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Original contribution

Inhibin-α and synaptophysin immunoreactivity in synovial sarcoma with granular cell features

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Summary We recognized immunoreactivity for the α subset of inhibin and synaptophysin in synovial sarcomas with granular cell features. Histologic findings of 90 cases of synovial sarcoma were reviewed. Two (2.2%) of the 90 cases had granular cell features, showing sheet or nested proliferation of characteristic epithelioid cells with abundant eosinophilic and granular cytoplasm, in addition to the typical spindle cell component. The 2 cases were both female (aged 86 and 76 years). The tumors were located in the foot and the retroperitoneum and measured 3.5 and 14 cm in maximum diameter. Reverse transcriptase polymerase chain reaction analysis revealed *SS18-SSX1* transcripts in both cases. *SS18* gene rearrangement was detected in granular cells as well as spindle cells by chromogenic in situ hybridization. Immunohistochemistry found the granular cells to be positive for inhibin-α in both cases and for synaptophysin in 1 case, whereas spindle cells were not. Thirty-six cases (20 monophasic fibrous, 11 biphasic, and 5 poorly differentiated synovial sarcomas) were additionally examined for comparison; they showed no immunoreactivity for inhibin-α or synaptophysin. This is the first report of immunoreactivity for inhibin-α and synaptophysin in synovial sarcoma. These immunohistochemical findings might be characteristic of synovial sarcomas with granular cell features.

1. Introduction

Synovial sarcoma (SS) occurs primarily in the extremities, usually close to joints, although it does not arise from or differentiate toward the synovium. SS can also occur away from the extremities, such as in the lung, the head and neck, or the abdominal wall. Rare anatomical sites including intra-

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abdominal or retroperitoneal regions such as the kidney [1-3], prostate [4], fallopian tube [5], and vulva [6] have been reported. Some earlier studies have reported a series of intra-abdominal or retroperitoneal cases [7,8].

Histologically, there are 2 major subtypes of SS: biphasic SS and monophasic SS. Biphasic SS has both spindle cell and epithelial components in various proportions. The tumor cells in the epithelial component form a glandular structure and sometimes look papillary. In some poorly differentiated SSs, large epithelioid cells proliferate in sheets, but they usually have clear cytoplasms.

In this study, we report 2 cases of SS arising in the foot and the retroperitoneum, which demonstrated a distinct epithelioid component with a granular cell feature in addition to the typical spindle cell component, arising in a series of 90 cases of SS. In the diagnosis of the retroperitoneal case, its location and peculiar histologic features needed immunohistochemistry with a wide spectrum of antibodies. An immunohistochemical feature that seemed unusual for SS was positivity for inhibin-α, synaptophysin, and Wilms tumor protein 1 (WT1), and therefore, we compare the immunohistochemical expression of these proteins in granular SS cases and 36 cases of conventional SS.

2. Materials and methods

2.1. Patients and materials

Two tumors with similar histologic features were identified in 90 reviewed cases of SS. All cases were registered in the Department of Anatomic Pathology, Graduate School of Medical Sciences, Kyushu University, Japan, between 1980 and 2010. The remaining 88 cases comprised 59 monophasic fibrous, 23 biphasic, and 6 poorly differentiated SSs [9].

For immunohistochemistry, formalin-fixed and paraffin-embedded samples were obtained from these 2 cases with granular cell features and 36 control cases among the total of 90 cases. The control cases consisted of 20 monophasic fibrous, 11 biphasic, and 5 poorly differentiated SSs. A fresh-frozen sample obtained from case 1 and formalin-fixed, paraffin-embedded tissue from case 2 were used for reverse transcriptase polymerase chain reaction (RT-PCR) to detect *SS18-SSX* fusion transcripts. The institutional review board at Kyushu University approved this study (permission code: 22-74).

2.2. Immunohistochemistry

Formalin-fixed, paraffin-embedded tissue was cut at 3 μm, mounted on slides, and dried at 58°C overnight. The primary antibodies are summarized in Table 1. Antigen retrieval was carried out by incubating the slides in 0.1% (wt/vol) trypsin or by boiling the slides with 10 mmol/L sodium citrate buffer (pH 6.0, with or without 0.1% Tween-20) or with Target Retrieval Solution (TRS; DAKO, Carpinteria, CA). The immune complex was visualized with the DAKO EnVision Detection System. Adequate positive controls were applied in each series of immunostaining, and negative controls were obtained by skipping the primary antibodies.

2.3. RT-PCR and sequence analysis

Total RNA was extracted from the frozen (case 1) and paraffin-embedded (case 2) samples using a TRIzol reagent (Invitrogen, Carlsbad, CA) and was reverse transcribed using Superscript III reverse transcriptase (Invitrogen) to prepare the first-strand complementary DNA.

An *SS18-SSX* fusion assay was based on the previously reported primers [10] that specifically amplify the fusion gene transcripts of *SS18-SSX1* and *SS18-SSX2*. Each PCR product (5 μL) was loaded onto 2% agarose gel with ethidium bromide and visualized under UV illumination. The PCR products were also evaluated by direct sequence analysis using the Big-Dye terminator method (version 1.1; Applied Biosystems, Foster City, CA) to confirm the breakpoints of fusion transcripts.

2.4. Dual-color chromogenic in situ hybridization

The rearranged *SS18* gene was detected using a dual-color chromogenic in situ hybridization (dc-CISH). Dual-color break-apart probes were designed and generated as previously reported [11,12]. The dc-CISH procedure was previously described [11]. Briefly, 3-μm-thick formalin-fixed, paraffin-embedded sections were deparaffinized, dehydrated, and air dried. After proteinase digestion for 10 minutes, hybridization was carried out using a hybridizer (DAKO). Signals were visualized by Dako DuoCISH using an automated immunostainer (Autostainer plus; DAKO). As for signal interpretation, the rearrangement of *SS18* gene was judged as definitely positive when isolated red and blue signals and a paired signal were observed in individual nuclei of tumor cells [12].

2.5. Ultrastructural evaluation

Tissue samples from the 2 cases with granular cell features were examined by electron microscopy (JEOL JEM-1011 electron microscope; JEOL, Tokyo, Japan). Formalin-fixed tumor tissues were refixed in 2% glutaraldehyde and in 1% osmium tetroxide. The refixed sections were embedded in epoxy resins and stained with toluidine blue for trimming and were confirmed to contain granular cell components. Thin sections were placed on carbon-coated copper grids for observation under the electron beam.

3. Results

3.1. Patients' characteristics

The 2 cases that histologically showed granular cell features both occurred in women. Patients 1 and 2 were 86 and 76 years