Purpose:
Angiogenesis has been correlated with increased biologic aggressiveness in a variety of cancers including breast, gastrointestinal, lung, and gynecologic tumors (1,2). In a prior study, we demonstrated that angiogenesis in chondrosarcoma follows the same pattern of neovascularization found in carcinomas (3). Grades II and III chondrosarcoma had significantly increased neovascularization compared to grade I chondrosarcoma or benign cartilage tumors. The purpose of this study is to investigate angiogenic cytokines in chondrosarcoma. The hypothesis is that grades II and III chondrosarcoma have higher levels of the angiogenic cytokines vascular endothelial growth factor (VEGF) and basic fibroblastic growth factor (b-FGF) than grade I chondrosarcoma or benign cartilage tumors.

Materials and Methods:
RNA isolation: Human tumor specimens and corresponding normal tissues were flash frozen in liquid nitrogen. Total RNA was extracted by homogenization in 4 M guanidinium isothiocyanate buffer and ultracentrifugation through Cs-TFA.
Northern hybridization: Ten ug of total RNA were electrophoresed in agarose gel containing formaldehyde. RNA was transferred to nylon membranes, cross-linked with UV light, and hybridized with labeled probes for VEGF-A and GAPDH. After hybridization, blots were washed and autoradiographed.

Immunohistochemistry: Group 1 consisted of 9 grade II or III chondrosarcoma and group 2 consisted of 6 grade I or benign tumors, selected from our prior study. Each specimen was deparaffinized in xylene, followed by serial ethanol dilutions and rehydration. The tissue was incubated with an anti-VEGF antibody or anti-b-FGF antibody. Secondary biotinylated antibodies and DAB chromogen were used for visualization.

Cytokine expression was quantified by grading the percentage of cells with positive staining from 0-5 (0%, <1%, 1-10%, 11-33%, 34-67% and >67%) and the staining intensity was graded from 0-3 (none, weak, moderate and strong). The score for cytokine expression was the sum of the scores for intensity and percentage and ranged from 0-8. The results for each antibody were averaged for each group and compared with the student t-test.

Results:
VEGF-A expression: Northern blotting demonstrated expression of VEGF-A in 0/4 normal articular cartilage 1/4 benign, 0/2 grade I, 1/2 grade II, and 3/4 grade III chondrosarcoma.

Immunohistochemistry for b-FGF and VEGF: Group 1 consisted of 5 grade III and 4 grade II chondrosarcoma that had an average microvascular count of 64. Group 2 consisted of 4 grade I chondrosarcoma, 1 enchondroma, and 1 osteochondroma, which had an average microvascular count of 9. Both b-FGF and VEGF immunochemistry showed staining of cytoplasmic granules in the cartilaginous areas but not in surrounding normal tissues (Figure 1).

Discussion:
Our results show that VEGF is more highly expressed in grade II and III chondrosarcoma, but that b-FGF is uniformly expressed in all cartilage lesions. High and intermediate grade chondrosarcoma have a greater tendency to metastasize than low-grade chondrosarcoma or benign cartilage tumors. This may be related to the greater microvessel density found in grade II and III tumors, which in turn may be related to overexpression of VEGF. VEGF is the most common angiogenic cytokine overexpressed in carcinoma, and increased VEGF production is correlated with biologic aggressiveness in these tumors. Chondrosarcoma seems to follow the same pattern. VEGF expression shows promise as another variable to aid in the grading of chondrosarcoma, and investigation of antiangiogenesis treatment strategies may be warranted.

References:
2. Smith K et al, Upregulation of basic fibroblast growth factor in breast carcinoma and its relationship to vascular density, oestrogen receptor, epidermal growth factor receptor and survival, Ann Onc, 10:707-13, 1999

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