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"Differential Expression of Fibrogenic Biomarkers in Naïve Human Synoviocytes Cultured in Synovial Fluid from Knee Osteoarthritis Patients"

The synovium maintains normal joint function by secreting the fundamental components of synovial fluid (SF), but is also susceptible to inflammatory and fibrotic thickening during osteoarthritis (OA), contributing to disease symptomology. We recently published evidence that synovial fibrosis (SFb) presents heterogeneously in a large sample of patients with end-stage OA in association with deficits in range of motion (ROM). During OA, diseased chondrocytes and synoviocytes undergo a dynamic crosstalk that influences severity of cartilage degradation and synoviopathy. Therefore, we aim to test the responses of validated commercial human fibroblast-like synoviocytes (HFLS) after stimulation with SF from high or low SFb patients that has been pre-screened for levels of key fibrogenic ligands known to regulate SFb. Furthermore, we aim to compare the HFLS responses to the fibrogenic transcript expression of patient fibroblastic synoviocyte cells (FSC). We hypothesize that HFLS challenged with OA patient SFs will upregulate fibrogenic transcripts relative to corresponding histological severity of SFb.

The HFLS line was cultured for 24h followed by 24h stimulation with SF from patients classified with high or low SFb, as percentage of collagen deposition over tissue area, at a 1:4 dilution in 24 well plates. OA FSCs were isolated from patient synovium, banked after total knee arthroplasty, and grouped by low (<40%; n=2) and high (>54%; n=2) histological SFb. After cell homogenization, RNA was extracted and purified, cDNA synthesized, and assayed by qPCR using a validated primer array of genes related to collagen synthesis and cross-linking (*Plod2*, *Tgfb1*, *Timp1*, *Ctgf, Col1a1*), myofibroblast differentiation (*Acta2*), and matrix degradation (*Mmp1, Mmp13*) against *Hprt1* and *Actb* housekeeping genes. *Smad7* will be screened to test negative regulation of master pro-fibrotic TGFβ1. *Urotensin2* and *PGFalpha2* will be tested for their role in cardiac/pulmonary fibrosis and *Plod2* upregulation, respectively. Data will be processed by comparative Cp method. Unpaired t-test with $\alpha=0.05$ will be applied to comparisons and results log scaled.

We expect HFLS stimulated with SF from high SFb patients to have a more robust fibrogenic gene expression response than those stimulated with SFs from low SFb patients. We also expect FSCs from high SFb OA patients to express higher levels of fibrogenic *Plod2, Timp-1, Tgfb-1, Ctgf, Col1a1,* but lower expression of *Smad7* when compared to FSCs from the low SFb cohort. Our overarching goal is to determine if high SFb SFs will increase collagen and secretion of fibrogenic factors by FSCs, which will aid us in evaluating knee OA SFb status non-invasively by screening inflammatory and fibrogenic factors in the SF linked to histological kOA SFb.