Synovial Sarcoma, Histologically Mimicking Primitive Neuroectodermal Tumor/Ewing's Sarcoma at Distant Sites

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We report a case of synovial sarcoma (SS) showing unusual histology at distant sites. A 47-year-old man was aware of a tumor on the sole of his left foot. After preoperative chemotherapy with a diagnosis of SS, wide excision was performed. During postoperative chemotherapy, multiple tumorous lesions developed in the bone (including the whole spine) and both lungs. The patient died 1 year later. Histologically, the excised tumor of the foot showed a biphasic cellular pattern typical of SS, whereas at autopsy the bone and lung lesions were composed only of undifferentiated small round cells with cytoplasmic fibrillar processes. Homer-Wright rosettes were also observed. Immunohistochemically, 80% of the bone and lung tumor cells expressed MIC2 protein homogeneously. To clarify whether the bone and lung round cell tumors were metastatic lesions or second malignancies, especially primary primitive neuroectodermal tumor (PNET)/Ewing's sarcoma (ES), we performed reverse transcription-polymerase chain reaction (RT-PCR) analysis of tumor type-specific fusion gene transcripts. The SYT/SSX fusion transcript was identified in both the foot and lung lesions, whereas the EWS/FLI1 transcript was not detected in either lesion. Therefore, we concluded that the multiple bone and lung tumors were poorly differentiated metastatic tumors, which arose from the SS of the foot. We also conclude that the identification of chimeric fusion transcripts can be successfully applied to poorly differentiated sarcomas and will help in the differential diagnosis of tumors that cannot be distinguished by conventional morphological examinations. Also, it should be remembered that cytoplasmic staining for MIC2 protein may occur in sarcomas other than PNET/ES.

Key words: synovial sarcoma – reverse transcription-polymerase chain reaction – Homer-Wright rosette – MIC2 protein

INTRODUCTION

Synovial sarcoma (SS) is a clinically and histologically well-defined entity. This tumor type accounts for about 10% of all soft tissue sarcomas (1) and is subclassified into three subtypes: monophasic, biphasic and poorly differentiated (1,2). The poorly differentiated subtype accounts for about 20% of SSs and shows a more aggressive clinical behavior than the other subtypes. Tumors of this subtype sometimes consist of diffuse small round tumor cells and show rosette-like structures or a hemangiopericytomatous pattern (2). Therefore, it is often very difficult to distinguish a poorly differentiated SS from a hemangiopericytoma, primitive neuroectodermal tumor (PNET)/Ewing’s sarcoma (ES) and other small round cell malignancies.

Expression of the MIC2 gene product is reported to be characteristic of PNET/ES (3–6). Moreover, many types of sarcoma are characterized by specific chromosomal translocations (4). For instance, the t(11;22) (q24;q12) and t(21;22) (q22;q12) translocations are considered to be specific for PNET/ES and the t(X;18) (p11;q11) translocation is specific for

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SS (4). Thus, immunohistochemical and genotypic analyses have turned out to be effective diagnostic tools.

We report a case of biphasic SS of the foot, which showed histological and immunohistochemical features reminiscent of PNET/ES at the metastatic sites.

**CASE REPORT**

A 47-year-old man had been aware of a tumor on the sole of his left foot for approximately 1 year before it was resected intracapsularly at a local hospital. Five months later it recurred locally. The patient visited the National Cancer Center Hospital and a needle biopsy was performed. Biopsy specimens showed a tumor with a distinct biphasic pattern of epithelioid polygonal cells and fibroblast-like spindle-shaped cells that was diagnostic of biphasic SS. The lesion did not decrease in size despite two courses of neoadjuvant chemotherapy, so wide local excision followed by reconstruction using a skin and bone graft was performed. Three months later, multiple lytic bone lesions and bilateral nodular lung lesions were found roentgenographically, which enlarged rapidly. Needle biopsy of the involved bone revealed small round cell tumor. The patient died of disseminated disease 3 years after initial presentation.

**MATERIALS AND METHODS**

**HISTOLOGICAL AND IMMUNOHISTOCHEMICAL ANALYSIS**

The tissues obtained at both surgery and the autopsy examination were fixed in 10% formalin for light microscopic examination. Paraffin-embedded tissue blocks were cut into 5 μm thick sections and stained with hematoxylin and eosin. Immunohistochemical staining to detect MIC2 protein (013, dilution 1:50; Signet Laboratories, Dedham, MA), synaptophysin (dilution 1:20; Dakopatts, Glostrup, Denmark), vimentin (dilution 1:100; Dakopatts) and cytokeratin (CAM5.2, dilution 1:100; Dakopatts) was performed using the streptavidin-biotin-peroxidase complex method.

**REVERSE TRANSCRIPTION POLYMERASE CHAIN REACTION (RT-PCR)**

Fresh tumor tissues were immediately frozen in liquid nitrogen. Total RNA was isolated using the acid guanidinium thiocyanate-phenol–chloroform method (7). A 1 μg amount of total RNA was reverse-transcribed to cDNA using random 9-mers and 100 U of reverse transcriptase (Superscript, Gibco BRL, Gaithersburg, MD) in a total volume of 20 μl according to the manufacturer’s recommendations. The putative EWS/FLI-1 and SYT/SSX cDNAs were amplified by PCR. The PCR products were sequenced using a Perkin-Elmer ABI Prism sequence analyzer (Applied Biosystems, Foster City, CA). The sequences of the primers used for RT-PCR were 5'-TCCTACAGCCAAGGCTCC-AAGTC-3' (8) for EWS, 5'-ACTCCCGGTGTCCTCCCTCC-3' (8) for FLI-1, 5'-CAACAGCAAAGATCGTACCA-3' (9) for SYT and 5'-CATGGTCAATGCACCTGATG-3' (9) for SSX. For the direct sequence analysis, the primer for SYT, 5'-CAGGGACAA-GGTCAACGTA-3', was used.

**RESULTS**

**MACROSCOPIC AND MICROSCOPIC FINDINGS**

On gross examination, the surgically resected specimen from the foot contained a 3 × 2 cm whitish nodular tumor. Microscopically, the specimen showed a distinctive biphasic pattern of epithelioid polygonal cells and fibroblast-like spindle-shaped cells. The polygonal cells contained round or oval nuclei and abundant cytoplasm and had distinct cellular borders. The surrounding spindle-shaped cells contained small amounts of cytoplasm and their cellular borders were not clear (Fig. 1). A sheet-like arrangement of round or oval cells with smaller amounts of cytoplasm and stromal fibrillar material was observed in some areas (Fig. 2).

At the autopsy examination the whole spine, right 12th rib, left clavicle, sternum and both lungs were seen to be invaded by the tumor tissue. Histological examination of these lesions revealed small round cell proliferation. Tumor cells with fibrillar cytoplasmic processes formed Homer–Wright rosettes with a central solid core, mimicking PNET/ES (Fig. 3).

From the morphological observations described above, at least two possibilities were considered in this case: primary SS in the foot with metastasis or double primary malignancy consisting of...
biphasic SS of the foot and small round cell malignancy (especially PNET/ES) at an unknown primary site.

IMMUNOHISTOCHEMICAL FINDINGS

The polygonal cells in the foot lesion expressed cytokeratin, MIC2 protein and vimentin, whereas the spindle-shaped cells expressed only vimentin and MIC2 protein. By contrast, the round or oval cells seen both in small areas of the foot lesion and throughout in the lung and bone lesions showed no distinct immunohistochemical reaction to anti-cytokeratin or anti-vimentin antibodies. Synaptophysin was not detected in any lesion. MIC2 protein was detected in the cytoplasm of 10% of the tumor cells (polygonal cells, spindle-shaped cells and small round or oval cells) in the foot lesion. By contrast, it was detected in 80% of the tumor cells (small round or oval cells) in the lung and bone lesions (Fig. 4, Table 1).

Table 1. Immunohistochemical profiles of the lesions by cell type

<table>
<thead>
<tr>
<th>Site</th>
<th>Tumor cell type</th>
<th>Immunoreactivity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CK</td>
<td>Vim</td>
</tr>
<tr>
<td>Left foot</td>
<td>Polygonal cells</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>Spindle-shaped cells</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Small round or oval cells</td>
<td>-</td>
</tr>
<tr>
<td>Lung/bone</td>
<td>Small round or oval cells</td>
<td>-</td>
</tr>
</tbody>
</table>

CK, cytokeratin; Vim, vimentin; MIC2, MIC2 protein; Syn, synaptophysin; +, positive; -, negative.

RT-PCR

We performed RT-PCR and sequence analysis for both foot and lung lesion RNA samples. SYT/SSX chimeric transcripts specific for SS were detected in both lesions (Fig. 5), whereas EWS/FLI-1 chimeric transcripts specific for PNET/ES were not detected in either lesion (data not shown).

In the light of these data showing that both the foot and lung tumors were SS and not PNET/ES, the final diagnosis of the presented case was biphasic primary SS in the foot, which showed histological conversion to poorly differentiated SS with metastasis to the lungs and bones.

DISCUSSION

In the present case, the primary lesion showed a distinctive biphasic cellular pattern, whereas the metastatic bone and lung lesions were composed of small round cells that formed Homer–Wright rosettes. The histological features of the metastatic lesions were similar to those of PNET/ES. Moreover, 80% of the cells of the metastatic lesions expressed MIC2 protein. Therefore, it was difficult to determine whether these lesions were metastatic SS or primary PNET/ES.
MIC2 protein is the product of the MIC2 gene (3). The MIC2 gene is mapped on the human X and Y chromosomes and encodes a cell surface glycoprotein with a molecular mass of 30,000–32,000 Da (11,12). Immunohistochemical analyses have detected MIC2 protein in PNET/ES (3–6). Some authors (3,11) have reported that MIC2 protein is evenly distributed throughout the PNET/ES cells, unlike its distribution in other positive sarcomas. However, other tumors of small round cells, including rhabdomyosarcoma, small cell osteosarcoma and desmoplastic small round cell tumors (S), have also been reported to express this antigen. In addition to hemangiopericytomas, SSs (13) have further been reported to express this antigen. In the present case, MIC2 protein was detected in the cytoplasm and we have recently observed a rhabdomyosarcoma with homogeneous expression of this antigen in the cytoplasm (data not shown). Therefore, cytoplasmic staining for MIC2 protein may be a point of distinction between PNET/ES and other MIC2 protein-positive sarcomas.

Monophasic or poorly differentiated SS is an MIC2 protein-positive sarcoma that should be differentiated from PNET/ES. Renshaw (13) reported that the MIC2 protein-positive cells of SS express epithelial markers that PNET/ES cells do not express. However, poorly differentiated SS often lacks epithelial antigen expression (2), as seen in the present case.

Recently, many types of sarcomas have been characterized by specific chromosomal translocations (4). The t(11;22) (q24;q12) and t(21;22) (q22;q12) translocations are considered to be specific for PNET/ES and the t(X;18) (p11;q11) translocation is specific for SS (4). In the present case, we identified the same chimeric fusion transcript specific for SS in both the primary and metastatic sites by RT-PCR followed by sequence analysis. We therefore concluded that the present case was SS with poorly differentiated components.

In addition to the difficult morphological interpretation, poorly differentiated sarcomas often lack the immunophenotype that is characteristic of the better differentiated sarcoma and pose a special problem in diagnosis. The present report proves that identification of chimeric fusion transcripts by RT-PCR is a sensitive, specific and practical diagnostic method in this clinical setting.

Although poorly differentiated SS is a relatively well-known entity, its natural history is not known. In the series reported by van de Rijn et al. (14), only two cases showed histological change from monophasic or biphasic SS to poorly differentiated SS among seven cases for which the primary and recurrent tumor were available for review. Those two patients had received radiotherapy before the histological change was observed. However, in the present case radiation therapy was not administered, so other causes of the disease progression and loss of morphological differentiation in SS remain to be clarified.

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References