Correlation of hypoxic signaling to histological grade and outcome in central chondrosarcoma

INTRODUCTION:

Conventional central chondrosarcoma are malignant cartilaginous tumors arising centrally within the medullar cavity of bone. They are classified in 3 grades according to Evans (1) from grade I chondrosarcoma which metastasize only very rarely to grade III chondrosarcoma for which metastasis formation is observed in 71% of cases. Grade I chondrosarcoma display a hyaline cartilage matrix and low vascularity whereas the matrix of grade III chondrosarcoma is mainly mucoid-myxoid with high vascularization. The biologic behavior of chondrosarcoma, thus, correlates to differences in both the composition and morphology of the tumor matrix and its vascularisation pattern, suggesting an important role for angiogenic and matrix remodeling processes during the development and progression of these tumors. The molecular mechanisms responsible for the regulation and control of these processes are so far poorly understood. The aim of this project was to identify genes involved in the progression of chondrosarcoma by comparison of gene expression profiles and correlation of gene and protein expression to histological grade and clinical outcome, the ultimate standard of biological behavior.

METHODS:

Gene expression profiles of 18 central chondrosarcoma samples (5 grade I; 8 grade II; 5 grade III) were assessed on a customized cDNA array representing 230 cartilage and chondrogenesis-relevant genes (2). Genes differentially expressed between histological grades were extracted by Significance Analysis of Microarrays (3) and results for these genes were confirmed by quantitative RT-PCR. The expression of selected molecules was furthermore evaluated by immunohistochemical analysis on a panel of further 68 central and peripheral chondrosarcoma samples. The expression of the proteins galectin 1, hypoxia-inducible factor 1α (HIF1α) and carbonic anhydrase IX (CA IX) was assessed and was correlated to grade and clinical outcome.

The studies were approved by the local ethics committee. Specimens from Leiden were handled according to the ethical guidelines described in "Code for Proper Secondary Use of Human Tissue in The Netherlands" of the Dutch Federation of Medical Scientific Societies. For cases from Heidelberg, informed consent was obtained from all individuals included in the study.

RESULTS:

Significant differences in expression of genes for cartilage matrix molecules were in accordance with the histology of the tumors. Beside these genes, array analysis revealed significant higher expression in grade III compared to grade I chondrosarcoma of the matrix metalloprotease MMP2 (5-fold), galectin 1 (3.47-fold) and the CD74 antigen (2.29-fold). Among these genes, MMP2 and galectin 1 have both been described as hypoxia-regulated genes with potential role in tumor progression (4,5), indicating possible hypoxic signaling in high-grade chondrosarcoma.

For this reason, the expression of galectin 1 and of the hypoxia markers HIF1α and CA IX was assessed by immunohistochemical analysis on a panel of 68 central and peripheral chondrosarcoma samples. Galectin 1 expression was overall weak. In central chondrosarcoma, it was detected in 10 samples (10/33), of which 9 were high-grade tumors. However, no correlation with grade or outcome was found.

Nuclear expression of HIF1α was highly variable. In some tumor samples, expression in the vicinity to blood vessels was seen (figure 1). Nuclear protein localization in central tumors was practically restricted to high histological grades and, thus, showed a significant positive correlation to grade (p=0.002). Kaplan-Meier analysis revealed a trend for longer total survival and longer metastasis-free survival in HIF1α-negative central chondrosarcomas. In peripheral chondrosarcoma, a trend for higher expression in high-grade tumors was observed.

REFERENCES: