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Case study

NUTM2A-CIC fusion small round cell sarcoma: a genetically distinct variant of *CIC*-rearranged sarcoma☆,☆☆

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Summary *CIC*-rearranged sarcoma is a new entity of undifferentiated small round cell sarcoma characterized by chimeric fusions with *CIC* rearrangement. We report a *NUTM2A-CIC* fusion sarcoma in a 43-year-old woman who died of rapidly progressive disease. Histologic analysis revealed multinodular proliferation of small round tumor cells with mild nuclear pleomorphism. The sclerotic fibrous septa separated the tumor into multiple nodules. Immunohistochemistry showed that the tumor cells were diffusely positive for vimentin, focally positive for cytokeratin, and negative for CD99 and NKX2.2. Tumor cells were also negative for ETV4, which was recently identified as a specific marker for *CIC*-rearranged sarcoma. High-throughput RNA sequencing of a formalin-fixed, paraffin-embedded clinical sample unveiled a novel *NUTM2A-CIC* fusion between *NUTM2A* exon 7 and *CIC* exon 12, and fluorescence in situ hybridization identified *CIC* and *NUTM2A* split signals. This case shared several clinicopathological findings with previously reported *CIC*-rearranged cases. We recognized the tumor as a genetically distinct variant of *CIC*-rearranged sarcomas with a novel *NUTM2A-CIC* fusion.

1. Introduction

The diagnosis of small round cell sarcomas (SRCSs) can be challenging for pathologists because many candidates must be considered to obtain a differential diagnosis, and genetic confirmation is often needed. Ewing sarcoma (ES) is the most representative SRCS of soft tissue and bone and bears a specific fusion between TET/FET (*EWSRT1* and *FUS*) and ETS family genes, mainly *EWSR1-FLI1* or *EWSR1-ERG* derived from t(11;22)(q24;q12) or t(21;22)(q22;q12), respectively [1]. In addition, it is known that "Ewing-like sarcoma" has some

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fusions between TET/FET family and non-ETS family genes resulting in *EWSR1-ZSG*, *EWSR1-POU5F1*, and other such fusions. Furthermore, recent next-generation sequencing (NGS) has revealed new SRCSs that possess characteristic fusions between non-TET/FET family genes and other genes, including SRCSs with *CIC-DUX4*, *CIC-DUX10*, *CIC-FOXO4*, *BCOR-CCNB3*, and *ETV6-NTRK* fusions [1]. Among them, *CIC* rearrangement including *CIC* gene in 19q13 has given rise to the term "*CIC*-rearranged sarcoma." *CIC-DUX4* fusion sarcoma between *CIC* gene and *DUX4* gene in 4q35 is the most well-known *CIC*-rearranged sarcoma [2,3]. A recent study with 200 cases of ES and Ewing-like sarcoma revealed a low incidence of *CIC-DUX4* fusion sarcoma, which consisted of 3% of the cohort [4]. Furthermore, another study indicated that *CIC*-rearranged sarcomas account for up to 68% of non–*EWSR1*-rearranged SRCSs [5]. Thus, *CIC*-rearranged sarcomas might represent most non–*EWSR1*-rearranged SRCSs, although the precise incidence of *CIC*-rearranged sarcomas is not known. A recent cohort study with 20 *CIC*-rearranged sarcomas identified some distinct clinical and pathological features and revealed a significantly worse prognosis for patients [3]. Therefore, we should recognize that *CIC*-rearranged sarcoma is an entirely distinct entity among the SRCSs and focus on differentiating these tumors from other SRCSs during routine examinations.

However, it can be very difficult for pathologists to confirm a diagnosis of *CIC*-rearranged sarcoma by morphology and immunohistochemistry (IHC) alone because the small round cell morphology and some IHC findings are shared by many types of SRCSs. Genetic analyses including fluorescence in situ hybridization (FISH) or reverse-transcription polymerase chain reaction (RT-PCR) to detect *CIC* rearrangement is required to obtain a pathological diagnosis. We report here a case of a middle-aged female patient with an SRCS of the right buttock. We first detected *CIC* rearrangement by FISH, although neither *CIC-DUX4* nor *CIC-FOXO4* fusion [6] was found in the tumor. We attempted NGS to look for an unknown *CIC* rearrangement, and discovered a new fusion between the *NUTM2A* (also known as *FAM22A*) and *CIC* genes. We have described the *NUTM2A-CIC* fusion sarcoma as a genetically distinct variant of *CIC*-rearranged sarcoma.

2. Materials and methods

2.1. Case representation

A 43-year-old woman had a rapidly growing mass in her right buttock about half a year ago. She was given a clinical diagnosis of perianal abscess and underwent an incisional biopsy of the mass at another hospital. Pathological examination of the biopsy specimen revealed an undifferentiated sarcoma, and the patient was subsequently admitted to Sapporo Medical University Hospital for resection of the mass. Magnetic resonance imaging showed a well-demarcated multilobulated tumor (9 × 8 × 7.5 cm) mainly located in the subcutaneous fat of the right buttock. The tumor had low intensity on the T1-weighted image and high intensity on the T2-weighted image, and had focal high intensity areas on both T1- and T1-weighted images. Chest x-ray photography demonstrated multiple masses in both lungs, which were suspected of being metastatic lesions. The patient underwent resection of the tumor with marginal margins. Adjuvant chemotherapy could not be performed after the operation because the patient had developed thrombocytopenia due to chronic disseminated intravascular coagulation associated with metastatic and locally residual tumors. She was given palliative treatment for pain control and died 6 months after the operation.

2.2. Immunohistochemistry

We chose representative sections from formalin-fixed and paraffin-embedded (FFPE) tissue sliced into 3-μm-thick sections and examined them by IHC with an automated IHC system at Sapporo Medical University Hospital. All slides were loaded into a PT Link module (Dako, an Agilent Technologies Company, Glostrup, Denmark) and subjected to a heat-induced and/or enzyme-induced antigen-retrieval protocol with EnVision FLEX Target Retrieval Solution and the Autostainer Link 48 instrument (Dako). We used FLEX RTU antibodies (Dako) against the following antigens: vimentin (V9), cytokeratin (AE1/AE3), epithelial membrane antigen (E29), CD34 (QBEnd10), α-smooth muscle actin (1A4), muscle-specific actin (HHF35), desmin (D33), S-100 (polyclonal), CD99 (MIC-2: 12E7), synaptophysin (SY38), and neurofilament protein (2F11). We also used antibodies against these antigens: INI1 (H-300; Santa Cruz Biotechnology, Santa Cruz, CA; 1:50), NUT (C52B1; Cell Signaling Technology, Denver, MA), WT1 (C-terminal; polyclonal; Abnova, Taipei, Taiwan; 1:500), MKX2.2 (74.5A5; Developmental Studies Hybridoma Bank, Iowa City, IA; 1:250), and PEA3 (ETV4) (16; Santa Cruz Biotechnology; 1:20).

2.3. RNA sequencing and RT-PCR

Total RNA was isolated from 10-μm-thick sections of FFPE tumor tissues using NucleoSpin FFPE RNA XS Kit (Takara Bio, Tokyo, Japan). The quality and quantity of the RNA were determined using a Bioanalyzer 2100 (Agilent, Santa Clara, CA). The RNA sequencing library was prepared using TruSeq RNA access library kit (Illumina, San Diego, CA) as per the manufacturer's protocol. The library was subjected to paired-end sequencing of 76-base-pair (bp) fragments on the MiSeq and NextSeq sequencer (Illumina). Paired-end reads were mapped and aligned to known RNA sequences in the RefSeq, Ensembl, and LincRNA databases with the BWA-MEM program.

Total RNA was reverse-transcribed into complementary DNA (cDNA) with iScript (Bio-Rad, Hercules, CA). cDNA was subjected to PCR amplification with GXL-Taq (Takara Bio) and