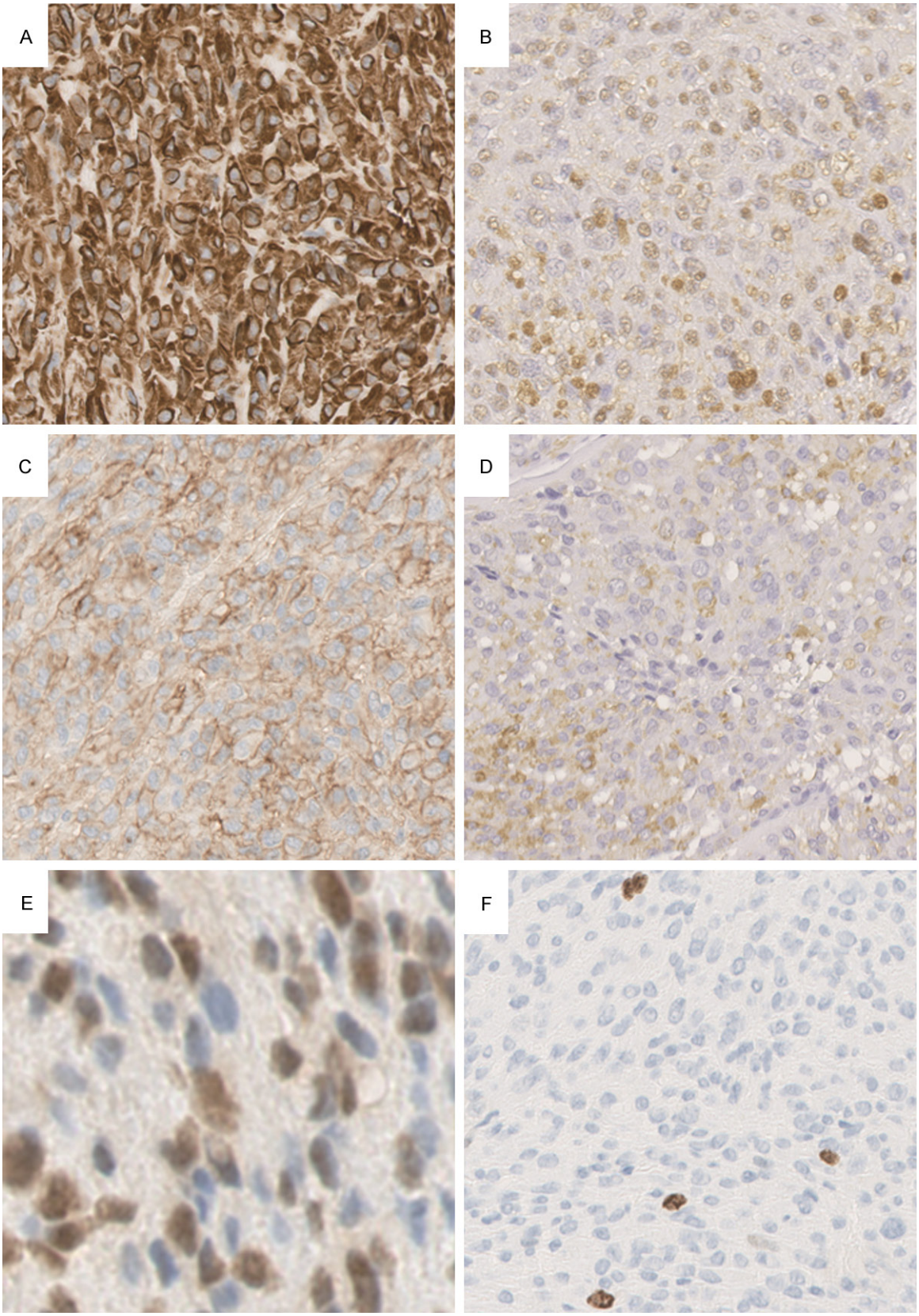

Figure 3. Immunohistochemical findings. A. Diffuse and strong expression of vimentin (× 400); B. Widespread expression of S-100 protein with low to high intensity (× 400); C. Patchy expression of CD10 (× 400); D. Focal and

weak expression of αSMA (× 400); E. Characteristic mosaic loss of INI-1 expression (× 1000); F. Sparse expression of Ki-67 (× 400).

Ventana). The Ki-67 (MIB-1, 1:100; Dako, Glostrup, Denmark) labeling index was 2.4% in 1000 cells (24 positive cells of 1000 counted cells; **Figure 3F**).

These results indicate a diagnosis of atypical OFMT. In the minor component of the hypocellular and myxoid areas, tumor cells were reminiscent of typical OFMT manifesting itself in a cord-like arrangement. IHC results also suggested a diagnosis of typical OFMT. However, the predominant hypercellular area and some tumor cells showing high-grade nuclear atypia were apparently different from typical OFMT. However, the tumor was not classified as malignant because mitotic activity was not so prominent, consistent with the low Ki-67 labeling index.

Discussion

A subset of OFMT displays atypical histological features, which often include nuclear atypia, increased cellularity, and elevated mitotic activity. A study exploring parameters for malignancy found that an increased mitotic activity, > 2 mitotic figures/50 HPFs, is a risk factor of local recurrences, whereas tumor size and necrosis are not significant risk factors [2]. Tumors with a high nuclear grade or cellularity and elevated mitotic activity (> 2/50 HPFs) have a substantial risk of aggressive behavior In a study by Folpe et al., in which 6 of 10 cases developed local recurrences (60%) and 6 of 10 cases developed metastases (60%) [5]. Likewise, in a study by Graham et al., 2 of 15 such cases developed local recurrences (13%) and 3 of 15 such cases developed metastases (20%) [8]. Tumors in this subgroup were initially designated as malignant OFMTs by Folpe et al. [5]. Cases that deviate from typical OFMT but do not meet malignant criteria can be designated as atypical OFMT. In the study by Folpe et al., 2 of 16 atypical OFMT cases developed local recurrences (13%) and 1 of 16 (6%) case developed metastases [5]. In the study by Graham et al., none of 5 atypical OFMT cases developed either local recurrence (0%) or metastasis (0%) [8]. The frequency of local recurrences and metastases of atypical OFMT was not significantly different from those of typical OFMT. In the study by Folpe et al., 3 of 25 typical OFMT cases (12%) and 1 of 25 cases (4%) developed local recurrence and metastasis, respectively [5]. In the study by Graham et al., none of 26 cases developed either local recurrence (0%) or metastasis (0%) [8]. A summary of the results from large studies [1, 4, 10] indicates that the rates of local recurrences and metastases of typical OFMT are approximately 17% and 5%, respectively, and are not very different from atypical OFMT. These findings suggests that typical and atypical OFMTs may be considered as tumors of intermediate malignancy, and for the purpose of management, it would be reasonable to recognize them as one group of tumors with histopathological variation [5]. Based on these facts, our case might be distinctively separated from malignant OFMT for the purpose of management, since it is classified as an atypical OFMT without the mitotic activity meeting malignant criteria in spite of relatively high nuclear atypia and a predominant hypercellular area. Indeed, it has not recurred for 6 years.

Considering that most OFMTs are positive for vimentin and S-100 protein on IHC, we predicted that this case would also be positive for these markers [1, 2, 8]. The expression level of S-100 protein tends to decrease as the tumor grade increases as follows: 88% for typical cases, 75% for atypical cases, and 42% for malignant cases [8]. Although CD10 expression on IHC has only been examined in one study, the rate of positive expression in OFMT was also high, similar to those of vimentin and S-100 protein [2]. The present case was also positive for CD10. This finding is especially important here, as it allowed us to differentiate it from a Schwann cell tumor. Although this case arose from the sympathetic trunk and appeared to be a kind of Schwann cell tumor based on its expression of S-100 protein, Schwann cell tumors are known to be S-100 protein-positive but CD10-negative [2]. Other markers such as cytokeratin, GFAP, desmin, and αSMA are positive only in a few cases of OFMTs [2]. Detection of αSMA, as seen in this case, is a relatively rare event, as expression was only found in about 2%-7% of cases in large studies [1, 2, 8]. Particularly striking here was the mosaic loss of INI-1 expression. Although

the same primary antibody was used, two different studies had contradictory results [8, 9]. Graham et al. frequently observed mosaic loss of INI-1 expression in typical, atypical, and malignant OFMTs, without a correlation to tumor grade [8], whereas Gebre-Medhin et al. did not detect it at all [9]. By using the same primary antibody, we also detected mosaic loss of INI-1 expression, similar to all the 3 cases of atypical OFMT (100%) showing the mosaic loss of INI-1 expression in the study of Graham et al. [8]. Although mosaic loss of INI-1 expression was not observed in the cases reported by Gebre-Medhin et al., INI-1 expression was occasionally weak [9]. The mosaic loss of INI-1 expression seen here is extremely unusual, documented only in rare schwannomas associated with familial schwannomatosis [11] and in some synovial sarcomas [12]. INI-1-deficient tumors such as renal medullary carcinomas, epithelioid sarcomas, a subset of epithelioid malignant peripheral nerve sheath tumors, myoepithelial carcinomas, and extraskeletal myxoid chondrosarcomas, in addition to malignant rhabdoid tumor of infancy, have been documented [13]. However, mosaic deficiency has not been observed in these tumor types.

INI-1 is a putative tumor suppressor gene located on chromosome 22q11.2, which encodes a protein whose expression is essential to all nucleated cells. This protein plays a role in genomic stability and regulation of cell-cycle progression [14]. Graham et al. recently proposed that OFMT develops principally through inactivation of *INI-1* [8]. Hemizygous deletion of *INI-1* was found in 5 of 7 examined cases (71%) by using interphase FISH. Mosaic loss of INI-1 expression was observed in 14 of 19 cases (74%) [8]. Although FISH was not conducted in our case, a similar mechanism leading to tumorigenesis and mosaic loss of INI-1 expression was suspected. Meanwhile, Gebre-Medhin et al. has much more recently demonstrated that tumorigenesis of OFMT is frequently related to rearrangement of the *PHF1* gene in interphase FISH, regardless of typical, atypical, and malignant OFMTs [9]. The PHF1 protein interacts with the polycomb-repressive complex 2 (PRC2), which regulates expression of various developmental genes [15]. Deregulation of such genes influenced by PRC2 is critical for tumorigenesis of OFMT. There is an antagonistic relationship between polycomb group proteins and SWI/SNF complexes, in which INI-1 is a core subunit [16, 17]. Therefore, a plausible mechanism of tumorigenesis of OFMT could increase PRC2 activity either by deregulation of *PHF1* or by inactivation of *INI-1* [9].

In conclusion, we present a case of atypical OFMT that occurred in an extremely unusual site, in the mediastinum. For its clinical management, using the same treatment as that of typical OFMT was reasonable. Mosaic loss of INI-1 expression was a characteristic finding in this case. In some OFMTs and other exceptionally rare cases, mosaic loss of INI-1 expression has also been reported. Inactivation of *INI-1* and deregulation of *PHF1* are proposed to be involved in OFMT tumorigenesis. We speculate that the mosaic loss might be related to a kind of abnormality of *INI-1*, which is sometimes observed in OFMT.

Disclosure of conflict of interest

None.

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