TUMOR ANGIOGENESIS IN CHONDROSARCOMA

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Introduction: Tumor induced angiogenesis is necessary to sustain radial growth of tumors beyond 1-5 mm in diameter and reflects the ability of tumors to recruit microvessels from surrounding normal tissue. Increased microvascularity is correlated with increased metastatic potential in breast, gastrointestinal, and gynecologic tumors. Tumors which induce the formation of leaky microvessels allow tumor cells to embolize and metastasize. Grade II and III chondrosarcoma have greatly increased metastatic potential compared to grade I tumors. One reason for this may be pathologic neovascularization. The purpose of this study is to quantify the microvessel density of cartilage tumors. The hypothesis is that grade II and III chondrosarcoma have higher densities of microvessels than grade I chondrosarcoma or benign cartilage tumors.

Materials and Methods: Cartilage tumor specimens were retrieved from pathologic archives. Hematoxylin and eosin stained slides of each specimen were reviewed to confirm pathologic grade and to select areas containing tumor. Sections were deparaffinized in xylene, followed by serial ethanol dilutions and rehydration. The tissue was then incubated with a murine anti-CD34 antibody (Biogenex, San Ramon, CA), which stains endothelial cells as well as erythroblast precursors, which are present in the early stages of neovascularization. A secondary biotinylated horse anti-mouse antibody, and diaminobenzidine chromogen were used for visualization. A malignant gastric stromal tumor was used for a positive control, and negative controls were performed with isotype matched nonimmune antibody. Microvessel density (MVD) was determined on the CD34 stained specimens using two standard techniques in a blinded fashion. In the Weidner method, the number of vessels in the three high powered fields (400x) with the most intense staining are counted. In the Chalkley method, a 25 point Chalkley reticle is used to estimate MVD. Two observers agreed upon the resultant count for each specimen. The results were then grouped by pathologic grade, and statistically analyzed using ANOVA and Students t-tests with Bonferroni correction for multiple comparisons. P< 0.05 was considered significant.

Results: Seven grade III, seventeen grade II, eight grade I chondrosarcoma, and twenty-two benign cartilage tumors (11 enchondroma, 8 osteochondroma, 3 synovial chondromatosis) were studied. A representative photomicrograph of a high grade chondrosarcoma stained for CD 34 (Figure 1) shows a large number of microvessels.

Mean MVD counts for grade III and II chondrosarcoma were 45.9 and 46.2 per high powered field. Counts for grade I and benign tumors were 9.3 and 10.3 respectively (Figure 2).

MVD of grade III and II tumors were each greater than grade I and benign tumors (p <0.05 and <0.01 when comparing grade III tumors to grade I and benign tumors; p <0.01 and <0.001 when comparing grade II tumors to grade I and benign tumors). Figure 3 shows the microvessel density of the more aggressive tumors (grades III and II combined) was greater than the nonaggressive tumors (grade I and benign combined) (p <0.00003). Raw MVD of the aggressive group did not overlap those of the nonaggressive group.

Chalkley estimation results paralleled those of the MVD counts (Figure 4). Mean Chalkley counts for grade III and II chondrosarcoma were 7.1 and 9.3 per high powered field and counts for grade I and benign tumors were 3.0 and 3.7 respectively. The biologically aggressive cartilage tumors (grade III and II combined) had more microvessels than nonaggressive tumors (grade I and benign combined) (p<0.00001).

Discussion: High and intermediate grade chondrosarcoma behave differently than low-grade chondrosarcoma or benign cartilage tumors. One of their greatest differences is metastatic potential, which is rare in low-grade chondrosarcoma. Grade II and III chondrosarcoma display significantly greater MVD than low-grade or benign lesions, as demonstrated by direct MVD counting as well as by the Chalkley estimation technique. This difference in vascularization may explain the increased metastatic potential of grade II and III chondrosarcoma. Grade I chondrosarcoma had no statistical difference from benign cartilage tumors by these methods, which correlates with their lack of metastatic potential. Assessment of MVD in chondrosarcoma shows promise in grading these difficult to classify neoplasms.

Increased pathologic neovascularization has been correlated with biologic aggressiveness and metastatic potential in many tumors, most notably breast carcinoma. It is theorized that the pathologic neovessels are leaky and allow tumor cells to embolize, thereby providing one mechanism of metastasis. Antiangiogenesis chemotherapeutic agents are therefore being developed. Since current chemotherapeutic regimens are not effective for intermediate or high-grade chondrosarcoma, antiangiogenesis protocols may prove useful for patients with these tumors.

References: