VEGF expression and neovascularization in the epiphyseal extension of osteosarcoma in skeletally immature patients

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Introduction: The epiphyseal cartilage in skeletally immature patients was believed to be a strong barrier against the extension of metaphyseal osteosarcoma (OS) to the epiphysis. However, Enneking(1) revealed that OS often invaded the epiphysis breaking through the phyleseal cartilage. In spite of some reports(2,3) after Enneking’s, the clinical significance of the epiphyseal extension by OS and the mechanism of breaking through the phyleseal cartilage remains unclear. The authors examined the exact incidence and prognostic value of epiphyseal extension of OS in skeletally immature patients at a single anatomical site. Moreover, we studied the expression of VEGF and MMP9 to investigate the mechanism of epiphyseal extension.

Materials and Methods: Patients: Eligible patients for this analysis had a newly diagnosed primary osteosarcoma, had not received irradiation or chemotherapy before, and completed treatment protocols consisting of wide or radical tumor excision with adjuvant chemotherapy. Forty-seven patients with osteosarcoma treated between 1976 and 1987 at Memorial Sloan-Kettering Cancer Center were studied. The location of the osteosarcoma was limited to the distal femoral metaphysis. All patients were classified clinically as Enneking’s stage 2B (tumors were histologically high grade malignant, had extra-cortical penetration, and were non-metastatic at diagnosis). Twenty-seven patients were male and 20 patients were female. The age at first presentation ranged from 5 to 17 years, with median age of 11.2 years. All patients were treated with definitive surgery. Twenty-one patients were amputated and 26 patients had limb saving procedures. All patients had histopathologically negative surgical margin and underwent high dose methotexate chemotherapy.

Pathologic evaluation: The resected tumor specimen was fixed in formalin and sectioned. The hematoxylin-eosin (H&E) stained histologic sections were taken from the largest dimension of the tumor. According to the histopathologic examination, the degree of extension into the epiphysis was classified into 3 categories; 1) no extension, 2) minimal extension and 3) massive extension. Minimal extension was defined as tumor that extended into the epiphysis, but the penetration depth was less than or equal to 2mm from the caudal margin of the phyleseal cartilage. Massive extension was defined as tumor that extended into the epiphysis and the penetration depth was more than 2mm.

Immunohistochemical staining: Consecutive 4μm sections were recut from each block and were immunostained for VEGF and MMP9. We carried out immunohistochemical detection of VEGF and MMP9 using the avidin-biotin complex method. The polyclonal antibody reactivity for VEGF and MMP9 with individual tissue sections was considered positive if equivalent staining was seen in the membrane or the cytoplasm of more than 30% of tumor cells.

Results: The Degree of Epiphyseal involvement: In 10 cases, no tumor extended into the epiphysis. Eighteen cases had minimal tumor extension to the epiphysis and 19 cases had massive tumor extension to the epiphysis. In 5 of the massive extension cases (11% of all cases), the tumor extended to the joint cavity breaking through the joint cartilage.

The Type of Epiphyseal Extension: There were two routes for the epiphyseal extension by OS; transphyseal and perichondral routes. Twenty-two cases had only transphyseal extension without perichondral involvement. Five cases had both of transphyseal and perichondral extension. In 10 cases, we were undecided on the exact tumor extension route due to the amount of the tumor. There was no case with perichondral extension alone.

The histopathologic examination to establish the route of epiphyseal extension: This process formed the vascular channels between the epiphysis and the metaphysis. Whenever sarcoma crossed the growth plate there was a proliferation of fibrous tissue and new blood vessels. The vascular channels were dilated resulting in a relatively wide disruption of the phyleseal cartilage. Following the fibrovascular invasion of the phyleseal cartilage, the tumor extended into the epiphysis through this disruption. Perichondral extension also occurred through a typical route in a predictable sequence. Tumor first elevated the perichondral ring tissue in centrifugal direction. New vessel formation and fibrous tissue epiphyseal proliferation filled the potential space. Finally, tumor invaded the epiphysis through the vascular channel.

Association of VEGF and MMP2 expression with epiphyseal extension: VEGF staining was defined as positive in 25 cases out of 38 cases studied. Only 2 cases out of 8 cases (25.0%) of which tumor did not extend into epiphysis were positive, whereas 23 cases out of 30 cases (76.7%) of which tumor extended into epiphysis were positive, and in this group, all of 5 tumors that extended into joint cavity were positive. There was significant difference between the two groups. MMP staining was defined as positive in 11 cases out of 38 cases (28.9%). There was no significant difference between the groups.

Association of epiphyseal extension and prognosis: Thirty patients were continuously disease-free at the last follow-up. Fifteen patients died of the disease. Two patients were alive after thoracotomy of the metastatic lesion. Statistically, the prognosis depended neither on the degree of epiphyseal extension, on the method of operation nor on the immunoreactivity of vascular endothelial growth factor (VEGF) and matrix metalloproteinase 2 (MMP2).

Discussion: The current study revealed that 78% of all cases had epiphyseal extension to some degree and 40% had massive extension. This result was consistent with Enneking’s(1) observation that metaphyseal OS often extended into the epiphysis. The prognosis of the patients did not depend on the degree of epiphyseal extension. As long as the OS tumors are excised with sufficient surgical margin, the degree of the epiphyseal extension is not a prognostic factor for metaphyseal OS.

With regard to the exact route of extension, we clarified the transphyseal and perichondral routes. We could not find the perichondral route OS extension without the transphyseal route OS extension. Transphyseal extension will precede the perichondral extension.

Our study demonstrated that the tumor sometimes extended into the phyleseal cartilage, but complete invasion into the epiphysis through the phyleseal cartilage is unlikely without vascular channel formation between the metaphysis and the epiphysis. The angiogenesis by OS is likely to be more significant than the inherent resistance of phyleseal cartilage against OS tumor.

We demonstrated the significantly higher expression of VEGF in OS that showed epiphyseal extension than in OS that did not. VEGF expression is thought to be predictive of pulmonary metastasis and poor prognosis in OS patients(4). We think that VEGF is one of the most important angiogenic factors for OS tumor cells to invade the epiphyseal cartilage.