Chemokine ENA-78/CXCL5 is Produced by OA Synovial Fibroblasts in Response to a Matrix Damage Stimulus and is Found in Synovial Fluid Collected from Patients After ACL Injury

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Introduction

- Cytokines/chemokines are critical upstream mediators of catabolic activity that promote both a decrease in matrix production and an increase in MMPs and other proteases.
- Proteolytic matrix degradation generates bioactive matrix fragments including fibronectin fragments (FN-f) found in OA cartilage and synovial fluid (1). FN-f induce chondrocytes to produce a host of proinflammatory chemokines and proteases that promote a positive feed-forward loop of cartilage matrix degradation (1-2).
- Cartilage matrix degradation products released into the synovial fluid can potentially act on cells in the synovium.
- Interactions between joint tissues through pro-inflammatory cytokine networks may contribute to OA pathogenesis.

Assess the production of proinflammatory mediators, including cytokines and MMPs, by human synovial fibroblasts from OA tissue treated with a bioactive fragment of fibronectin (FN-f) in order to discover new factors that may promote OA.

Results

**A.** FN-1 treatment Induces production of MMP1, MMP13, and IL-6 by OA chondrocytes and MMP-1 and IL-6 by OA synovial fibroblasts. Confluent human primary articular chondrocytes and passage 3 synovial fibroblasts were treated overnight in serum-free media with 1µM 42kd recombinant FN-1 (FN7-10) which contains the RGD α5β1 integrin binding region or PBS as control. Conditioned media (to study MMPs and IL-6) and cell lysates (to study β-actin) were collected for immunoblotting. A. Representative immunoblots from n=4 independent donors. B. Densitometric quantification of immunoblot bands normalized to actin. Data are mean ± s.d (n=4); *p<0.05; significant difference compared to PBS treated controls.

**B.** FN-1 stimulation of pro-inflammatory cytokines and chemokines by OA synovial fibroblasts. OA synovial fibroblasts in serum-free media were stimulated with FN-1 overnight or PBS control. A. Conditioned media was collected and incubated on cytokine protein arrays (RayBio). Representative array of n=2 shown. B. Arrays were quantified by densitometry and results expressed relative to mean of the 6 positive control spots on the array. Selected results shown in bar graphs. High basal production of IL-6 and MCP-1 were noted. The chemokine Epithelial Neutrophil-Activating Peptide-78 (ENA-78, CXCL5) was one of the most highly upregulated in response to FN-1 and was selected for further study as a novel chemokine produced by OA synovial fibroblasts.

Fig. 2. FN-1 stimulation of pro-inflammatory cytokines and chemokines by OA synovial fibroblasts. OA synovial fibroblasts in serum-free media were stimulated with FN-1 overnight or PBS control. A. Conditioned media was collected and incubated on cytokine protein arrays (RayBio). Representative array of n=2 shown. B. Arrays were quantified by densitometry and results expressed relative to mean of the 6 positive control spots on the array. Selected results shown in bar graphs. High basal production of IL-6 and MCP-1 were noted. The chemokine Epithelial Neutrophil-Activating Peptide-78 (ENA-78, CXCL5) was one of the most highly upregulated in response to FN-1 and was selected for further study as a novel chemokine produced by OA synovial fibroblasts.

Fig. 3. FN-1 stimulated production of ENA-78/CXCL5 by articular chondrocytes and synovial fibroblasts. A. Representative immunoblots of conditioned media after overnight treatment with FN-1 or PBS control. B. ELISA results using conditioned media from n=4 overnight FN-1 or PBS control treated synovial fibroblast cultures. (p<0.05; significant difference compared to PBS controls).

Table 1. Spearman correlation analysis of ENA-78/CXCL5 concentrations in synovial fluid samples from ACL injured patients with selected outcomes. ENA-78 synovial fluid samples collected for a previously reported study (3) aspirated from patients within 2 weeks of an acute ACL injury were measured by ELISA (n=141). The synovial fluid samples were graded 1-4 for the amount of blood staining as described (4). ENA-78 concentrations ranged from less than 10 pg/mL to 4.7 ng/mL. Spearman correlation was performed with clinical outcomes that included T1rho MR values and KOOS scores. Greater T1rho relaxation times are indicative of decreased proteoglycan content in the cartilage and therefore cartilage matrix loss. Lower KOOS scores are indicative of worse reported patient outcomes. MFC= Medial femoral condyle, LFC= Lateral Femoral condyle, ACLR= ACL reconstruction.

Conclusions

- The bioactive cartilage matrix degradation product FN-1 (FN7-10 which binds the α5β1 integrin) induces OA synovial fibroblasts to produce a host of proinflammatory mediators that include cytokines such as IL-6, chemokines (ENA-78/CXCL5, MCP1-3, RANTES, MIP) and matrix degrading enzymes (MMP-1).
- ENA-78/CXCL5 is a novel chemokine involved in neutrophil activation that is produced by OA synovial fibroblasts in response to FN-1.
- The presence of ENA-78/CXCL5 in the synovial fluid of patients who sustained an ACL injury is associated with decreased cartilage proteoglycan density and worse KOOS scores providing preliminary evidence that ENA-78/CXCL5 may play a role in PTOA development.

References