OVEREXPRESSION OF MMP-14 IN HUMAN OSTEOARTHRITIC JOINT IS MEDIATED BY SAF-1

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INTRODUCTION
Osteoarthritis is a crippling disease that is the result of progressive damage to articular cartilage and is a major cause of activity limitation and physical disability in older population. More than 250,000 total knee replacements are performed annually in the United States to treat the joint destruction and ensuing symptoms related to osteoarthritis.

In weight-bearing joints, one role of articular cartilage is to protect the underlying bone against shearing forces generated by joint movement and compressive forces that result from joint loading. In the healthy cartilage, chondrocytes maintain a balance of anabolic and catabolic activities to maintain the cartilage stability. In osteoarthritic cartilage, an increase in the catabolic matrix metalloproteinase (MMP) activity shifts the balance toward net degradation and is believed to be the major reason of the degradation of articular cartilage.

The membrane-type 1 matrix metalloproteinase also known as MMP-14 is a member of the novel cell membrane anchored MMPs that plays an important role in the localized degradation of articular cartilage because of its broad substrate specificity, cell membrane location, ability to be synthesized in an active form and its involvement in the processing and activation of other members of the MMP family, including MMP-2, MMP-13 and MMP-9. MMP-14 level is shown to be increased in several pathogenic disorders associated with progressive extra-cellular matrix loss such as those seen in tumor progression, degenerative joint arthroplasties and rheumatoid arthritis. To date limited data are available regarding the expression level of MMP-14 during osteoarthritic condition.

METHODS
Human osteoarthritic cartilage was obtained from patients with the diagnosis of osteoarthritis, who had radiographic evidence of joint destruction in one knee or another. We excluded patients with a history of inflammatory or systemic arthritis. The study was reviewed and approved by our Institutional Review Board.

Explanted cartilage consisted of the distal femoral condyles, the articular surface of the patella, and the proximal tibia. These tissues are routinely removed during total knee replacement surgery. Tissues were fixed in formalin, embedded in paraffin, and tissue sections (5 microns) were cut for immunohistochemical (IHC) analysis. Antibodies against MMP-14 and SAF-1 were used in IHC. Cartilage cell extracts were prepared by first freezing the tissues in liquid nitrogen and the frozen tissues were ground in a mortar-pestle, resuspended in lysis buffer. Cells were lysed by three rounds of freeze-thaw cycles, centrifuged to collect cell lysate which were used in electrophoretic mobility shift assay (EMSA). Radiolabelled MMP-14 promoter region from -140 to +1 which contains a SAF-binding element was used in the EMSA. Promoter function analysis using 0.2MMP-14CAT reporter was performed by transient transfection of cartilage-specific chondrocyte cells (HTB94) with the wild-type 0.2MMP-14 CAT and a mutant derivative containing mutation at the SAF-binding site of the promoter located within nucleotide position -122 and -77. In some assays, HTB-94 cells were co-transfection with expression plasmid pcDSAF-1.

RESULTS
Presence of MMP-14 in cartilage tissue was examined by IHC which revealed that the normal cartilage tissue contain very low level of this enzyme (Fig. 1, panel a). Similar analysis was performed with cartilage samples from 24 OA patients with varying degrees of pathogenecity. MMP-14 protein level is markedly increased in all OA tissues albeit at variable level. Three representative samples are shown in Fig. 1, panels b-d. Similar analysis for SAF-1 was performed using anti-SAF-1 antibody (Fig. 2). Normal cartilage tissue contains low but detectable level of this protein (Fig. 2, panel a) but it was highly prevalent in the cartilage tissue of OA patients (Fig. 2, panels b-d).

To test whether the DNA-binding activity of SAF-1 with MMP-14 promoter is also increased during OA, EMSA was performed using MMP-14 promoter DNA as a probe (Fig. 3). DNA-protein complex formation was increased in the cartilage extracts of OA patients and the bound protein was identified as SAF-1 (Fig. 3, lane 6).

DISCUSSION
In this investigation, we show by immunohistochemical analysis that MMP-14 is abundantly expressed in the chondrocytes of human osteoarthritic cartilage tissues while chondrocytes of normal cartilage showed only negligible level of MMP-14 staining. MMP-14 expression was increased in all layers of the cartilage but most increase was seen in the chondrocytes present in the middle to deep layer. We also show that osteoarthritic cartilage-derived chondrocytes which express increasing amounts of MMP-14 protein also express the transcription factor SAF-1 at a higher level. Recent studies have indicated that MMP-14 expression under inflammatory conditions is regulated by SAF-1 transcription factor. Consistent with this finding, SAF-1 in the chondrocyte cells was shown to augment MMP-14 promoter activity. Taken together these results indicate that increased synthesis of MMP-14 in human OA patients is mediated via induction of SAF-1 that plays a key role in cartilage degradation during osteoarthritis.

Fig. 1. IHC analysis of MMP-14 in human joint cartilage. Cartilage from normal cadaver (panel a) and those of three representative OA patients collected from the medial femoral condyle or tibial plateau or patella were subjected to IHC. Cells positive for MMP-14 show brown color.

Fig. 2. IHC analysis of SAF-1 in human joint cartilage. Tissues same as those described in Fig. 1 were subjected to IHC for SAF-1. Cells stained positive for SAF-1 show intense brown color.

Fig. 3. EMSA for SAF-1 DNA-binding activity in human joint cartilage tissue. Cartilage extracts were incubated with 32P-labeled MMP-14 promoter DNA and the DNA-protein complexes were fractionated a 6% nondenaturing polyacrylamide gel.

Fig. 4. MMP-14 promoter function. Wild-type and mutant MMP-14 promoters were co-transfected with pcDSAF-1 expression plasmid in HTB94 chondrocyte cells. Data represent an average of 3 experiments.