

Differences in Cartilage Biomarkers Related to Histologic Degradation Severity in the Osteoarthritic Knee

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Introduction: Osteoarthritis (OA) is a multifactorial disease often progressing from an initial insult or injury to whole-joint inflammation and degeneration causing pain and dysfunction. The development and progression of OA are highly variable, resulting in significant regional variation in architecture, biomechanical properties, and metabolism of the articular cartilage. While the regional anatomical variation in OA is well established, mechanistic characterization of the development and progression of OA in specific regions of the joint based on metabolic responses has not been fully elucidated. This study was designed to characterize OA-related changes in cartilage structure with respect to regional differences in pro-degradative and anti-degradative biomarkers in articular cartilage at the time of recovery from patients undergoing total knee arthroplasty (TKA) for the treatment of OA. It was hypothesized that there would be significant differences in pro-degradative and anti-degradative biomarker protein content in OA cartilage based on the region of collection (femoral condyle or tibial plateau, medial or lateral) and histopathologic degradation severity.

Methods: Tissue recovery and culture: Procedures were performed with IRB approval (IRB #1208392) and informed patient consent. Excised femoral condyle (FC) and tibial plateau (TP) 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, n=159) were created from the anterior (A) and center weight bearing (C) regions the medial and lateral FC and TP, as well as the posterior (P) region of the FC. 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-1 α , 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); and the STR, PRO, and COL were used for an extracellular matrix score (ECM), and the Pro and Col were used for the PRO\COL score. **Statistical Analysis:** Biomarker concentrations were standardized to protein content and log transformed for analysis. Samples were grouped based on the score of each component of the OARSI system, or the summed scores outlined above, and significant (p<0.05) differences in tissue biomarker concentrations were determined using a one-way ANOVA and Tukey post-hoc test.

Results: Differences based on individual Cartilage Histology Scores: Numerous significant differences were identified between samples based on the individual cartilage scores of the OARSI system. (Table 1) Samples with low STR, CHON, PRO, and/or COL scores had significantly higher Leptin, MMP-1, Gro- α , IL-6, IL-8, MIP-1 α , and OPN, and significantly higher MMP-9, MMP-13, DKK-1, and SOST, compared to samples with higher scores. This indicates a decrease in tissue inflammatory signaling, but an increase in specific degradative enzymes and bone remodeling proteins, occurs in the cartilage tissue as degradation progresses. **Differences based on Histology Sum Score Groups:** Numerous significant differences were identified between samples based on the sum histology score groups. (Table 2) Similar to the data from the individual cartilage histology scores, samples with low summed scores typically had significantly higher Leptin, MMP-1, Gro- α , IL-6, IL-8, MIP-1 α and OPN, and significantly higher MMP-9, MMP-13, and DKK-1, compared to samples with higher scores. However, the differences between high and low scores were not a consistent with the sum groups as with the individual score groups, with differences often occurring between middle score ranges of the sum score, and not including the highest or lowest score groups. For example, leptin was significantly higher in the Total 1-8 score group compared to samples in the 11-13 score group, but not the 14-20 score group. Further, there were some interesting differences noted between the various score groups. For example, adiponectin was significantly higher in samples with higher scores for the TOTAL, ECM, and PRO\COL scores, but was significantly lower in samples with higher scores for the CART score.

Discussion: The data from this study indicate that inflammatory, degradative enzyme, and bone metabolism-related biomarker concentrations have important and potentially clinically relevant associations with histological assessments of cartilage degradation. The concentration of the degradative enzyme MMP-13 was highest in tissues with severe STR, CHON, and PRO changes. However, when categorical histologic scores were summed into a total histopathology severity score, significant differences in biomarker profiles were only observed in samples with the highest scores compared with samples in the middle range. Surprisingly, the concentrations of Gro- α , IL-6, IL-8, and MIP-1 α all decreased as the histological severity of cartilage degradation increased, indicating a waning of inflammatory processes within the articular cartilage as OA progresses.

Significance: Taken together, the results of this study elucidate potentially important changes in inflammatory, degradative enzyme, and bone metabolism related proteins in osteoarthritic cartilage that may serve as biomarkers for severity of articular cartilage degradation. 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.