CHEMOKINE STROMAL CELL-DERIVED FACTOR-1 INDUCES MATRIX METALLOPROTEASE 13 mRNA IN OA CHONDROCYTES: DEPENDENCY ON P38 MAP KINASE

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Introduction: Chemokines are a family of soluble peptides that regulate cell movement, morphology, and differentiation. A recent study has shown that a chemokine Stromal Cell-Derived Factor-1 (SDF-1) is involved in regulating cartilage catabolism by synovium in the joint (1). SDF-1 is synthesized by synovial fibroblasts and secreted into synovial fluid. SDF-1 then interacts with its receptor CXCR4 in chondrocytes. Such interaction results in stimulation of Matrix Metalloprotease (MMP)-3 release from chondrocytes. However, it is not known whether SDF-1 also induces release of other types of MMPs from chondrocytes. Furthermore, the intracellular pathway that transduces SDF-1 signals in chondrocytes remains unclear. Previously it has been shown that mitogen activated protein kinases (MAPKs) are involved in transmitting SDF-1 signals in lymphocytes. The purpose of this study is to determine whether SDF-1 induces chondrocyte release of MMP-13, one of the major catabolic proteases involved in osteoarthritis pathogenesis, and if so, which MAP kinase, ERK, JNK, or p38 MAPK, is involved in transducing SDF-1 signals in OA chondrocytes.

Method: Chondrocytes were isolated from human OA cartilage and cultured in DMEM containing 10% FBS. Cells were treated with SDF-1 at different concentrations and time points in serum-free medium for 24 h. Sometimes p38 MAPK inhibitor, SB203580 (10 µM) was added 2 hours before SDF-1 treatment. Control cells were treated with DMSO. p38 MAPK kinase, JNK and ERK activity as well as their protein levels were determined by western blot analysis with polyclonal Ab against p-p38, p-JNK and monoclonal Ab against p-ERK, respectively. Western blot data were quantified by image acquisition and analysis software (UVP bioimaging systems). Total RNA was isolated from chondrocytes with RNeasy isolation kit (Qiagen). The mRNA level of MMP-13 was determined by Real time PCR. Distribution of p38 MAPK activity and MMP-13 in cartilage from OA and normal patients was examined by immunohistochemistry with a Histostain SP kit (Zymed, San Francisco, CA) using anti-p-p38 MAPK and MMP-13 polyclonal Ab as primary antibodies.

Result: SDF-1 up-regulated p38 MAPK activity in OA chondrocytes in a dose-and time-dependent manner (Fig.1). In contrast, the p38 MAPK protein levels remain unchanged. However, SDF-1 treatment did not affect activities of ERK and JNK within chondrocytes. Inhibiting p38 activity with specific inhibitor SB 203580 before SDF-1 treatment completely abolishes SDF-1 induction of p38 MAPK activity in chondrocytes. In parallel to the activation of p38 MAP kinase, SDF-1 treatment increases MMP-13 mRNA levels in chondrocytes. Furthermore, inhibition of p38 MAPK suppressed SDF-1 induced increase of MMP-13 mRNA in OA chondrocytes (Fig. 2). In addition, both MMP-13 protein and p38 MAPK activity were detected in chondrocytes from superficial and middle zones of OA cartilage. Neither MMP-13, nor activated p38 MAPK was detected in normal cartilage.

Discussion: Elevation of MMP-13 level is one of the hallmarks of OA cartilage. In this study we show that this elevation can be caused by SDF-1 treatment of chondrocytes. Furthermore, p38 MAPK activation is essential for transducing SDF-1 signals to stimulate MMP-13 mRNA synthesis by chondrocytes. This signal transduction pathway is specific, because other two major MAP kinases, ERK and JNK are not activated by SDF-1 in OA chondrocytes. This observation may have significance in vivo, since MMP-13 and activated p38 MAPK are co-localized in OA cartilage. It is interesting to note that SDF-1 in synovial fluid from OA patients is 5 times higher than that from normal adults. Thus, elevated levels of SDF-1 in OA patients may cause excessive activation of the SDF-1/CXCR4 pathway in chondrocytes. This excessive activation may induce high levels of MMP-13 production in chondrocytes and ultimately lead to breakdown of cartilage matrix. Based on data from this study, inhibition of p38 MAPK activation in chondrocytes may be considered for treatment of OA by preventing excessive production of MMP-13 resulted from SDF-1 signaling.