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Case study

Sclerosing epithelioid fibrosarcoma presenting as intraabdominal sarcomatosis with a novel *EWSR1-CREB3L1* gene fusion

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Summary We report a case of intraabdominal sclerosing epithelioid fibrosarcoma (SEF) with a t (11;22)(p11.2; q12.2) *Ewing sarcoma breakpoint region 1–cAMP-responsive element-binding protein 3–like 1* translocation. A 43-year old man presented with massive ascites and shortness of breath. Imaging studies revealed a large mesenteric-based mass with extensive omental/peritoneal disease. After resection and cytoreductive surgery, the tumor recurred with metastasis to the lungs; the patient is still alive with disease. Histologically, there was a uniform population of epithelioid cells arranged in cords and nests, embedded in a dense collagenous matrix; no areas of low-grade fibromyxoid sarcoma were identified. All immunohistochemical markers were nonreactive. Fluorescence in situ hybridization studies showed rearrangement of *Ewing sarcoma breakpoint region 1*. Genomic profiling by clinical grade next-generation sequencing revealed a fusion gene between intron 11 of *Ewing sarcoma breakpoint region 1* (22q12.2) and intron 5 of *cAMP-responsive element-binding protein 3–like 1* (11p11.2). This is the first report of "pure" or true SEF presenting as intraabdominal sarcomatosis with confirmation of the recently described unique *Ewing sarcoma breakpoint region 1–cAMP-responsive element-binding protein 3–like 1* gene fusion in SEF without areas of low-grade fibromyxoid sarcoma.

1. Introduction

Sclerosing epithelioid fibrosarcoma (SEF) is a rare, distinctive variant of fibrosarcoma, typically occurring in middle-aged adults. Despite its bland appearance, it is a fully malignant neoplasm usually characterized by local recurrences and late metastases. Since its formal description by Meis-Kindblom et al [1] in 1995, SEF has been the subject of considerable interest with regard to its relationship to low-grade fibromyxoid sarcoma (LGFMS) [2-4]. The classic histologic picture of SEF is that of a sclerosing or densely hyalinized tumor with nests and cords of angulated to round epithelioid cells with scant cytoplasm that ultrastructurally have been shown to have features of fibroblasts [1]. However, the morphologic diagnosis of SEF continues to be challenging,

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particularly in biopsy specimens, owing to its typically low cellularity, abundant collagen, and banal cytologic features.

A possible genetic relationship between SEF and a subset of LGFMS possessing SEF-like histology has been postulated based on the identification of a t(7;16)(q33;p11) FUS-CREB3L2 [2,3,5-9]. However, rearrangements of *fused in sarcoma* (*FUS*) were later shown to be rare in cases showing a predominantly SEF morphology [4]. SEF-like areas can be seen in other sarcomas besides LGFMS. To date, no cases of true or "pure" SEF (ie, cases showing primarily SEF histology) have shown the specific translocations seen in LGFMS [2-4,10].

Most SEFs have been reported to occur in the deep musculature, associated with fascia and periosteum. Review of the literature has revealed rare cases occurring within the abdominal cavity [1,4,11-15]. The differential diagnosis of intraabdominal cases, particularly those associated with disseminated peritoneal disease, includes gastrointestinal stromal tumor (GIST), mesothelioma, and disseminated carcinoma.

Herein, we report a case of "pure" or true SEF presenting as sarcomatosis harboring a novel gene fusion involving Ewing sarcoma breakpoint region 1 (*EWSR1*) and *cAMP-responsive element-binding protein 3–like 1* (*CREB3L1*).

2. Materials and methods

The histologic slides of this previously unpublished case were received in consultation at the University of Texas MD Anderson Cancer Center (MDACC), and the patient was subsequently referred for further treatment. All subsequent biopsies and resection specimens in addition to clinical data were reviewed.

Immunohistochemical studies were performed on formalin-fixed, paraffin-embedded tissue sections using the streptavidin-biotinylated horseradish peroxidase complex method in a Dako AutoStainer (Carpinteria, CA). The primary antibodies used were AE1/AE3 (1:4; DAKO), calretinin (1:20; Zymed, South San Francisco, CA), Cam 5.2 (1:50; BD Biosciences, San Jose, CA), CD34 (my10, 1:40; BD Biosciences), CDX2 (CDX2-88, 1:50; BioGenex, San Ramon, CA), CD117 (c-kit) (A4502, 1:200; DAKO), carcinoembryonic antigen (1:200; Lab Vision, Freemont, CA), D2-40 (1:50; Signet Laboratories, Dedham, MA), CK5/6 (D5/16B4, 1:50; DAKO), desmin (D33, 1:75; DAKO), discovered on gist 1 (DOG1) (K9, 1:200; Novacastra, Newcastle, UK), epithelial membrane antigen (EMA) (1:100; DAKO), MDM2 (1 F2, 1:50; Calbiochem, San Diego, CA), MOC31 (1:50; DAKO), pancytokeratin (1:100; DAKO), S-100 protein (1:500; DAKO), smooth muscle actin (1:250; Ventana-Roche Diagnostics, Tuscon, AZ, USA), WT1 (6 F-H2, 1:40; DAKO), and vimentin (V9 or VIM 3B4, 1:400; DAKO).

Rearrangements of *FUS* on chromosome 16p11 were assessed using the LSI *FUS* dual-color, break-apart probe (Abbott Molecular, Des Plaines, IL), and rearrangements of *EWSR1* on chromosome 22q12 was assessed using the LSI EWSR1 dual-color, break-apart probes (Abbott Molecular) according to the manufacturer's recommendations. Tissue sections and procedure were carried out as previously described [4].

Next-generation sequencing was performed using 200 ng of genomic DNA extracted from formalin-fixed, paraffin-embedded tissue sections cut at 10-μm thick for this specific case. DNA sequencing was performed for 3769 exons of 236 cancer-related genes and 47 introns of 19 genes frequently rearranged in cancer on an indexed, adaptor-ligated, hybridization-captured library, and fully sequenced using 49 base-paired reads (Illumina HiSeq 2000, Hayward, CA, USA) to an average depth of 877X with high, uniform coverage. The specimen was evaluated for genomic alterations including base substitutions, insertions, deletions, copy number alterations (amplifications and homozygous deletions), and select gene fusions/rearrangements as previously described [16]. Sequence reads were mapped to the reference human genome (hg19) analyzed for all classes of genomic alterations. The bioinformatics process included Bayesian algorithms to detect base substitutions, local assembly algorithms to detect short insertions and deletions, a comparison with process-matched normal control samples to detect gene copy number alterations, and an analysis of chimeric read pairs to identify gene fusions.

3. Results

3.1. Clinical features

A 43-year-old man presented with a 2-month history of increasing abdominal girth and pain associated with shortness of breath. Imaging studies detected multiple masses within the abdominal cavity with diffuse ascites. Computed tomography–guided fine needle aspiration of the intraabdominal mass was diagnosed at the outside institution as a low-grade mesenchymal neoplasm. The patient sought treatment at the MDACC after 4 weeks, where reimaging studies (Fig. 1A and B) showed progression of disease with extensive involvement of the small bowel, omentum, mesentery, and peritoneum as well as increased ascites. The primary tumor was centered in the mesentery with multiple lesions and implants throughout the abdomen. Core biopsies at MDACC revealed a sclerosing sarcoma, after carcinoma and mesothelioma were excluded. The patient subsequently underwent complete omentectomy, resection of mesenteric nodules, pelvic peritonectomy, and en bloc resection of a pelvic mass and segment of small bowel, right colon, and portion of psoas muscle involved with tumor as well as intraperitoneal cisplatin chemotherapy. Eight months after resection, there were local recurrences in the diaphragm, mesentery, and peritoneum with clinical metastases to the left upper lobe of the lung and pericardium.

3.2. Gross features

Tumors diffusely involved the omentum, mesentery, peritoneum, and serosal surfaces of bowel with greater than 100 intraabdominal tumor deposits identified,