VEGF Expression in Osteosarcoma Correlates with Vascular Permeability by Dynamic MRI

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Introduction: Osteosarcoma (OS), the most common primary malignancy of bone, carries a 5-year survival rate near 70% in patients who present with no clinically detectable metastases. The histologic response to preoperative chemotherapy, which may take several months to assess, is still the most powerful predictor of outcome in this disease. At present, there is a need to stratify patients early according to their potential for disease progression. Despite intensive efforts, however, biological markers for disease progression have not been proven. Recently, Vascular Endothelial Growth Factor (VEGF) has been implicated as an important early marker for metastasis in OS. The utility of dynamic enhanced magnetic resonance imaging (DEMRI) in detecting viable tumor has been established and has also used in estimating VEGF expression.(1,2) In this study, we examine whether tumor vascular permeability (as estimated by DEMRI) can be correlated with VEGF expression in OS. We hypothesize that a non-invasive estimate by DEMRI can provide information on tumor angiogenic activity as reflected by VEGF expression. In this report, we provide the first evidence linking DEMRI vascular parameters with the degree of VEGF staining in OS tissues. Given that VEGF portends a poor prognosis in OS, DEMRI may serve as a means to stratify OS patients early according to their tumor VEGF status. As such, we suggest that DEMRI should be investigated further in a clinical setting as a surrogate measure of tumor angiogenesis as reflected by VEGF expression.

Methods: From 1998 to 2002, 55 patients with the pathologic diagnosis of OS at Memorial Sloan-Kettering Cancer Center (MSKCC) were enrolled in a treatment protocol that included DEMRI. In 15 of these patients, fresh-frozen tumor tissues were available for immunohistochemical studies. Five specimens were obtained at initial biopsies prior to chemotherapy and 10 were from definitive en-bloc resection following chemotherapy. MR Imaging studies were acquired on a 1.5T GE Signa Horizon or LX scanner. Gadopentetate dimeglumine (Gd-DTPA) was used as a paramagnetic contrast agent at a concentration of 0.1 mM/kg followed by a saline flush. Dynamic perfusion studies were acquired using a fast multi-phase spoiled gradient echo sequence (FMPSPGR). The entire tumor was covered contiguously with 10-12 mm thick sections yielding 5-9 slices depending on tumor extent. Acquisition parameters included a 9 ms repetition time (TR), a 2 ms echo time (TE), 30° flip angle, 15.63 kHz receive bandwidth, 20-24 cm field of view (FOV) and a 256 x 128 matrix yielding a voxel resolution of 12-20 mm^3. Data were acquired from a total of 20-40 time points in scan times of less than 5 minutes. Software was written to display and analyze the data using IDL (Research Systems Inc., Boulder,CO). Time intensity curves were analyzed for each voxel within the entire tumor using the two-compartment model proposed by Hoffman, based on that of Brix. The model contains three parameters: A (signal amplitude), k_{ep} (exchange constant between plasma and tumor compartments in min^{-1}), and k_{el} (elimination constant in min^{-1}). The product A x k_{ep} (Ak_{ep}) represents the DEMRI estimate of tumor vascular permeability used in this study. This allows a correlation between the MRI signal intensity over time and that of vessel permeability on a per voxel basis. A histogram summarized the values of Ak_{ep} for each voxel in tumor. All histograms were normalized to the number of voxels contained within each ROI to account for variability in the size of tumor burden between patients. Histograms used an optimized curve fitting procedure to extract the asymmetric distribution mean, amplitude, and width.

Immunohistochemical detection of VEGF was performed using the ImmunoCruz Staining System (Santa Cruz Biotechnology, Santa Cruz, CA) according to the manufacturer's protocol. Briefly, 4-µm sections were cut from fresh-frozen OS tissues and placed on silane-coated slides. A mouse monoclonal antibody for the N-terminus of human VEGF (Santa Cruz Biotechnology, Santa Cruz, CA) was used as the primary antibody (1:50 dilution) and recognized all variants of the protein. For a negative control, all reagents except for the primary antibody were used. Evaluation of VEGF immunoreactivity was carried out independently by a musculoskeletal pathologist who was blinded to the clinicopathologic and DEMRI data. Immunohistochemical staining of tumor cells was classified as 0 (negative), 1+, 2+ or 3+ according the intensity of staining and the number of positively stained cells.

Results: There was no significant correlation between VEGF expression and histologic necrosis following neoadjuvant chemotherapy in this group of patients ($p = 0.49$). In addition, there was no significant association between VEGF expression and gender, age, site of disease, or histologic subtype of OS ($p > 0.1$) The mean pharmacokinetic estimate of vascular permeability (Ak_{ep}) was 1.84 min^{-1} for the entire studied group. Analysis of the estimate for vascular permeability Ak_{ep} revealed that VEGF-positive tumors exhibited higher average Ak_{ep} value than VEGF-negative tumors. As shown in Figure 1, the mean Ak_{ep} for the VEGF-positive and VEGF-negative tumors were 2.17 min^{-1} and 1.2 min^{-1}, respectively ($p < 0.07$). Figure 2 shows the raw data for the two groups. In addition, the degree of VEGF staining significantly correlates with the mean value for Ak_{ep} ($r^2 = 0.92$, $p < 0.04$) when tumor samples were stratified into 4 groups (0 or negative, 1+, 2+, 3+ immunostaining).

Discussion: In osteosarcoma, VEGF may be an important prognostic indicator for disease progression (3). Patients with VEGF-positive tumors have poorer disease-free as well as overall survival compared to those with VEGF-negative tumors. Furthermore, VEGF expression in pre-treated OS specimens is predictive of eventual development of pulmonary metastasis. (3) In OS, the DEMRI estimate of k_{ep} has been reported as a potential prognostic factor by Reddick et al showing that a higher k_{ep} sustained a correspondingly inferior disease-free survival.(4) In summary, we examined a series of 15 osteosarcomas for the expression of VEGF and correlated this expression with model parameters for vascular permeability by DEMRI. Results from this study suggest an important role for DEMRI in assessing tumor angiogenic factor in a non-invasive manner. As such, the physiologic basis of DEMRI as a surrogate measure for tumor neovascularization in OS can be explained partly by tumor VEGF expression. The potential use of DEMRI to stratify OS patients according to angiogenic and metastatic potential of their tumors may be of clinical significance.

1) George ML, Dzik-Jurasz AS, Padhani AR, et al: Br J Surg. 2001;88:1628-36. 2) Knopp MV, Weiss E, Sinn HP, et al. J Magn Reson Imaging. 1999;10:260-6.
3) Kaya M, Wada T, Akatsuka T, et al: Clin Cancer Res. 2000;6:572-7. 4) Reddick WE,Wang S, Xiong X, et al. Cancer. 2001;91:2230-7.