Cytokine Production by Synovial Fibroblast using the Fibronectin Fragment (FN-f) In Vitro Model of Osteoarthritis

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Abstract

Osteoarthritis (OA) progression is characterized by a disruption in homeostasis that drives the degradation of cartilage’s extracellular matrix (ECM) and promotes an increase in matrix fragments. Cartilage degradation is primarily due to the overproduction of secreted matrix metalloproteinases (MMPs) by chondrocytes, however synoviocytes also contribute to this activity by releasing OA mediators that exacerbate joint destruction. The cross-talk between synovial cells and chondrocytes could be crucial, due to further secretion of key cytokines that intensify catabolic signaling and activity in both cell populations. Indeed, synovial fibroblasts have been hypothesized to express cytokines and MMPs in response to ECM protein fragments found in the cartilage and synovium of human OA. We test this hypothesis by measuring the production of MMPs and IL-6 in synovial fibroblasts in comparison to chondrocytes, using a fibronectin fragment (FN-f) in vitro model.

Introduction

- OA is a deteriorating joint disease that affects not only the cartilage but the synovial and subchondral bone.
- Due to the lack of effective disease-modifying treatments, recent research has focused on the signaling pathways involved in the pathology of the disease.
- An imbalance of catabolic and anabolic activity is seen in OA cartilage cells, known as chondrocytes, that is driven by interactions between joint tissues through pro-inflammatory cytokine networks.
- Cytokines are critical upstream mediators of catabolic activity that promote both a decrease in matrix production and an increase in MMPs.
- Synovial fibroblasts are a sub-population of synovial cells that have been shown to highly express several cytokines, including IL-6, that regulate the phenotypic changes in OA chondrocytes.
- To study OA, we stimulate synovial fibroblasts and chondrocytes with a FN-f, present in human OA cartilage and synovial fluid, to examine pathways responsible for cytokine release and MMP production.

Tissue Damage Due to Risk Factors

Figure 1. Signaling pathway involved in the progression of osteoarthritis. Risk factors such as weight, age, and prior joint trauma lead to an imbalance of catabolic and anabolic signaling and activity, including an increase of MMPs and cytokine production. This disruption contributes to the degradation of cartilage and synovium that is seen in the phenotype of OA and it is amplified through the cross-talk of sub-populations of the joint.

Objective

Assess catabolic activity of synovial fibroblast from OA donors, following overnight treatment with FN-f by measuring cytokine and MMP production.

Results-1

Figure 2. Analyzing synovium-derived cell population using flow cytometry.

- a) Microscopic representative images of human OA articular chondrocytes (left) and OA synovial fibroblasts (right). Representative sample of flow cytometry is shown. OA synovial fibroblasts are stained with DAPI, CD45-FITC, CD73-PE Cy7, and/or CD105-APC at either cell passage 3 (top) or passage 4 (bottom) (n=3). b) DAPI staining for live/dead cells. G1 gates for live cells. c) Quantification of negative CD45 sub-populations (G2) based on FMO staining shows homogenous populations of non-hematopoietic cells for both passages. d) Quantification of double positive synovial fibroblast’s cell markers (CD73 and CD105) within G2 shows high expression of markers. e) Values from flow cytometry data of CD73 and CD105 were separately plotted in a log graph. Decrease in expression value of data from passage 3 to passage 4 is evident in CD73 marker for OA Donor 79 and CD105 marker for OA Donor 74.

Results-2

Figure 3. FN-f treatment induces production of MMP-1 and IL-6 in articular chondrocytes and synovial fibroblasts.

- Confluent primary articular chondrocytes and passage 3 synovial fibroblasts are changed to serum-free media for three hours and then treated overnight with FN-f. Conditioned media (to study MMPs and IL-6) and cell lysates (to study β-actin) are collected for immunoblotting. BCA is used on β-actin to quantify protein concentration. a) Representative immunoblotting of proteins from media and cell lysates with or without FN-f, for MMP-1 and IL-6. b) Quantification of the change in both chondrocytes and fibroblasts following FN-f stimulus, data are mean ±1 sd normalized to β-actin (n=4). MMP-1 and IL-6 are overproduced following overnight FN-f treatment.

Results-3

Figure 4. FN-f stimulation induces the expression of several pro-inflammatory cytokines in synovial fibroblasts.

- Conditioned synovial fibroblasts are changed to serum-free media for three hours and then stimulated with FN-f, overnight. Conditioned media is collected and cytokine protein arrays (RayBio) are performed on OA donor 93 (a,b) and donor 95 (c,d). Cytokines were quantified on the basis of visible increase following FN-f stimulus compared to the unstimulated. Cytokines including MCP family, IP-10, ENA-78, and RANTES, were normalized to positive control and compared to unstimulated also normalized to positive control.

Conclusions

- Isolated synovial populations are homogeneous for non-Hematopoietic cell lines and differences between cell passages were not significant.
- Synovial fibroblast cell markers are loss between cell passage 3 and passage 4.
- Passage 3 synovial fibroblasts is best to use for the FN-f in vitro model of OA.
- Overnight treatment of FN-f induces synovial fibroblast to produce MMP-1 and IL-6, but no MMP-13 production was observed.
- Pro-inflammatory cytokines that are measured in OA synovial fluid, are overproduced following FN-f treatment of synovial fibroblasts.

Future Directions

Further experiments will look to study the signaling pathway involved with FN-f treatment in synovial fibroblasts. Immunoblotting experiments will be performed, further measuring production of many of the cytokines that were increased in the cytokine arrays following stimulation.