INTRODUCTION
Chondrosarcomas, malignant tumors of cartilage cells, present very difficult clinical problems. Relatively common benign cartilage tumors, enchondromas and osteochondromas, can undergo malignant transformation and become chondrosarcomas. Unfortunately, there is no way to reliably predict which enchondromas and osteochondromas will become malignant. The unpredictable behavior of the human chondrosarcoma underscores the need for a better understanding of the factors that contribute to malignant progression of this tumor. Cellular immortalization depends on telomere maintenance and is believed to be necessary for malignant progression. Telomeres in ~90% of all malignancies are maintained by telomerase, a polymerase that catalyzes telomere elongation (1). Telomerase expression is associated with poor prognosis in diverse cancers however, the role of telomerase in chondrosarcomas remains controversial. One recent attempt failed to detect telomerase activity in 29 chondrosarcoma biopsies (2). However, another study showed telomerase expression in ~80% (5/6) of grade I chondrosarcomas and 100% (4/4) of grade III chondrosarcomas (3). Analysis of a small set of chondrosarcomas (n=5) by Sangiorgi et al showed an apparent correlation between high telomerase activity and recurrence or metastasis (4). These studies employed a PCR-based telomerase activity assay that requires fresh tissue specimens. The method is sensitive but is subject to interference by proteoglycans present in tumor extracts. Here we describe an immunohistochemical technique to assess telomerase expression in paraffin embedded chondrosarcoma specimens. This method obviates activity assay interference problems and is able to detect telomerase-positive cells even if these constitute a small fraction of the tumor mass. Moreover, since patient histories are available for archived pathology specimens our approach can be used to determine if telomerase expression correlates with chondrosarcoma malignancy. The preliminary data reported here suggests that histologic grade correlates with the frequency of telomerase-positive cells in chondrosarcomas.

METHODS
Formalin-fixed, paraffin embedded chondrosarcomas (n=55) obtained from the University of Iowa Department of Pathology. The specimens were graded previously by qualified pathologists. Sections were mounted on Superfrost Plus® slides (Fisher Scientific). De-paraffinized tissues were graded previously by qualified pathologists. Sections were increased with grade (Figure 2). The frequency of positive cells was lowest in grade 1 tumors and increased with grade (Figure II-IV). Semi-quantitative analysis of 4 tumors from each grade indicated that grade III specimens (75%), and 5 of 5 grade IV specimens (100%) were telomerase-positive cells. Grade I specimens (67%), 9 of 11 grade II specimens (82%), 9 of 12 chondrosarcomas analyzed (75%). Positive tumors included 18 of 27 telomerase-positive cells were detected in 41 of the 55 chondrosarcomas analyzed (75%). Positive tumors included 18 of 27 grade I specimens (67%), 9 of 11 grade II specimens (82%), 9 of 12 grade III specimens (75%), and 5 of 5 grade IV specimens (100%). Semi-quantitative analysis of 4 tumors from each grade indicated that the frequency of positive cells was lowest in grade I tumors and increased with grade (Figure 2).

DISCUSSION
The preliminary data reported here suggests that histologic grade correlates with the frequency of telomerase-positive cells in chondrosarcomas. Representative tumor samples from each grade (I-IV) were semi-quantitatively scored for the frequency of positive stain (see Methods). Columns and error bars represent means and standard deviations based on 4 samples.

REFERENCES