ABSTRACT

Ewing's sarcoma (ES) is a small round cell tumor of adolescents or young adults that usually arises in the deep soft tissues of the extremities. The tumor cells have uniform round nuclei, fine powdery chromatin and indistinct nucleoli. CD99 (O13) is a product of the MIC 2 gene that is highly sensitive to ES but not specific. A panel of markers should be used for the differential diagnosis of small round cell tumors because nearly all others, on occasion, show membranous staining for CD99. One of the defining feature of ES is the presence of 22q12 gene rearrangement. The presented case is a 6 year-old boy complaining of swelling on his right leg. The biopsy was compatible with classic ES in terms of histopathological, immunohistochemical and cytogenetic criteria. Wide surgical resection was performed after chemotherapy. The posttreatment specimen was composed of uniformly small round cells mixed with areas of ganglion cells embedded in neurophil-like fibrillary background. Immunohistochemically, neoplastic cells revealed strong CD99 (O13) and NSE staining and the tumor had EWSR1 gene rearrangement. Morphologic alterations due to treatment are commonly seen in pediatric tumors. Single case reports have defined neural differentiation in ES but to the best of our knowledge this is the first report of ES in the literature with all histopathological, immunohistochemical, and cytogenetic criteria evaluated in both pretreatment and posttreatment specimens.

Key Words: Ewing's sarcoma, Therapy, FISH, EWSR1, Neural differentiation

INTRODUCTION

Ewing's sarcoma (ES) represents 6% to 8% of all primary malignant bone tumors. Young adults and children are those most commonly affected. Radiological features include an infiltrative, ill-defined lesion and bone destruction and periosteal reaction of the diaphysis of a long tubular or flat bone. ES is composed of small round cells with scant cytoplasm and indistinct cytoplasmic borders. Nearly all tumors show immunohistochemical positivity for CD99 (O13). This group of tumors contains a 22q12 gene rearrangement (1) and may exhibit rosette-like structures and/or variable markers of neural differentiation (excluding ganglion cells) with a fibrillary background (2). The aim of this case presentation and literature review was to discuss the characteristics and treatment for ES.

CASE REPORT

A 6-year-old Syrian boy presented to another university hospital with a mass in his right thigh and inability to bear weight for the last 6 months in October 2013. A truncut biopsy was performed and ES without any metastases diagnosed at that center. The patient underwent neoadjuvant chemotherapy with the VIDE protocol for 4 months. After neoadjuvant chemotherapy, the patient had been referred to our center for surgical treatment. Radiological staging of the patient had been done and a lytic-sclerotic lesion of the entire femoral diaphysis causing a partially healed pathological fracture with a massive soft tissue mass which had extensive sunburst-like calcifications of soft tissue and formation of a Codman triangle involving the full length of the femoral diaphysis (Figure 1A-C). Magnetic resonance imaging of the thigh showed a huge soft tissue mass involving both anterior and posterior compartments with central necrosis and heterogeneous contrast enhancement originating from the femoral diaphysis, from the proximal femoral metaphysis (without physeal involvement) to the entire femoral diaphysis and the distal physis (Figure 2A,B). No sign of metastasis was seen in PET/CT and bone scan (Figure 3). The pathological specimens were reviewed again at our pathology department. The tumor was composed of uniform small round cells with fine chromatin and scant cytoplasm with diffuse immunopositivity to CD99 (O13; 1:50; Biogenex, Fremont, CA, USA); but negative to leukocyte common antigen (LCA) (1:100; Dako, Glostrup, Denmark), desmin (1:50; Dako, Glostrup, Denmark), and myeloperoxidase (MPO) (1:300; Leica, IL, USA), terminal deoxynucleotidyl transferase (TDT) (1:50; Dako, Glostrup, Denmark). Vysis LSI EWSR1 (22q12) Dual Color Break Apart Rearrangement Probe (Abbott, Des Planes, IL, USA) was used to detect tumor cells with one green and one orange
signal (Figure 4A,B). The EWSR1 gene rearrangement was detected by fluorescence in situ hybridization (FISH) in this biopsy. The tumor was interpreted as ES. The patient was discussed at the musculoskeletal oncology council and because of extension of the disease through the whole femoral diaphysis with a huge soft tissue mass, the age of the patient and the patient’s socioeconomic status (he was a Syrian immigrant living in an immigrant camp and unable to have the adequate follow-up after a complex biological reconstruction), a hip disarticulation was performed in February 2014. Grossly, a 15×7×7 cm tumor mass was detected. Necrotic areas represented 5% of the whole lesion. The viable tumor was composed of uniformly small round cells mixed with areas of ganglion cells embedded in a neurophil-like fibrillary background. The surgical margins were negative. Immunohistochemistry (IHC) revealed strong expression of CD99 and patchy expression of neuron-specific enolase (NSE) (1:200; Dako, Glostrup, Denmark), but not LCA, desmin, neuroblastoma marker (Nb84a; 1:100; Leica, New Castle, UK), or MPO. The EWSR1 gene rearrangement was detected by fluorescence in situ hybridization (FISH) in the surgical material (Figure 5A,B). The patient completed the adjuvant chemotherapy in September 2014. After a follow-up of 1 year, the patient is

Figure 1: A) Clinical view of the patient. B) AP and C) Lateral radiography of the right femur after neoadjuvant chemotherapy.

Figure 2: A) Coronal and B) Axial T1 fat sat postcontrast MRI images.
alive with no evidence of the disease and is able to walking with two crutches.

**DISCUSSION**

Morphologic alterations due to chemotherapy are commonly seen in pediatric tumors such as rhabdomyosarcomas, osteosarcomas, and Wilms’ tumors (3-5). Some studies have indicated that complete neural differentiation in ES may be due to treatment (6-9).

We have herein presented a case of classic alteration of ES with areas of ganglion cells and a fibrillary neurophil-like background intermingled with small round cells after four courses of chemotherapy. Both pretreatment and posttreatment specimens showed CD99 immunopositivity and presence of the EWSR1 gene rearrangement. This case differs from other cases in the literature because the morphological, immunohistochemical, and molecular features of the ES persisted after therapy. Maeda et al. (7) presented a case involving widespread replacement of an ES by a tumor with a lower MIB-1 index and neuroendocrine differentiation composed of ganglion cells following preoperative chemotherapy and radiotherapy. Knezevich et al. (8) presented an extraosseous ES that exhibited a well-differentiated neural tumor composed of ganglion cells following chemotherapy and radiotherapy. The initial biopsy showed EWS/FLI1 fusion, but wide resection lacked the EWS/FLI1 fusion transcript. Collini et al. (10) presented a case of CD99-positive ES; after treatment, the surgical specimen lacked CD99, resembling differentiating neuroblasts. Both pretreatment and posttreatment specimens showed the EWS/FLI1 gene fusion transcript, the hallmark of ES (Table I).

The possible scenarios of neural differentiation in ES are summarized below.

1. Cells with neural differentiation survive. Primitive round cells are killed by cytotoxic drugs (7).
2. Chemotherapeutic drugs induce active neural differentiation in tumor cells (7).
3. Slowly growing clones of the primary tumor lacking the EWS/FLI1 gene fusion transcript are less sensitive to chemotherapy and radiation (8).

**Figure 3:** PET/CT after neoadjuvant chemotherapy.

**Figure 4:** A) Initial tru-cut biopsy showing uniformly small round neoplastic cells with scant cytoplasm (H&E; x40). B) Initial biopsy, EWSR1 gene rearrangement transcript (FISH; x600).
4. Neural differentiation could represent a direct phenotypic feature of surviving cells or may be induced by therapy (8).

5. EWS/FLI1 and related oncoproteins may inhibit neural differentiation and maintenance of cells in a proliferative state within the cell cycle (8).

<table>
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<th>Clinical findings</th>
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<tr>
<td><strong>Needle biopsy</strong></td>
<td>Usual type ES.</td>
<td><strong>Needle biopsy</strong></td>
<td>Vimentin (+), O13 (+), SYN (+), NSE (+), Leu7 (–), MIB-1 (5)</td>
<td><strong>Autopsy</strong></td>
</tr>
<tr>
<td><strong>Surgical specimen</strong></td>
<td>Widely necrotic areas were detected. Residual tumor nests mimicking ganglion cells were intermingled with foamy macrophages.</td>
<td><strong>Needle biopsy</strong></td>
<td>Vimentin (+), O13 (+), NF (+), SYN (+), NSE (+), Leu7 (+), MIB-1 (2.2)</td>
<td><strong>Autopsy findings</strong></td>
</tr>
<tr>
<td><strong>Autopsy findings</strong></td>
<td>Metastatic tumors were composed of mainly uniform small round cells. Ganglion-like large cells were not detected.</td>
<td><strong>Surgical specimen</strong></td>
<td>Vimentin (+), O13 (+), NF (+), SYN (+), NSE (+), Leu7 (+), MIB-1 (2.2)</td>
<td><strong>Autopsy findings</strong></td>
</tr>
<tr>
<td><strong>Needle biopsy</strong></td>
<td>Usual ES with occasional neural-type rosettes. Ultrastructural analysis revealed primitive cells with glycogen accumulation.</td>
<td><strong>Biopsy</strong></td>
<td>O13 (+), NSE weakly (+), SYN occasionally (+), S100 (–), MSA (–), desmin (–), EMA (–)</td>
<td><strong>Surgical specimen</strong></td>
</tr>
<tr>
<td><strong>Surgical specimen</strong></td>
<td>Well-differentiated peripheral neural tumor with ganglion cell differentiation, no foci of typical ES. Ultrastructurally, neurosecretory granules were detected. Necrotic areas were undetermined.</td>
<td><strong>Biopsy</strong></td>
<td>O13 (+), NSE weakly (+), SYN occasionally (+), S100 (–), MSA (–), desmin (–), EMA (–)</td>
<td><strong>Surgical specimen</strong></td>
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<td><strong>Needle biopsy</strong></td>
<td>Usual ES.</td>
<td><strong>Needle biopsy</strong></td>
<td>CD99 (+), vimentin occasionally (+), SYN (+), NSE focal (+), S100 focal (+), Hu (–), Nb (–), chromogranin A and B (–), cytokeratin (–)</td>
<td><strong>Surgical specimen</strong></td>
</tr>
<tr>
<td><strong>Surgical specimen</strong></td>
<td>Necrotic areas, 40%. Ganglion cells in a fibrillary background resembled a differentiating neuroblastoma.</td>
<td><strong>Biopsy</strong></td>
<td>CD99 (+), vimentin occasionally (+), SYN (+), NSE focal (+), S100 focal (+), Hu (–), Nb (–), chromogranin A and B (–), cytokeratin (–)</td>
<td><strong>Surgical specimen</strong></td>
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<td><strong>Needle biopsy</strong></td>
<td>Usual ES.</td>
<td><strong>Needle biopsy</strong></td>
<td>CD99 diffusely (+), NSE (–), LCA (–), MPO (–), Nb (–)</td>
<td><strong>Surgical specimen</strong></td>
</tr>
<tr>
<td><strong>Surgical specimen</strong></td>
<td>Necrotic areas, 5%. Uniformly small round cells mixed with areas of ganglion cells embedded in a neutrophil-like fibrillary background.</td>
<td><strong>Needle biopsy</strong></td>
<td>CD99 diffusely (+), NSE (–), LCA (–), MPO (–), Nb (–)</td>
<td><strong>Surgical specimen</strong></td>
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**IHC:** Immunohistochemistry, **NF:** Neurofilament protein, **SYN:** Synaptophysin, **NSE:** Neuron-specific enolase, **GFAP:** Glial fibrillary acidic protein, **MSA:** Muscle-specific actin, **Nb:** Neuroblastoma marker, **MPO:** Myeloperoxidase, **LCA:** Leukocyte common antigen, **RT-PCR:** Real-time polymerase chain reaction, **FISH:** Fluorescence in situ hybridization, **CT:** Chemotherapy, **RT:** Radiotherapy
Collini et al. and Maeda et al. reported the EWS/FLI1 gene fusion transcript following therapy (7,10). However, the patient described herein contained the EWSR1 gene rearrangement; therefore, the results are not consistent with the suggestion by Knezevich et al. (8). Our patient’s initial and post CT-RT specimen exhibited CD99-positive staining and presence of the EWSR1 gene rearrangement transcript. From this aspect, this case differs from the other cases in terms of the IHC and cytogenetic features.

Our patient’s surgical specimen contained neural cells following therapy, which is consistent with hypotheses #2 and #4 described above. Furthermore, he had the same IHC and FISH results regardless of therapy. The best of our knowledge this is the first report of ES in the literature with all histopathological, immunohistochemical, and cytogenetic criteria in both pretreatment and posttreatment specimens. Pathologists must be aware of therapy-induced alterations to avoid misdiagnosis of these tumors.

**CONFLICT of INTEREST**

The authors have no conflict of interest to declare.

**REFERENCES**


