Matrikine-activated Synovial Fibroblasts Promote Inflammatory Responses and Impair Chondrocyte Function

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Disclosures: The authors have nothing to disclose.

INTRODUCTION: Osteoarthritis (OA) is characterized by the degradation of cartilage matrix proteins, including fibronectin, leading to the formation and accumulation of fibronectin fragments (FN-f) found in OA cartilage and synovial fluid. A specific FN-f, FN7-10, has been found to act as a matrikine by binding to the chondrocyte α5β1 integrin to recapitulate an OA phenotype. FN-fs in synovial fluid could also interact with synovial fibroblasts, potentially promoting pathological crosstalk between the synovium and cartilage. The objective of this study was to examine the effects of bioactive FN7-10 on synovial fibroblast activation and to explore how these cells interact with chondrocytes and immune cells to promote OA pathogenesis.

METHODS: Synovial fibroblasts were isolated from OA synovial tissue obtained from knee replacements (n=34, 20 females and 14 males). Normal human chondrocytes were from cadaveric talar cartilage (n=6 males due to donor availability). RNA and conditioned media were collected after overnight stimulation of fibroblasts with FN7-10 or PBS in serum-free media. RNAseq was analyzed with DESeq2. Conditioned media (CM) were used for cytokine protein arrays. For crosstalk studies and transwell migration experiments, fibroblasts were stimulated for 4 hours with either FN7-10 or PBS and then incubated in fresh media to remove FN7-10 followed by overnight incubation to allow secreted factors to accumulate. The CM and RNA were collected the following day. CM was centrifuged before being used in THP-1 migration assays or to treat normal chondrocytes, naive THP-1 cells, and PMA stimulated THP-1 cells. Chemokine receptor inhibitors for CCR2, CXCR2, and CXCR3 were used in the migration assay. Alcian blue dye was used to assess chondrocyte micromass glycosaminoglycan (GAG) levels. CXCL5 was measured in the synovial fluid of patients (n=38, 21 females and 17 males) with an anterior cruciate ligament (ACL) injury. Human subjects research was IRB approved.

RESULTS: RNA-seq analysis of FN7-10 stimulated synovial fibroblasts revealed a significant shift in gene expression compared to PBS controls with 3,409 genes differentially expressed. We identified 22 upregulated chemokine genes (Fig. 1), many of which were confirmed as secreted proteins induced by FN7-10 in a cytokine protein array, including CCL4, CCL5, CCL7, CCL8, CCL20, CXCL5, and CXCL10. In a transwell migration assay (Fig. 2A), THP-1 cells increased migration toward the CM from FN7-10 stimulated synovial fibroblasts compared to the control CM (Figs. 2B and 2C). Pre-treating THP-1 cells with a CCR2 inhibitor or CXCR2 inhibitor reduced migration by about 50% (Fig. 2C). THP-1 cells incubated with CM from FN7-10 stimulated synovial fibroblasts demonstrated increased expression levels of *CD86* (2.9-fold increase) and *CD163* (3.8-fold). PMA-treated THP-1 cells, incubated with FN7-10-stimulated synovial fibroblast CM displayed a significant increase in *IL1B* (9.0-fold) and *TNFA* (4.7-fold), along with a decrease in *TGFB* (0.7-fold), indicative of a pro-inflammatory macrophage phenotype. Normal chondrocytes treated with CM from FN7-10 stimulated synovial fibroblasts demonstrated a downregulation of *COL2A1* and *ACAN* across all donors, along with a modest increase in catabolic and inflammatory markers (Fig. 3A). Chondrocyte micromasses treated with the same CM exhibited decreased GAG levels compared to controls (Fig. 3B). CXCL5 levels were identified in synovial fluid after an ACL injury and higher levels correlated with worse KOOS pain (Spearman rho = -0.464, p-value = 0.005) and greater T1rho relaxation times in the medial femoral articular cartilage (Spearman rho = 0.652, p-value = 0.001), which measured lesser proteoglycan density at 6 months post-ACLR.

DISCUSSION: Our findings reveal that the FN7-10 matrikine stimulated synovial fibroblasts to secrete chemokines which attract monocytes through chemokine receptors and could thereby promote synovitis in OA. This is supported by the finding that CM from activated synovial fibroblasts promoted macrophage phenotypes associated with inflammation. In addition, the matrikine-activated synovial fibroblasts exhibited crosstalk with chondrocytes, resulting in decreased anabolic markers, increased catabolic enzymes, and elevated inflammatory mediators. CXCL5/ENA78 was a prominent chemokine produced by synovial fibroblasts in response to FN7-10 and chondrocytes in response to synovial fibroblast CM. CXCL5 was found in synovial fluid samples from patients who had sustained an anterior cruciate ligament injury, which increases the susceptibility and likelihood of developing OA. The association of CXCL5 levels with worse preoperative pain KOOS scores and early decreased articular cartilage proteoglycan density suggests it may contribute to posttraumatic OA.

SIGNIFICANCE: This study demonstrates the effects of FN7-10, a matrikine derived from matrix breakdown, on synovial fibroblasts, making it a useful in vitro model for studying inflammation in synovial fibroblasts associated with OA. CXCL5, a potent chemokine expressed by all cell types used in this study, may serve as an indicator of early OA development. Although limited to one matrikine, this research highlights the importance of understanding bioactive cartilage products in the development of OA, which could inform new therapeutic strategies targeting chronic joint inflammation.

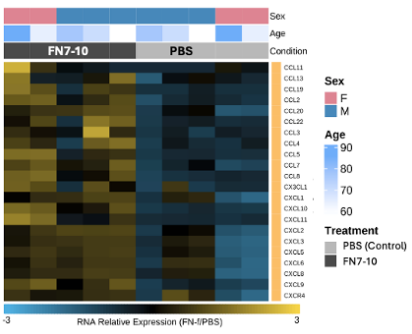

Figure 1: Heatmap of upregulated chemokines in synovial fibroblasts stimulated with fibronectin fragment (FN7-10) compared to a PBS control.

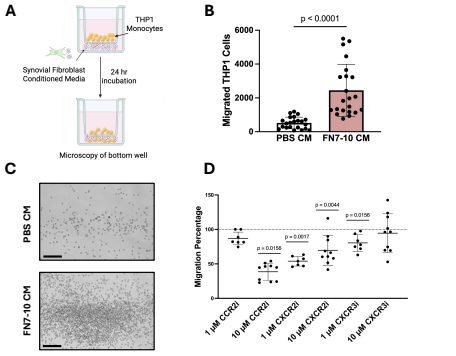

Figure 2: THP-1 transwell migration in response to synovial fibroblast CM. A) Experimental schematic. B) Graph of THP-1 migration across a transwell insert. C) Representative images of THP-1 cells migrated to the bottom of the well. D) Migration percentage of THP-1 cells pre-treated with chemokine receptor inhibitors.

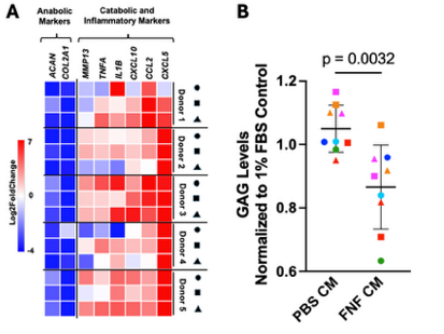

Figure 3: Response of normal chondrocytes to synovial fibroblast signals. A) Heatmap of chondrocyte gene expression comparing the effects of FN7-10 stimulated synovial fibroblast CM to PBS-treated synovial fibroblast CM. B) GAG levels from chondrocytes treated with synovial fibroblast CM spiked with 1% FBS.