Comparison of Osteoarthritic Infrapatellar Fat Pad and Synovial Tissue Biomarkers

Kayla R. Schweitzer, Kathleen A. Harris, Cole C. Stevens, Ahmed S. Fouad, James A. Keeney, James L. Cook, Aaron M. Stoker
Thompson Laboratory for Regenerative Orthopedics, University of Missouri, Columbia, MO
stokera@health.missouri.edu

Introduction

Osteoarthritis (OA) is a significant cause of disability in patients, characterized by pain, effusion, and dysfunction of affected joints. The joint is considered an organ, such that the infrapatellar fat pad (IPFP) and synovium (SYN) in the knee contribute to the development and progression of OA. While the contributions of IPFP and SYN in OA development and progression have been initially characterized, it is not yet clear how these two tissues differ in their OA-response profiles or how patient demographic factors such as age, BMI, and sex may influence differences in IPFP and SYN tissue responses. Distinguishing these and influences may allow for new insight regarding pathomechanisms for OA development and progression, which could allow for identification of new biomarkers for diagnosis, staging, and treatment monitoring, as well as new targets for preventative and therapeutic interventions. Therefore, this study was designed to characterize differences in pro-inflammatory and pro-degradative biomarker concentrations between IPFP and SYN tissues obtained from patients undergoing total knee arthroplasty (TKA) for treatment of symptomatic knee OA. It was hypothesized that IPFP tissue would be associated with significantly higher pro-inflammatory and pro-degradative protein concentrations compared to SYN tissue from patients with knee OA, and that key patient demographics (age, sex, BMI) and pain scores would correspond to differences in clinically relevant biomarkers.

Methods

Tissue collection: With IRB approval (IRB# 1208392) and informed patient consent, resected IPFP and SYN tissues that would otherwise be discarded after surgery were recovered from the knees of 61 OA patients (mean age 64.04 years, sex: 25 female, 6 male, mean BMI 33.97) undergoing total knee arthroscopy (TKA). One tissue explant (6mm diam) of the IPFP and SYN were created using a dermal biopsy punch and stored at -80°C. *Tissue Protein extraction*: The protein content of the tissue samples was extracted using the T-PER protein extraction reagent (Fisher) with protease inhibitors included. The IPFP and SYN tissue explants were homogenized using a mini-bead beater, the homogenate was centrifuged to pellet tissue debris, and the supernatant was stored at -80°C until used for analysis. *Protein Biomarker Analysis:* The BCA assay was used to determine the protein concentration of the tissue extract and media. The concentration of MMP-1, MMP-2, MMP-3, MMP-9, MMP-13, TIMP-1, TIMP-2, TIMP-3, TIMP-4, GRO-α, MCP-1, MCP-3, PDGF-AA, IL-6, IL-8, MIP-1α, MIP-1β, RANTES, TNF-α, VEGF, Leptin, Adiponectin, Adipsin, CRP, and Resistin were determined using commercially available Luminex assays according to the manufacturer's protocol. *Statistical analysis:* The concentration of each biomarker was standardized to the protein content of the tissue extract and log transformed for analysis. Significant ($p<0.05$) differences in tissue biomarker concentration between IPFP and SYN tissues were determined using an independent samples t-Test and a univariate linear model accounting for patient age, sex, BMI, and VAS pain score at the time of surgery.

Results

The IPFP had significantly higher tissue concentrations of TIMP-4, MIP-1α, and Leptin, and significantly lower IL-6, IL-8, RANTES, CRP, and Resistin, compared to the SYN recovered from OA patients. When accounting for patient demographic factors (age, sex, BMI, VAS pain score), the IPFP had a significantly higher tissue concentrations of TIMP-4, MCP-3, MIP-1α, and Leptin, and significantly lower MMP-3, TIMP-1, PDGF-AA, IL-6, IL8, RANTES, CRP, and Resistin, compared to SYN recovered from OA patients. Patient age was a significant factor in the model for MMP-3, TIMP-1, MIP-1α, and CRP. Patient sex was a significant factor in the model for TIMP-1, MCP-3, IL-6, Leptin, and Resistin. Patient BMI was a significant factor in the model for Leptin and Resistin.

Discussion

The data from this study indicate significant differences in inflammation- and degradation-related proteins present in IPFP versus SYN recovered from OA patients at the time of TKA. Further, patient age, sex and BMI significantly influenced differences between IPFP and SYN concentrations for many of the targeted proteins. The observation that the IPFP had higher Leptin concentrations compared to SYN, and that patient BMI significantly influenced Leptin concentrations, is in agreement with previous studies that indicate that fat is a primary source of Leptin and is increased as the BMI of the patient increases. Importantly, Leptin can stimulate the production of IL-6 in the synovium, which may account for the higher IL-6 concentrations in the SYN observed in this study and result from Leptin production by the IPFP. Ongoing studies in our lab are aimed at determining how these differences in IPFP and SYN protein concentrations directly contribute to the development and progression of OA in order to delineate biomarkers for diagnosis, staging, and treatment monitoring, as well as new targets for preventative and therapeutic interventions.

Significance

This study highlights potentially important differences in concentrations of pro-inflammatory and degradative enzyme biomarkers in infrapatellar fat pad and synovium recovered from osteoarthritic knees at the time of TKA. Characterizing tissue-specific contributions to the development and progression of OA based on differences in clinically relevant biomarkers is critical for elucidating whole-organ pathomechanisms that can be targeted for advancing more effective preventative and therapeutic strategies.