

Original Article

Rosette-forming epithelioid osteosarcoma in childhood: a case report

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Abstract: Osteosarcoma with epithelioid appearance and a rosette-like configuration is a rare, recently reported variation in osteogenic sarcoma, which is thought to be associated with a poor prognosis. We report an unusual pediatric case of rosette-forming epithelioid osteosarcoma with poor response to neoadjuvant chemotherapy and repeated local recurrence following surgical resection. A 6-year-old boy presented with a progressive swelling and pain around the right knee for 25 days before admission. Plain X-ray and MRI showed an ill-defined, expansile, and osteolytic lesion involving the cortical and medullary region of right distal femur with varying degrees of mineralization, and periosteal reaction, extending to surrounding soft tissue. Punch biopsy and subsequent surgical specimens showed a tumor composed of epithelioid cells predominantly arranged in a rosette-like structure, or between dilated blood vessels showing a hemangiopericytoma-like appearance, occasionally with lacelike osteoid deposits. This tumor showed immunoreactivity for epithelial membrane antigen, CD56, CD99, Fli-1, TTF-1, and vimentin. Because of its peculiar morphology, rosette-forming epithelioid osteosarcoma should be differentiated from small cell osteosarcoma, metastatic carcinoma and other tumor with similar morphology, especially neuroblastoma, as well as Ewing's sarcoma, and vitally can be distinguished by the presence of osteoid matrix. Given its poor prognosis, awareness of rosette formation in osteosarcoma is important to avoid misdiagnosis and guide further clinical treatment. This patient experienced twice recurrence during a 15-month period in spite of surgery with wide surgical margins and systemic chemotherapy.

Keywords: Osteosarcoma, epithelioid, rosette-forming

Introduction

Rosette-forming epithelioid osteosarcoma (RF-EOS) is a rare type of conventional osteoblastic osteosarcoma that appears epithelioid, arranged in a rosette form or chrysanthemum-like structure. The tumor, like conventional osteosarcoma, arises primarily in the metaphysis of long bones in adolescents and young adults under 30 years of age, and males were predominant in the sex distribution, it accounts for 5.7% of all osteosarcoma [1]. Currently, less than 30 cases have been reported [1-6]. Because the tumor can express EMA, NSE, CD56 and CD99 [1], it can be easily misdiagnosed as small cell osteosarcoma, neuroblastoma, Ewing's sarcoma, or metastatic carcinoma.

Herein, we reported an unusual pediatric case of such tumor occurred in the metaphysis of

the distal femur, with special attention to its intense and diffuse nuclear expression of Fli-1 and TTF-1, and the differential diagnosis between these osteosarcomas from Ewing's sarcoma and metastatic carcinoma. To our knowledge this is the first report of RF-EOS with such unexpected immunoprofile.

Case presentation

A 6-year-old, previously healthy boy had gradually increasing pain developed in his right knee for approximately 4 weeks before seeking medical attention in December 2013. He had no history of cancer, irradiation, trauma, or infection. Computerized tomography (CT) scan taken at an outside hospital revealed a disruptive change of the lower right femur with surrounding soft tissue swelling around right knee, highly suggesting malignancy, possibly osteosarcoma. The patient was referred to our institution for

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Figure 1. Radiographs of the distal femur tumor. A. Anteroposterior radiograph showing an osteolytic and destructive lesion located in the right distal femur with an ill-defined margin, small amount of fluffy internal mineralization, and thick periosteal reactions forming a typical Codman triangle. B. Coronal magnetic resonance imaging (MRI) scan showed a destructive lesion of the distal femur involving the surrounding soft tissues. Note the abnormal signal intensity of the bone marrow in the metaphysis of the femur, the cortical destruction, and the prominent soft-tissue mass with the surrounding edema or reactive zone.

additional evaluation and treatment. Physical examination revealed a mildly tender, warm, and markedly swollen area on the posterior aspect of the right distal femur without skin redness. Laboratory tests showed increased serum alkaline phosphatase of 437 IU/L. Anteroposterior and lateral plain X-ray radiographs of the right lower extremity revealed a radiolucent lesion in the metaphysis of the left distal femur, with cortical destruction, a Codman's triangle, and soft tissue mass (**Figure 1A**). Chest radiographs and CT of the lungs did not show evidence of metastatic disease. A bone scan was positive for the lesion in the left distal femur, but not elsewhere. Magnetic resonance imaging (MRI) and CT scans of the right lower extremity revealed a destructive lesion in the right distal femur measuring approximately 11 cm in length and abutting the articular surface with a soft tissue mass posterolaterally and periosteal elevation, which shows different degrees of calcification shadow (**Figure 1B**). A CT-guided biopsy of the right distal femur was interpreted as a high-grade osteoblastic osteosarcoma. The patient was started on six cycles of neoadjuvant chemotherapy consisting of lobaplatin and adriamycin, which was used for

the first time, and changed to ifosfamide. He subsequently underwent segmental resection of the right distal femur. The margins were negative for tumor. The patient also completed seven cycles of postoperative chemotherapy consisting of ifosfamide, lobaplatin, and pirarubicin hydrochloride without complication. Despite repeat courses of chemotherapy and wide resections of the tumor, local recurrence developed twice within 15 and 20 months after the initial surgery, respectively, and then he underwent wide surgical re-excision as a treatment for the recurrent lesions.

Materials and methods

The tissues were fixed in 4% buffered formalin and embedded in paraffin. Immunohistochemistry was performed on a Ventana Benchmark autostainer (Roche Diagnostics, Basel, Switzerland) using the polymer/HRP-linked secondary antibodies (Dako EnVision™ Peroxidase/DAB Detection System kit (Dako Corp., Carpinteria, CA, USA) after antigen retrieval. Peroxidase activity was developed using hydrogen peroxide as a substrate and 3, 3'-diaminobenzidine tetrahydrochloride (DAB) (Dako) as chromogen. The antibodies used in this study included AE1/AE3, CAM5.2, CD56, CD99, CDK4, cytokeratin (CK), chromogranin A (CgA), E-cadherin, epithelial membrane antigen (EMA) (Santa Cruz), Fli-1, Ki-67 (Novocastra, UK), MDM2 (Diagnostic BioSystems, USA), neuron-specific enolase (NSE), p53, S-100 (polyclonal), synaptophysin (Syn) (polyclonal), thyroid transcription factor-1 (TTF-1), and vimentin. All primary antibodies used in this study are murine monoclonal antibodies obtained from DAKO Corporation (DAKO Corporation, Carpinteria, CA), unless otherwise stated.

Results

Initial tumor biopsy specimens consisted gray broken tissue, measured 1.0 × 1.0 × 1.0 cm in size. The resected specimen, measured 7.0 × 4.5 × 4.5 cm, showed extensive intraosseous and extraosseous tumor growth with broad cortical destruction. The cut section showed a

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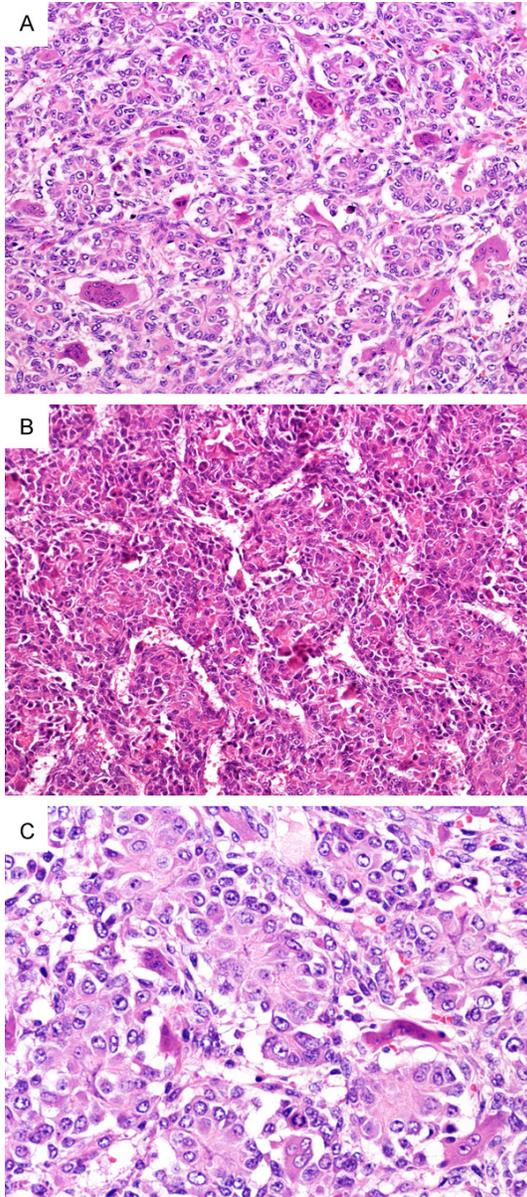


Figure 2. Histological features of epithelioid osteosarcoma of the femur. A. Osteosarcoma with rosette-like structures showing a small multinodular growth pattern with osteoid deposits in the center. B. Epithelioid-appearing osteoblastic cells dilated blood vessels showing a hemangiopericytoma-like appearance. C. Epithelioid tumor cells showing abundant pale cytoplasm, vesicular nuclei, and prominent nucleoli in a rosette configuration, associated with scattered osteoclast-like multinucleated giant cells.

white and gritty appearance tumor, size 3.0 × 2.5 cm. First recurrence of resection specimen, 2.5 × 2.0 × 2.0 cm; Second recurrence resection specimens, 8.0 × 4.5 × 4.5 cm. Necrosis and hemorrhage were occasionally seen within the tumor.

Histologically, the initial biopsy, surgical resection, and two recurrence of specimens showed virtually identical morphology, characterized by obvious epithelioid-appearing osteoblastic cells in a small multinodular growth pattern arranged in rosette-like structures (**Figure 2A**), nests, or between prominent, often dilated blood vessels in a hemangiopericytoma-like appearance (**Figure 2B**). The tumor cells were noncohesive, short-spindled, round to polygonal, larger more than 20 μm in diameter, with relatively abundant eosinophilic cytoplasm, round to oval eccentrically located vesicular nuclei and prominent nucleoli. The nuclei showed a finely distributed chromatin pattern, and each contained 1 or 2 prominent nucleoli with minimal nuclear pleomorphism. Mitotic figures were abundant, more than 28 mitoses per 10 high-power fields. This tumor contained a little amount of stellate-shaped or fine lace-like osteoid matrix, focally deposited in broad sheets between the tumor cells within the nodules (**Figure 2C**). Osteoclast-like multinucleated giant cells were scattered and frequently associated with tumor osteoid within the nodules. The area of visible tumor tissue necrosis after chemotherapy tumor was of < 10% in resection specimens.

Immunohistochemically, the tumor cells were stained positively for EMA, CD56, CD99, Fli-1, TTF-1, and vimentin. CD56 was strongly in the cell membrane of epithelioid cell and weakly in area spindle cells (**Figure 3A**). Among these, EMA was positive in the membrane and cytoplasm of individual tumor cell, CD99 stained weakly in both epithelioid and spindled cells (**Figure 3B**), while Fli-1 (**Figure 3C**) and TTF-1 (**Figure 3D**) reactivity was observed in the nucleus of the majority of these cells. AE1/AE3, CAM5.2, NSE, p53, CgA, Syn, MDM2, CDK4, E-cadherin and S-100 were negative. The Ki-67 (MIB-1) labeling index was approximately 25% (**Figure 3E**).

Discussion

We describe an unusual case of pediatric osteosarcoma showing epithelioid appearance in rosette-like configuration, expressing CD56, CD99, Fli-1, and TTF-1. The case of tumor was highly aggressive, recurrence twice in the short term after surgical resection. Such clinical, pathological and unexpected immunophenotypic characteristics of osteosarcoma have not been reported previously.

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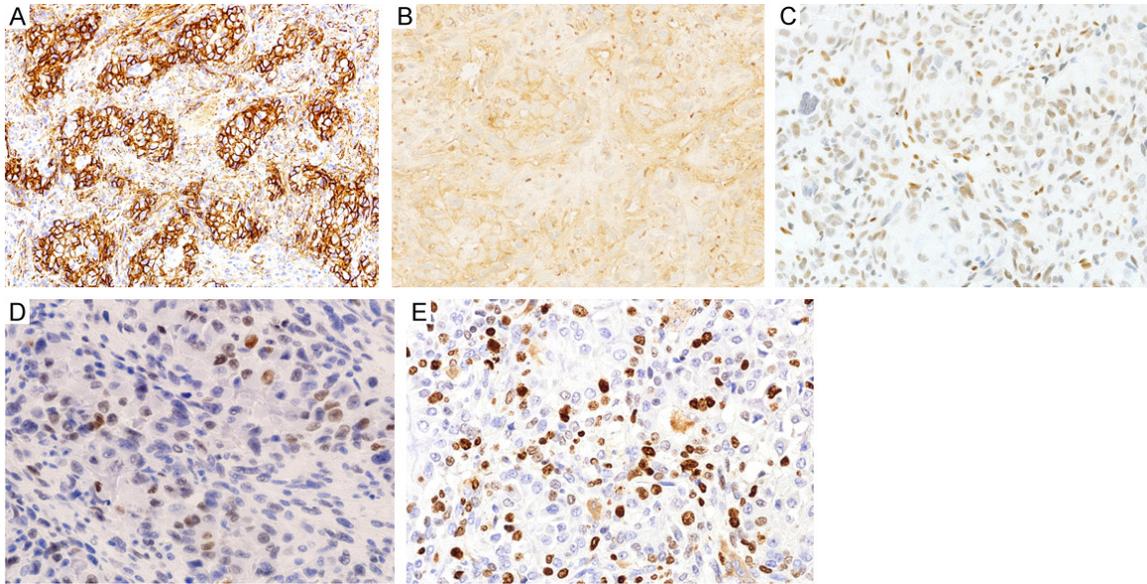


Figure 3. Immunohistochemical findings of epithelioid osteosarcoma of the femur. A. The epithelioid cells shown positivity in the cytoplasm for CD56. Note that diffuse and strong immunoreactivity in the epithelioid tumor cells of osteosarcoma with rosette like structures. B. CD99 was weakly expressed in the cytoplasm of neoplastic cells. C. Nuclear Fli-1 protein immunoreactivity in the tumor cells. D. Nuclear TTF-1 immunopositivity in the neoplastic cells. E. The proliferative index Ki-67 was about 25%. Dako Envision, original magnification, each $\times 400$.

Osteosarcomas have a variety of clinicopathologic types and histological patterns. Rarely, this tumor may appear epithelioid, including a rosette like configuration simulating glands. Epithelioid osteosarcoma is first described by the Scranton and others [7]. The term epithelioid is used to denote a variant of various tumors formed predominantly by cells with epithelial-like morphology, characterized by abundant eosinophilic cytoplasm, large vesicular nuclei with prominent nucleoli, at least two times larger when referring to its maternal cells. With regard to osteosarcoma, a variant of epithelioid osteoblastic osteosarcoma has been described that is characterized by rosette-like structures surrounding a central nidus of stellate-shaped or fine netlike osteoid [8]. A previous study of 16 cases of rosette-forming osteosarcoma showed that most cases occurred in men in the second decade with a predilection for the lower extremity (femur or tibia) [1]. At the time of diagnosis, the ages of the patients ranged from 8 to 26 years (mean age, 15 years), and 69% (11 of 16) of the patients were in the second decade. Eleven of the patients were male, and 5 were female. The tumors involved mainly the metaphysis of long tubular bones, particularly of a lower extremity (13 femurs and 3 tibias), with some extension to the epiphysis or diaphysis,

occasionally in areas such as the shoulder and jaw [3-6]. The most common symptoms are pain, followed by swelling, but nonspecific. Such manifestations may be accompanied by other symptoms, including joint dysfunction, increased skin temperature, a small number of patients with pathological fracture. Radiographic findings vary but are principally similar to those of conventional osteosarcoma, mostly show an ill-defined, osteolytic, and destructive appearance, involving the surrounding soft tissue with varying degrees of mineralization. Microscopically, tumors are characterized by a small multinodular growth pattern between prominent, often dilated blood vessels showing a hemangiopericytoma-like appearance. The tumor cells are arranged, at least partially, in rosette like structures, and may be surrounded stellate-shaped or fine lacelike osteoid. In most cases, scattered osteoclast-like multinucleated giant cells were present and frequently associated with tumor osteoid within the nodules. The tumor can be associated with hemorrhage and necrosis [9, 10].

The diagnosis of osteosarcoma depends predominantly on the images of the periosteal reaction, osteogenesis, and histological finding of osteoid matrix, which can be irregular lace-

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like, ribbons or wide strip, somewhat refractile. However, attention should be taken between the osteoid and other eosinophilic extra-cellular materials such as fibrin and amyloid. Unequivocal discrimination between osteoid and non-osseous collagen may be difficult, or sometimes arbitrary. Up to now, there are no specific markers for osteosarcomas. In previous series, 50% and 88% of osteosarcomas with rosettes stained positively for NSE and CD56, respectively. Similarly, 80% and 60% of conventional osteoblastic osteosarcomas showed reactivity for NSE and CD56, respectively. Other neural markers such as chromogranin A, neurofilament, and synaptophysin, which are more specific markers than are NSE and CD56, are not expressed by osteosarcoma with rosettes or by conventional osteoblastic osteosarcoma [1]. Thus, positive staining for either NSE or CD56 cannot be regarded as absolute evidence of neural differentiation. Moreover, the MIC2 (CD99) was originally described as a marker for ES/PNET, but its reactivity can occasionally be seen in many types of bone and soft-tissue tumors, including 16% of small-cell osteosarcomas. As in other mesenchymal tumors, the epithelioid phenotype in osteosarcoma may be accompanied by the expression of epithelial antigens, albeit in a small number of cases [11-13]. Expression of epithelial antigens does not appear to correlate with histological subtype as this finding has been reported in osteoblastic, fibroblastic, chondroblastic, and epithelioid osteosarcomas [11, 12]. In line with such findings, the present case also showed expression of CD56 and CD99, low degree of expressing epithelial marker EMA, but not other neural markers. Unexpectedly, the tumor expressed TTF-1 and Fli-1, both of which rare research have been done in bone tumors [14, 15]. Fli-1 protein, a member of the ETS family of DNA-binding transcription factors, mainly appears in ES/PNET [16] and vascular tumors [17], represents a genomic marker for these tumors. However, it can also appear in lymphoma, desmoplastic small round cell tumor, and with lower frequency in other soft tissue tumors [18, 19]. TTF-1 (NKX2-1) is a tissue-specific transcription factor that plays a critical role in the normal development of embryonic epithelial cells of the thyroid and lung. Because TTF-1 expression is highly restricted to epithelial tumors arising in these organs, it is, at present, most commonly used to identify tumors of

thyroid or pulmonary origin [20]. It has been reported although much less frequently, to be expressed in some carcinomas arising in other organs. Thus, the clinical and pathological significance of this unexpected expression of TTF-1 and Fli-1 in rosette-forming epithelioid osteosarcoma needs to be further characterized through the accumulation of more cases.

Because of its epithelioid morphology and particular immunophenotype, such tumor should be differentiated from other tumors with similar morphology, including small cell type osteosarcoma, neuroblastoma, Ewing's sarcoma, and metastatic carcinoma. Small cell osteosarcoma shares many of the well-described cytomorphologic features of classic osteosarcoma, but the relatively small cells, round hyperchromatic nuclei, and scant osteoid constitute the common denominator [21], most of the cells had a nuclear size of 6.5 to 7 μm , ranging up to 12.5 μm , and in rare case these small cells may be arranged in a rosette-like figure [8]. However, in our cases, the tumor cell size was larger, and had the characteristics of conventional osteoblastic osteosarcoma. In addition, small cell osteosarcoma and mesenchymal chondrosarcoma lack Fli-1 immunoreactivity [22]. Neuroblastoma metastatic to bone may mimic rosette-forming epithelioid osteosarcoma with its morphologic features and proclivity to young patients, particularly if the presence of a primary adrenal tumor is not known. Neuroblastoma is the most common malignant disease in early childhood and is distributed along sympathetic ganglia in addition to the adrenal medulla. Microscopically, neuroblastomas have a broad spectrum of differentiation varying between undifferentiated tumors composed only of small rounded blue cells and ganglioglioma composed uniquely of ganglia and Schwann stromal cells [21]. Neuroblastoma with varying degrees of differentiation containing Schwann and ganglion cells and/or neuropil is easily distinguished from osteosarcoma. Moreover, Homer-Wright rosettes, containing a central solid core of neurofibrillary material surrounded by neuroblasts, can be found in both NB and in Ewing's sarcoma. Immunohistochemically, CD99 antigen demonstrates a strong membranous expression in a large majority of Ewing's sarcoma [23]. Neuroblastoma cells are characterized by an intense expression of the 140-kd neural cell adhesion molecule, neural

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cell adhesion molecule CD56 [24]. Both tumors stain positive for neural markers, including neuron-specific enolase, and synaptophysin, but they do not appear tumor osteoid matrix. Also, Chromogranin, synaptophysin, and NSE were negative in our case, greatly aiding in distinguishing these tumors from neuroblastoma, the primary entity to be excluded in this age group. The limited expression of CD99 combined with Fli-1, and cytokeratin negativity in this case similarly differentiate this variant of osteosarcoma from two diseases.

Thus far, no standard therapy for epithelioid osteosarcoma has yet been established due to the rarity of the disease. The potential advantages of preoperative chemotherapy plus surgery plus postoperative chemotherapy have not been approved. Rosette-forming epithelioid osteosarcoma is reported to be associated with aggressive behavior and poor prognosis [25]. The estimated cumulative 5-year survival rate was 15%, significantly worse than the rate of 55% in 70 cases of conventional osteoblastic osteosarcoma without rosette like structures arising in long tubular bones [1, 25]. In addition, if the tumor > 10 cm, or response to chemotherapy is poor, is also one of the factors of poor prognosis [26].

In summary, epithelioid osteosarcoma is a rare and special type of osteosarcoma, especially when accompanied by a rosette formation, prognosis is poorer. Since the rosette-like structure can be easily recognized in routine diagnostic work, an osteosarcoma should be considered in the diagnostic list, to guide clinical treatment further. But, whether it should be a separate type, because now reported cases is less, has yet to be further accumulated cases.

Disclosure of conflict of interest

None.

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