STROMAL CELL-DERIVED FACTOR-1 INDUCES CARTILAGE DESTRUCTION IN OSTEOARTHRITIS AND RHEUMATOID ARTHRITIS VIA CXC CHEMOKINE RECEPTOR 4 THROUGH STIMULATION OF MATRIX METALLOPROTEINASE 3 RELEASE

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Introduction: During osteoarthritis and rheumatoid arthritis, synovium may be involved in the induction of catabolic activities of joint cartilage. Synovectomy is effective for preventing cartilage destruction and relieving pain from rheumatoid arthritis (RA) and osteoarthritis (OA) patients. However, the signaling pathway by which synovium regulates articular cartilage activity is unclear. The signaling molecules may be transported by fluid in the joints. In this study, we focus on a chemokine stromal cell-derived factor 1 (SDF-1) and its receptor CXCR4. The importance of SDF-1 signaling was illustrated by the phenotypes of SDF-1 and CXCR4 knockout mice. Both exhibit significant developmental abnormalities that lead to embryonic lethality. Very little is known about the role of SDF-1 in pathogenesis of OA and RA. Here we determined the possibility of such a role by quantifying SDF-1 protein levels in synovial fluid from both OA and RA patients. The aim of this study is to analyze the role of a chemokine stromal cell-derived factor 1 (SDF-1) and its receptor CXCR4 in rheumatoid arthritis and osteoarthritis by examining their gene expression in joint cartilage and synovium, and by determining the effect of their interaction on the release of matrix metalloproteases from chondrocytes.

Methods: Synovial fluid was collected from patients diagnosed with osteoarthritis (n=55, age range: 55 to 78 years old, mean age: 65 years old), rheumatoid arthritis (n=58, age range: 48 to 79, mean: 68), and no arthritis control (n=60, age range: 25 to 48, mean: 43). Synovial fluid from controls was taken at the time of knee arthroscopy from adults without any forms of arthritis. The concentration of SDF-1 in synovial fluid was quantified by ELISA according to manufacturer's instruction. An anti-human SDF-1beta monoclonal antibody and a secondary antibody with conjugates were used. Cartilage pieces from each category (RA, OA, and N) were finely minced, and chondrocytes were isolated by sequential enzymatic digestion. Isolated chondrocytes were cultured in 10% FBS in Dulbecco's modified Eagles's medium (DMEM). To culture synovial fibroblasts, synovium tissues were cut into small pieces and dissociated enzymatically overnight with Dispase I. Isolated synovial cells were plated into culture flasks in incubation medium under the same condition as described above. Total RNA was extracted from cultured primary cells using RNeasy mini kit. PCR primers were designed to amplify cDNA products within the respective coding regions according to GenBank sequences. Isolated chondrocytes were fixed in 4% paraformaldehyde, washed, and subsequently incubated with 10 µg/ml anti-CXCR4 or anti-CXCR5 monoclonal antibodies for flowcytometry. Chondrocytes were seeded in 24 well plates (4X105/well) and incubated for 24 hours in DMEM at 37oC in 5% CO2. SDF-1 beta, IL-1 beta, TNF alpha, or BCA-1 was then added to the wells, respectively. After 24-hour incubation, conditioned chondrocyte medium was collected for either MMP or PGE-2 assays. Levels of MMP-1, MMP-3, or PGE-2 within conditioned medium were determined by ELISA with antibodies from Biotrak. In some cases, chondrocytes were pretreated with anti-CXCR4, anti-CXCR1 or anti-CXCR5 monoclonal antibodies at indicated concentrations from 0 to 100 ng/ml for two hours before stimulation with 100 ng/ml SDF-1. These same experiments were performed with SDF-1alpha with similar outcomes. Mann-Whitney U tests or paired t-tests were used. P values less than 0.01 were considered significant.

Results: To determine whether SDF-1 was involved in OA and RA pathogenesis, we determined the concentration of SDF-1 in synovial fluid. From normal adults it was 70 ± 5 ng/ml (Figure 1). The concentration of SDF-1 in synovial fluid from OA patients was 250 ± 26 ng/ml, representing an average 257% increase over normal adults. In RA patients, the synovial concentration of SDF-1 was 750 ± 80 ng/ml, representing an increase of 971% over normal controls. To determine the source of SDF-1 in synovial fluid, we cultured chondrocytes and synovial fibroblasts in vitro. Neither normal chondrocytes, nor chondrocytes from OA or RA patients showed any production of SDF-1. In contrast, normal synovial fibroblasts secreted SDF-1 into the medium at 350 ± 40 ng/ml. Furthermore, OA synovial fibroblasts secreted 500 ± 58 ng/ml SDF-1, and RA synovial fibroblasts secreted 820 ± 89 ng/ml SDF-1. The SDF-1 mRNA level was the highest in RA synovial fibroblasts, high in OA, and low in normal. In contrast, SDF-1 mRNA was not detected in chondrocytes from any of the three groups. These data suggested that SDF-1 was synthesized de novo by synovial cells with elevated levels in RA and OA. Next, we determined which cells synthesize the receptor of SDF-1, CXCR4. While chondrocytes were positive for CXCR4 mRNA, synovial fibroblasts were negative. Furthermore flow cytometry analysis showed that about 50 % of the normal, OA, and RA chondrocytes contained CXCR4 receptor. SDF-1 increased the production/release of MMP-3 by chondrocyte in a dose-dependent manner, while it did not affect the concentration of MMP-1 (Fig. 2). The presence of an anti-CXCR4 monoclonal antibody before and during SDF-1 treatment of chondrocytes inhibited MMP-3 release in a dosage dependent manner. This indicated that SDF-1 stimulated MMP-3 release by specific interaction with CXCR4 in chondrocytes.

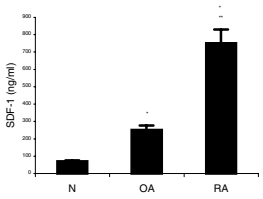

Fig. 1

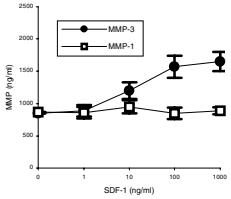

Fig. 2

Discussion: It has been shown previously that SDF-1 and its receptor CXCR4 play an important role in cell migration, embryonic development, and human immunodeficiency virus infection. In this study, we show that SDF-1/CXCR4 pathway may play a role in pathogenesis of RA and to a less degree, in pathogenesis of OA as well. We found a dramatic increase of SDF-1 concentration in synovial fluid from RA and OA patients. This suggests that there is a clear link between elevated SDF-1 level in synovial fluid and development of RA and OA. It also indicates that we may be able to use SDF-1 level in synovial fluid as a marker for diagnosis of RA and OA. Our data suggest that the stimulated expression of SDF-1 by synovial fibroblasts during RA and OA pathogenesis accounts for at least part of the SDF-1 increase in synovial fluid. Our data revealed a complementary gene expression pattern in which synovial fibroblasts expressed the ligand SDF-1 but not the receptor CXCR4, whereas chondrocytes expressed CXCR4 but not SDF-1. This complementary gene expression pattern suggests a paracrine regulatory mechanism in which SDF-1 made by synovial fibroblasts signals near-by chondrocytes by binding to their receptors. We demonstrated that CXCR4 activation through SDF-1 binding induced the release of MMP-3 from chondrocytes. MMP-3 is an effective member of the MMP family that is responsible for breaking down cartilage in vivo. Taken together, our data suggest that the release of MMP-3 induced by synovial SDF-1 for maintaining normal cartilage homeostasis may become excessive in RA and OA, due to the significantly higher levels of SDF-1 in synovial fluid from RA and OA joints. The excessive induction of MMP-3 by SDF-1 may be detrimental in RA and OA cartilage, thereby contributing to their characteristic degradation.

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