Site-Specific Biomarkers for Severity of Articular Cartilage Degradation in the Osteoarthritic Knee

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Introduction: There is significant variation in osteoarthritis (OA)-related changes in articular cartilage across the different anatomical surfaces of the knee within and among patients. The tibial plateau (TP) is often overlooked in terms of its potential contributions to symptomatic OA in the knee. While inflammation and degradation are mechanistic pathways that are consistently associated with the development and progression of OA in the whole-joint organ, the complex relationships among localized changes in articular cartilage and clinically relevant protein biomarkers have not yet been well characterized. It is possible that there are site-specific relationships that influence knee OA "phenotypes" with respect to mechanistic pathways and severity of disease. Therefore, this study was designed to characterize relationships among OA-related changes in cartilage structure with the concentrations of clinically relevant protein biomarkers in the TP. It was hypothesized that TP OA cartilage with more severe histological degradation would be associated with significantly higher levels of inflammation-related, degradation-related, and bone turnover-related biomarkers.

Methods: ***Tissue recovery:*** All procedures were performed with IRB approval (IRB#1208392) and informed patient consent. Excised tibial plateau articular surfaces that would otherwise be discarded after surgery were recovered from patients (n=22, 16F, 6M, age 65.9±8.8 years, BMI 34.9±6.0) undergoing TKA for symptomatic knee OA. Osteochondral explants (6mm diam, n=68) were created from the anterior (A) and center weight bearing (C) regions of the medial and lateral TP. The explants were cut in half and half was stored at -80°C for protein extraction, while the other half was formalin fixed for histological assessment. ***Tissue Protein Extraction and Testing:*** Protein was extracted from the cartilage tissue using the T-Per protein extraction reagent with protease inhibitors. The protein content of the extract was determined using the BCA assay, and the concentration of leptin, adiponectin, adipsin, CRP, MMP-1, MMP-2, MMP-3, MMP-9, MMP-13, TIMP-1, TIMP-2, TIMP-3, TIMP-4, GRO-α, MCP-1, MCP-3, IL-6, IL-8, MIP-1α, VEGF, OPG, OPN, and SOST was determined using commercially available Luminex assays. ***Histology:*** Stained sections of each osteochondral explant were evaluated by one blinded pathologist using a modified OARSI system that assesses cartilage structure (STR), chondrocytes (CHON), proteoglycan content (PRO), collagen integrity (COL), tidemark (TIDE), and subchondral bone plate (SUB). The sum of all scores was used for a total score (TOTAL); the TIDE and SUB were used for a bone score (BONE); the STR, CHON, PRO and COL were used for a cartilage score (CART); the STR, PRO, and COL were used for an extracellular matrix score (ECM), and the PRO and COL were used for an PRO\COL score. ***Statistical Analysis:*** Biomarker concentrations were standardized to protein content and log transformed for analysis. Significant ($p<0.05$) differences between samples grouped based side (TP-L, TP-M), region of recovery (TP-C, TP-A), and the various OARSI system score groups using a one-way ANOVA and Tukey post-hoc test or T-test based on number of groups in the comparison. A two-way ANOVA was performed using the histology sum score groups and the side (TP-L, TP-M) or region (TP-C, TP-A) of recovery to determine significant differences for each biomarker based on the interaction of the histology and location of recovery. Only significant differences between groups are discussed.

Results: ***Differences between Regions of the TP (Fig. 1):*** There was not a significant difference in the protein content of the cartilage between TP-M and TP-L samples. However, TP-A samples had lower VEGF, MCP-1, TIMP-2, and TIMP-4 compared to TP-C samples. ***Differences based on Histology Sum Score Groups (Fig 2):*** For the TOTAL score group: Total 7-8 scores had higher MMP-1 than 11-13 scores and MIP-1α than 14-20 scores, DKK-1 was higher in 11-20 scores than 1-6 scores, and MMP-9 was higher in 14-20 scores than 11-13 scores. For the CART score group, 12-15 scores had significantly higher MMP-13 than 8-11 scores, lower IL-6 and VEGF than 8-9 scores, and lower MIP-1α than 1-6 scores. DKK-1 was significantly higher in 10-11 scores than 1-5 scores. For the ECM score group, VEGF was higher 5-6 scores than 4 scores, and DKK-1 was higher in 10-11 scores than 1-4 scores. For the PRO\COL score group, group Leptin was higher in 1-3 scores than 4-5 scores, TIMP-3 was higher in 3 scores than 1-2 scores, IL-6 was higher in 4-5 scores than 1-3 and 6-7 scores, DKK-1 was higher in 6-7 scores than 1-3 scores, and OPN was higher in 3 scores than 4-5 scores. For the BONE score group, 4-5 had higher MMP-9 than 1-3 scores and higher levels of DKK-1 than 0 scores. ***Differences based on interaction of TP-M, TP-L, and Histology Sum Score Groups (Fig. 3):*** The concentration of IL-6 was higher in TP-M than TP-L at lower (7-8) TOTAL and CART (7) scores, and in TP-L than TP-M at mid-range CART (9-10) and PRO\COL (4-5) scores. IL-6 in the TP-L was higher in mid-range TOTAL (9-10), CART (8-9), and PRO\COL (4-5) scores. ***Differences based on interaction of TP-A, TP-C, and Histology Sum Score Groups (Fig. 3):*** The concentration of MMP-9 was higher in TP-A than TP-C at higher TOTAL (14-20), ECM (9-11), and PRO\COL (6-7) scores than lower scores. The TP-A had higher MMP-9 at higher TOTAL (13-20), ECM (9-11), and PRO\COL (6-7) scores than lower scores; and lower MIP-1α in higher CART (12-15) scores than lower scores.

Discussion: The data from this study indicated potentially important regional differences in tibial plateau articular cartilage biomarkers related to severity of degradation. More severe degradation in the TP was associated with increased DKK-1, indicating a shift towards increased cartilage calcification as knee OA progresses. Further, IL-6 concentrations were higher in the TP-M at earlier stages of cartilage degradation, while IL-6 concentrations were higher in the TP-L at later stages of cartilage degradation, suggesting that differences in IL-6 signaling may contribute to the consistently reported differential rates of disease progression in medial versus lateral tibiofemoral compartments of patients with knee OA.

Significance: Taken together, the results from this study suggest that severity of cartilage degradation related to OA is an important factor in regional differences in bone-related and inflammatory signaling biomarkers in the tibial plateau that may relate to knee OA "phenotypes" with respect to pathways and severity of disease. Ongoing studies in our lab are aimed at determining mechanistic relationships between changes in OA cartilage architecture and composition with proteins that may serve as biomarkers for disease development and progression.