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Small cell osteosarcoma with *Ewing sarcoma breakpoint region 1* gene rearrangement detected by interphase fluorescence in situ hybridization

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ABSTRACT

Because of its characteristic morphologic appearance, small cell osteosarcoma (SCO) can be confused with other small round cell malignancies of the bone, most importantly with Ewing sarcoma, making this distinction difficult. A specific tool used in separating SCO from Ewing sarcoma has been the detection of *Ewing sarcoma breakpoint region 1 (EWSR1)* gene rearrangements in Ewing sarcoma and their absence in SCO. However, there are rare case reports that have documented the existence of *EWSR1* gene rearrangement in SCO. In this report, we describe another case of SCO with an *EWSR1* gene rearrangement detected by interphase fluorescence in situ hybridization. Our finding adds support to the existing evidence that SCO is a tumor that can be characterized by *EWSR1* gene arrangements. Therefore, we caution the pathology community not to rely solely on molecular studies in distinguishing SCO from Ewing sarcoma.

1. Introduction

Small cell osteosarcoma is an uncommon variant of high-grade osteosarcoma (1.3%) [1] characterized morphologically by small-sized, uniform tumor cells with at least focal osteoid production. Although this variant of osteosarcoma has clinical features and a skeletal distribution similar to conventional osteosarcoma [1], due to its characteristic morphologic appearance, it can be confused with other small round cell malignancies of the bone, most importantly with Ewing sarcoma. As the treatment protocols and chemotherapy regimens for high-grade osteosarcoma and Ewing sarcoma are different (based on National Comprehensive Cancer Network guidelines), reaching a correct interpretation in challenging cases becomes paramount.

The correct diagnosis of small cell osteosarcoma cannot always be achieved based on the morphologic appearance of the tumor alone, as the presence of osteoid in small cell osteosarcoma is frequently focal and can be overlooked. Immunohistochemical stains have been developed to help distinguish small cell osteosarcoma from Ewing sarcoma [2], but some of them have proven to be nonspecific. More recently, a specific tool used in separating small cell osteosarcoma from Ewing sarcoma has been the detection of *Ewing sarcoma breakpoint region 1* (*EWSR1*) gene rearrangements in Ewing sarcoma and its absence in small cell osteosarcoma. However, even *EWSR1* gene rearrangements have not proven to be entirely specific for Ewing sarcoma, as these rearrangements have been described in several other unrelated soft tissue lesions [3]. Despite these new reports about the nonspecificity of *EWSR1* gene rearrangement, small cell osteosarcoma has not been considered a tumor to harbor this particular gene rearrangement. Yet, to date, 3 case reports have documented the existence of *EWSR1* gene rearrangement in small cell osteosarcoma [4-6]. In this report, we describe another case of small cell osteosarcoma with an *EWSR1* gene rearrangement detected by interphase fluorescence in situ hybridization (FISH). Our finding adds support to the existing evidence that small cell osteosarcoma is another tumor that can be characterized by *EWSR1* gene rearrangement. Therefore, we caution the pathology community not to rely solely on molecular studies in distinguishing small cell osteosarcoma from Ewing sarcoma.

2. Report of a case

A 17-year-old adolescent boy complained of right knee pain for approximately 2 months before developing a pathologic fracture through his right proximal fibula following minimal physical activity. On physical examination, he had a firm, nonmobile, painful mass over his right proximal fibula. He had a full range of motion of his right knee and ankle with no instability and had normal sensation to touch throughout his right lower extremity. His coordination and deep tendon reflexes were within normal limits, and he demonstrated a 2+ dorsalis pedis pulse.

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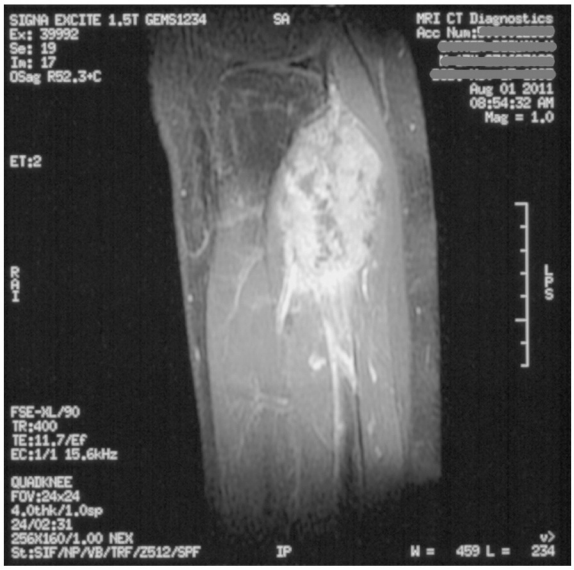

Fig. 1. Axial T2-weighted magnetic resonance imaging demonstrates the large soft tissue mass circumferentially around the right proximal fibula.

Conventional x-rays showed a permeative lesion associated with a pathologic fracture in the right proximal fibula with magnetic resonance imaging confirming the presence of a large soft tissue mass circumferentially around the right proximal fibula (Fig. 1).

The patient underwent incisional biopsy under fluoroscopic guidance of the right proximal fibula mass. The pathologic specimen from the incisional biopsy consisted of a 2.2 × 1.3 × 0.5 cm aggregate of pink-tan, soft tissue. The histologic sections showed a malignant neoplasm composed of relatively uniform tumor cells with increased nuclear-to-cytoplasmic ratio, minimal cytoplasm, and round-oval nuclei with coarse chromatin and 1 to 2 small nucleoli. Mitotic figures and apoptotic bodies were easily found. The tumor cells were arranged in nests separated by fibrovascular stroma. Tumor necrosis was observed in the center of the tumor nests. Pink, lace-like osteoid was focally identified percolating between the tumor cells (Fig. 2). This focal finding was better appreciated at higher magnification. A single focus of malignant cartilaginous matrix was identified (Fig. 3). Tumor cells were strongly and diffusely positive for CD99, with a distinct membranous pattern as well cytoplasmic staining (Fig. 4). A diagnosis of small cell osteosarcoma was rendered.

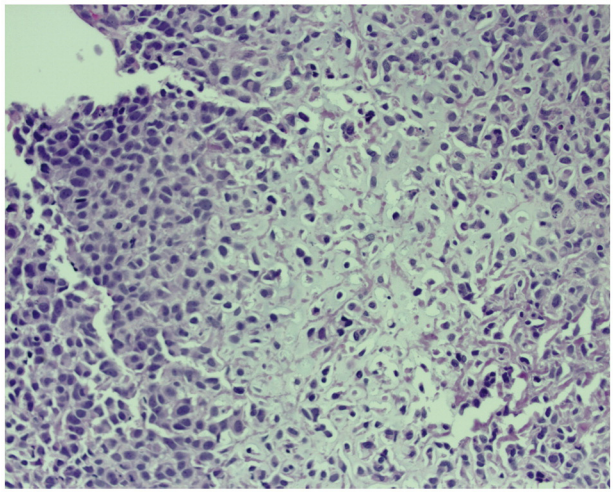

Fig. 3. Focally, malignant cartilaginous matrix is present in the biopsy material (medium power).

Tissue from the incisional biopsy that was fixed in 10% neutral buffered formalin and paraffin embedded was submitted for interphase FISH using a dual color-labeled break-apart probe specific for the *EWSR1* gene, which is localized to 22q12 (Abbott Molecular, Abbott Park, IL, USA). Following hybridization, 103 (41%) of 250 cells analyzed had an atypical FISH pattern consistent with the presence of a rearrangement of the EWSR1 probe. Specifically, 2 fusion signals and 1 green signal were seen (Fig. 5). Interestingly, one of the fusion signals was smaller than the other, suggesting that the breakpoint for the rearrangement was localized to the telomeric (3') portion of the probe. In the remaining cells, either a normal pattern or a random abnormal pattern was present.

To determine if this rearrangement might involve a translocation between chromosomes 11 and 22, a dual color fusion probe for FLI1 and EWSR1 (Cytocell, Cambridge, United Kingdom) was evaluated (Fig. 5). The FISH pattern seen with this probe was within normal limits (no fusion signals seen). However, because the breakpoint for the rearrangement in this tumor is distal (telomeric) to those often most described in patients having a t(11;22), the construct of the fusion probe may be uninformative for this case (breakpoint may lie outside the region targeted by the probe) (Fig. 5). Thus, one cannot

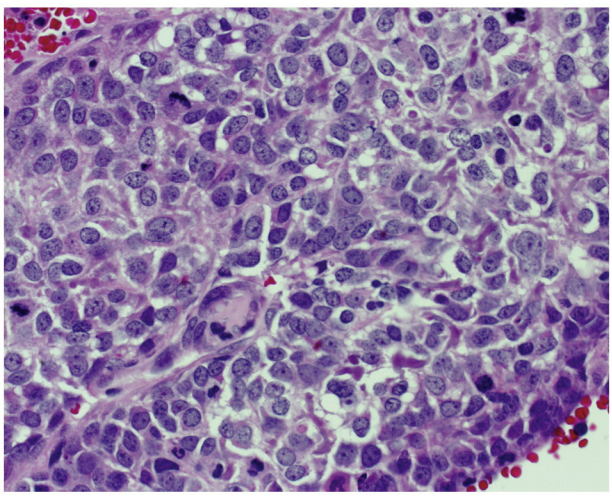

Fig. 2. The tumor consists of uniform, small-sized cells with oval, hyperchromatic nuclei and scant cytoplasm. Frequent mitotic figures are present. Pink, lace-like osteoid is focally noted between the tumor cells (high power).

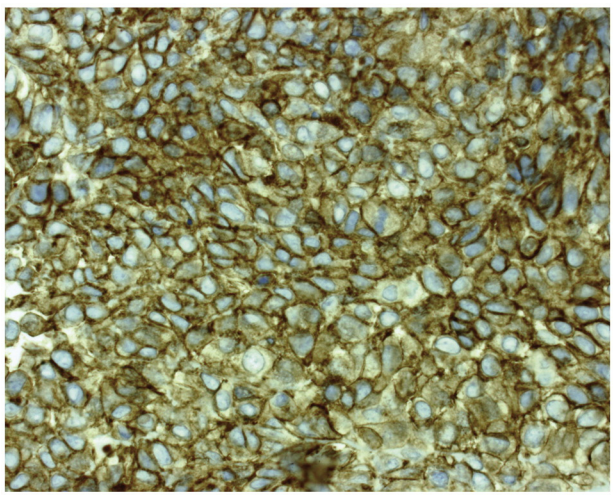

Fig. 4. Tumor cells are strongly and diffusely positive for CD99 with a distinct membranous pattern as well as cytoplasmic staining (high power).