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Case study

Ossifying fibromyxoid tumor presenting *EP400-PHF1* fusion gene☆,☆☆,★

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Summary Ossifying fibromyxoid tumor is a rare soft tissue tumor of borderline malignancy and uncertain differentiation. Recently, a novel fusion gene, *EP400-PHF1*, was discovered in ossifying fibromyxoid tumor; however, its relation to this type of tumor has been uncertain because the *EP400-PHF1* fusion gene has been successfully detected in only 1 case. We present an ossifying fibromyxoid tumor case with the *EP400-PHF1* fusion gene detected by reverse transcriptase polymerase chain reaction, along with compatible cytogenetic data showing a t(6;12)(p21;q24.3) translocation. Our results suggest that the *EP400-PHF1* fusion gene is a reproducible finding in ossifying fibromyxoid tumor.

1. Introduction

Ossifying fibromyxoid tumor (OFMT) is an uncommon soft tissue tumor classified as a tumor of uncertain differentiation with intermediate malignancy (rarely metastasizing) [1]. Histologically, OFMT is composed of uniform round-to-ovoid cells, which has led to speculation that OFMT is a translocation-associated sarcoma [2]. Recently, Gebre-Medhin et al [3] reported a novel fusion gene, *EP400-PHF1*, and recurrent rearrangement of the *PHF1* gene in OFMT. This would be quite a significant discovery for the diagnosis of OFMT; however, the *EP400-PHF1* fusion gene was found in only 1 case in their study [3]. Here, we present the second case of OFMT with the *EP400-PHF1* fusion gene definitely confirmed by reverse transcriptase polymerase chain reaction (RT-PCR) and direct sequencing, along with the compatible cytogenetic data of translocation, t(6;12)(p21;q24.3).

2. Case report

A 71-year-old woman was referred to our hospital, presenting with a mass on the little-finger side of the right palm, which had grown very slowly over the previous 20 years. Her health was good, except for diabetes mellitus, for which she took medication. Physical examination revealed a

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★ Conflicts of interest: None.
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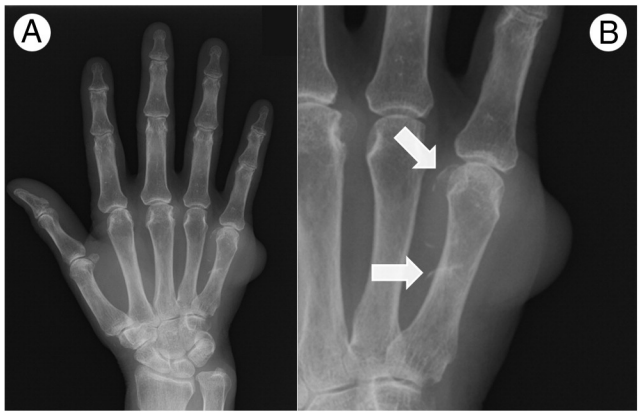

Fig. 1 Conventional radiograph of the right hand (A) and its magnification (B) reveal an oval lobulated mass with focal thin and curved mineralization (arrows) at the base of the little finger.

3-cm elastic hard mass that was negative for both tenderness and Tinel sign. Conventional radiographs revealed an oval lobulated mass (Fig. 1), measuring about 3 × 2.5 cm, with focal thin and curved mineralization (Fig. 1, arrows) in the subcutaneous area of the palm. The mass was removed by surgery. As of 3 years after surgery, the patient has remained free of any evidence of recurrence or metastasis. The patient was informed that data from the case would be submitted for publication, and she agreed.

3. Materials and methods

3.1. Immunohistochemistry

Immunohistochemistry for vimentin (1:25; Dako, Glostrup, Denmark), AE1/AE3 (1:1000; Dako), CAM5.2 (1:20; Becton Dickinson, Franklin Lakes, NJ), EMA (1:400; Dako), α-SMA (1:5000; Sigma-Aldrich, St Louis, MO), desmin (1:100; Dako), muscle-specific actin (HHF35) (1:50; Enzo Life Sciences, Farmingdale, NY), CD10 (1:100; Leica Biosystems, Nussloch, Germany), CD34 (1:50; Leica Biosystems), CD99 (1:100; Dako), S100 protein (1:400; Dako), NSE (HISTOFINE SAB-PO(R) kit; Nichirei Bioscience, Tokyo, Japan), NCAM (1:50; Leica Biosystems), GFAP (1:400; Dako), Leu7 (1:200; Becton Dickinson), SMARCB1 (also known as INI1; 1:250; BD Transduction Laboratories, San Diego, CA), and Ki-67 (1:100; Dako) was performed as described previously [4]. The immune complex was detected with the Dako EnVision Detection System (Dako). The sections were then reacted with a 3,3′-diaminobenzidine peroxytrichloride substrate solution and counterstained with hematoxylin.

3.2. Cytogenetic analysis

Viable tumor sample was obtained immediately after operation, disaggregated, cultured, harvested, and karyotyped with Giemsa banding, as described previously [5].

3.3. RT-PCR and direct sequencing for *EP400-PHF1* fusion gene

Total RNA was extracted from the frozen sample using a TRIzol reagent (Invitrogen, Carlsbad, CA) and was reverse transcribed using Superscript III reverse transcriptase (Invitrogen) to prepare the first-strand complementary DNA. Primers used for RT-PCR are listed in Table 1. PCR protocols are available on request. Each PCR product was loaded onto 2% agarose gel with ethidium bromide and visualized under UV illumination. The PCR products were also evaluated by direct sequencing using the Big-Dye terminator method (version 1.1; Applied Biosystems, Foster City, CA) to confirm the breakpoints of fusion transcripts.

4. Results

4.1. Pathologic and immunohistochemical findings

Grossly, the cut surface of the tumor was whitish in color and well demarcated in the subcutaneous tissue. Histologically, round-to-ovoid tumor cells proliferated in myxoid or fibrous stroma, presenting a multinodular growth pattern (Fig. 2A-C). Neither a hypercellular area nor mitotic figures were observed. A small, shell-like bone formation was found at the periphery of the tumor (Fig. 2D). Immunohistochemically, the tumor cells were positive for vimentin, S100 protein (Fig. 2E), CD10, and NSE, but negative for cytokeratins (AE1/AE3, CAM5.2), EMA, α-SMA, desmin, muscle-specific actin (HHF35), CD34, Leu7, NCAM, GFAP, and CD99. SMARCB1 (INI1) expression was reduced in a mosaic pattern (Fig. 2F). Based on these histologic and immunohistochemical analyses, the tumor was diagnosed as typical (nonatypical, nonmalignant) OFMT.

Table 1 Primers used for RT-PCR

Primer set		Sequence	Product size in our case (bp)
1	*EP400*ex37-F	5′-CAGGACGACAGCGACATCTA-3′	431
	*PHF1*ex2-R	5′-CAAAGTGAGGAGGCACCAGA-3′	
2	*EP400*ex38-F	5′-CCAACTTTTGCCAAACCCAC-3′	140
	*PHF1*ex2-R	5′-CAAAGTGAGGAGGCACCAGA-3′	