Introduction: Degradation of articular cartilage matrix is a prominent feature of osteoarthritis (OA). Matrix metalloproteinases (MMPs) play a critical role in this degradative process. MMP-1 is one such degradative enzyme that has been reported to be a major enzyme participating in the degradation of collagen type II in osteoarthritic cartilage. Tissue-specific and inducible transcription factors are implicated in the abnormal expression of MMPs that cause degradation of articular cartilage. This study focuses on the identification of specific transcription factors that are activated in the cartilage tissue of OA patients and involved in the expression of MMP-1. Our hypothesis was that the inflammation-responsive transcription factor serum amyloid A activating factor-1 (SAF-1) would regulate MMP-1 gene expression in OA.

Methods: All procedures were approved by the Institutional Review Board and University Animal Care and Use Committee. Articular cartilage specimens were obtained from human patients (n=10) undergoing total joint arthroplasty surgery and canine patients (n=5) undergoing surgery of the hip or knee. All patients had OA and severity was graded using the Outerbridge system and verified by histologic examination. Normal articular cartilage was obtained from human (n=1) and canine (n=2) cadavers. Chondrocytes were isolated and cultured from each specimen. Chondrocytes were transfected by calcium phosphate method. pSVβ-gal DNA was used to monitor the efficiency of transfection and to normalize the cell extracts used for CAT assay. Expression plasmid DNA for either SAF-1 or antisense-SAF-1 cDNA was included. Subsets of cells were stimulated with human IL-1β (500 U/ml, 5 ng/ml). Cell extracts containing equivalent amounts of β-galactosidase activity were used for CAT assay. All transfection experiments were performed at least three times. For fluorescence activated cell sorter (FACS) selection of transfected cells, cells were transfected with either pAAV-IRE-GFP vector or pAAV-SAF-1-IRE-GFP plasmid. These enriched populations of transfected cells were subsequently analyzed for MMP-1 protein expression.

Nuclear extract from normal and OA chondrocytes, both human and canine, was prepared. For DNase I foot-printing assay, DNA probes carrying MMP-1 promoter sequences from ~1200 to ~800, ~800 to ~517, ~517 to ~301, ~301 to +63 were radiolabeled with [32P]-ATP. The labeled probes were incubated with nuclear extracts and the DNase I-protected regions were determined. Radiolabeled probe for electrophoretic mobility shift assay was prepared by incorporating [γ-32P]-ATP from [γ-32P]ATP into a double-stranded 33-mer oligonucleotide, 5′-CCACCCTCTGCCCTGGGAGCAAGGTGTGTGGAG-3′ that represents human MMP-1 promoter sequence from ~386 to ~354. In some binding assays, the above oligonucleotide, unlabeled, was added as a homologous oligo competitor to assess the specificity of binding of the radioactive probe. Also, double-stranded SAF oligonucleotide, known binding site of SAF with the following sequence, 5′-GGCCCTCTCTCCACCCACAGCCCCCATGG-3′ that was used to characterize the protein in the DNA-protein complex. For antibody binding studies, either anti-SAF1 antibody or a pre-immune serum was added to the binding reaction mixture during preincubation. Immunohistochemical staining was performed using affinity-purified anti-SAF1 rabbit IgG as the primary antibody and pre-immune rabbit IgG as a control. Horseradish peroxidase conjugated goat anti-rabbit IgG was used as the secondary antibody. The slides were incubated in 0.1% trypsin solution to unmask antigens. Nonspecific rabbit IgG was used as the secondary antibody. The slides were incubated in 0.1% trypsin solution to unmask antigens.

Results: A novel promoter element was detected in the human MMP-1 gene in the specific region from nucleotide ~386 to ~354. The inflammation-responsive transcription factor SAF-1 was found to interact with this MMP-1 promoter region. The DNA-protein complexes formed between MMP-1 oligonucleotide and the OA chondrocyte nuclear extracts were efficiently competed by SAF-binding oligonucleotide and inhibited by anti-SAF1 antibody, but not by pre-immune serum (Fig.1). Immunohistochemical analysis revealed that normal articular cartilage had very few cells that were immunoreactive for SAF, while the majority of cells were SAF-positive in OA cartilage sections from both species (Fig. 2).

DISCUSSION: These data indicate that SAF-1 is involved in the regulation of MMP-1 gene expression and it is highly abundant in the chondrocytes of articular cartilage of OA patients. These data also demonstrate that control of SAF-1 activity can suppress induced expression of MMP-1. Therefore, SAF-1 could be a potential therapeutic target to control over-expression of MMP-1 associated with the pathogenesis of OA.

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