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Matrix Metalloproteinase (MMP)-12 Regulates MMP-9 Expression in Interleukin-1-Treated Articular Chondrocytes

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ABSTRACT

Limited information is available on the expression and role of matrix metalloproteinase (MMP)-12 in chondrocytes. We characterized the expression mechanism of MMP-12 and possible function in chondrocytes. Interleukin (IL)-1 induced the expression and activation of MMP-12 in primary culture chondrocytes and cartilage explants via mitogen-activated protein (MAP) kinase signaling pathways. Among MAP kinases, extracellular signal-regulated kinase and p38 kinase are necessary for MMP-12 expression, whereas c-jun N-terminal kinase is required for the activation of MMP-12. The possibility that MMP-12 acts as a modulator of other MMP was examined. MMP-12 alone did not affect other MMP expressions. However, MMP-12 enhanced expression and activation of MMP-9 in the presence of IL-1. Our results indicate that IL-1 in chondrocytes induces the expression and activation of MMP-12, which, in turn, augments MMP-9 expression and activation. J. Cell. Biochem. 105: 1443–1450, 2008.

KEY WORDS: CHONDROCYTES; IL-1; MMP-9; MMP-12; MAP KINASES

Cartilage is a complex tissue containing extracellular matrix (ECM) molecules that confer the ability to withstand mechanical stress associated with joint movement. Cartilage ECM is primarily composed of large quantities of proteoglycans (PG) and collagens [Eyre, 2002]. Collagen type II is the main isoform, while types VI, IX, X, and XI are found in small amounts in articular cartilage. PGs are large molecules containing a protein core with glycosaminoglycan branches. Maintenance of articular cartilage requires a precise balance between degradation and synthesis of the ECM by chondrocytes. Destruction of ECM homeostasis triggers degenerative joint diseases, such as osteoarthritis, characterized by the progressive loss of articular cartilage. Interleukin (IL)-1, a major pro-inflammatory cytokine, plays significant roles in cartilage destruction in arthritis [Martel-Pelletier et al., 1999; Choy and Panayi, 2001]. IL-1 is expressed at high levels in osteoarthritic cartilage, and modifies the physiology of joint and cartilage metabolism by altering the expression of a variety of genes. Moreover, IL-1 suppresses the expression of cartilage-specific ECM components, such as collagen type II and PGs, but promotes matrix metalloproteinase (MMP) expression.

MMPs are a family of proteolytic enzymes that share several structural and functional characteristics, with different substrate specificities [Brinckerhoff and Matrisian, 2002]. While proteolytic cleavage of ECM by MMPs is necessary for normal physiological processes such as embryonic development, pathological ECM breakdown plays an essential role in cartilage destruction [Burrage et al., 2006]. Accumulating evidence shows an association between the pathology of arthritis and MMPs. For instance, increased levels of several MMPs are present in arthritic joints, both in synovial fluid and cartilage. These include collagenase-1 and -3 (MMP-1 and -13), stomelysin-1 (MMP-3), membrane type-1 MMP (MT1-MMP), and gelatinases (MMP-9 and -27) [Okada et al., 1992; Mohtai et al., 1993; Imai et al., 1997; Tsuchiya et al., 1997; Fernandes et al., 1998]. MMP-1, -8, and -13 cleave triple helical collagens (types I, II,

Abbreviations used: DMEM, Dulbecco's modified Eagle's medium; ECM, extracellular matrix; GAPDH, glyceraldehyde 3-phosphate dehydrogenase; IL-1, Interleukin-1; JNK, c-Jun N-terminal kinase; MAP kinase, mitogen-activated protein kinase; MMP, matrix metalloproteinase; RT-PCR, reverse transcription-polymerase chain reaction.

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and III), whereas MMP-2 and -9 degrade non-triple helical collagen (gelatin). MMP-3 also activates proMMP-1, -8, -9, and -13 and is possibly involved in the degradation of PGs [Ogata et al., 1992]. MMP-12 is secreted as a 54 kDa pro-enzyme that is processed into 45 and 22 kDa active forms [Shapiro et al., 1993]. The protein was originally identified in medium conditioned with murine peritoneal inflammatory macrophages [Banda and Werb, 1981], and is expressed in several cell types, such as human airway smooth muscle cells [Xie et al., 2005], corneal epithelial cells [Lyu and Joo, 2005], and hypertrophic chondrocytes [Kerkela et al., 2001]. MMP-12 is able to degrade several ECM components, including elastin, collagen type IV, fibronectin, laminin, gelatin, vitronectin, entactin, PGs, heparan and chondroitin sulfate, plasminogen, and fibrinogen [Chandler et al., 1996; Gronski et al., 1997; Hiller et al., 2000].

While the roles of several MMPs including MMP-1, -3, -9, and -13 in chondrocytes and cartilage destruction are well characterized; little is known about the function of MMP-12 in chondrocytes. Here, we report that IL-1 induces MMP-12 expression and activation in chondrocytes via the mitogen-activated protein (MAP) kinase signaling pathway. MMP-12, in turn, upregulates MMP-9, suggesting its possible involvement in the regulation of cartilage ECM homeostasis.

MATERIALS AND METHODS

CULTURE OF PRIMARY CHONDROCYTES AND CARTILAGE EXPLANTS

Rabbit chondrocytes were isolated from the cartilages of 2-week-old New Zealand White rabbits to obtain larger number of cells, as described previously [Yoon et al., 2002]. Cartilage slices were dissociated enzymatically for 4 h in 0.2% collagenase type II (381 U/mg solid; Sigma, St. Louis, MO) in Dulbecco's modified Eagle's medium (DMEM; Gibco-BRL, Gaithersburg, MD). Individual cells were suspended in DMEM supplemented with 10% (v/v) fetal bovine serum (FBS), 50 g/ml streptomycin, and 50 U/ml penicillin, and plated on culture dishes at a density of 5×10^4 cells/cm^2. After 3 days in culture, cells were treated with various pharmacological reagents, including IL-1 (Calbiochem, La Jolla, CA) and MMP-12. MMP-12 was obtained from Abcam (Cambridge, MA), which was purified from stimulated human fibroblasts. MMP-12 is a mixture of the zymogen and active enzyme and is free of contaminating MMPs or TIMPs. Mouse rib chondrocytes were prepared from 3-day-old newborn ICR (imprinting control region) mice [Kim et al., 2007]. Briefly, cartilaginous rib cages were pre-incubated for 45 min in 0.2% type II collagenase, and rinsed with PBS. Tissues were further dissociated enzymatically for 4 h in 0.2% type II collagenase. Isolated chondrocytes were maintained in DMEM containing 10% (v/v) FBS, 50 g/ml streptomycin, and 50 U/ml penicillin. For cartilage explants culture, rabbit joint cartilage explants ($\sim$125 mm^3) were cultured in DMEM supplemented with 10% (v/v) FBS in the absence or presence of IL-1 for 72 h [Yoon et al., 2002].

POLYMERASE CHAIN REACTION (PCR)

Total RNA was isolated using TRI reagent (MRC, Cincinnati, OH), and treated with ImProm-IITM reverse transcriptase (Promega, Madison, WI). The cDNA obtained was amplified by PCR with *Taq* polymerase (TaKaRa Bio, Shiga, Japan). The PCR primers and conditions are summarized in Table I. PCR products were sequenced to confirm the specificity of primers. Quantitative real-time PCR (qRT-PCR) was performed using iCycler (Bio-Rad, Hercules, CA) and SYBR Premix Ex Taq^{TM} (TaKaRa Bio). All qRT-PCR reactions were performed in duplicate, and the amplification signal from the target gene normalized against glyceraldehyde-3-phosphate dehydrogenase (GAPDH) in the same reaction.

TABLE I. Oligonucleotide Primers and PCR Conditions

Gene	S/AS[a]	Primer sequence	Size (bp)	A_T[b] (°C)	Origin[c]
Col-II	S	5′-gac ccc atg cag tac atg cg-3′	370	62	Rabbit
(RT-PCR)	AS	5′-agc cgc cat tga tgg tct cc-3′			
Col-II	S	5′-ggg tct cct gcc tcc tcc tgc tc-3′	383	54	Rabbit
(In situ)	AS	5′-ctc cat ctc tgc cac ggg gt-3′			
GAPDH	S	5′-tca cca tct tcc agg agc ga-3′	299	62	Rabbit
(RT-PCR)	AS	5′-cac aat gcc gaa gtg gtc gt-3′			
MMP-1	S	5′-atg gac ctg aag gac agc tc-3′	534	55	Rabbit
(RT-PCR)	AS	5′-cct gca cag tcc agt act tg-3′			
MMP-2	S	5′-ccg tgt gaa gta tgg caa tgc-3′	493	55	Rabbit
(RT-PCR)	AS	5′-gcg gtc atc gtc gta gtt gg-3′			
MMP-3	S	5′-tgt acc cag tct acaacg cc-3′	550	55	Rabbit
(RT-PCR)	AS	5′-tcc agg gac tct ctc ttc tc -3′			
MMP-9	S	5′-cgc cgagat agg gaa caa gc-3′	542	62	Rabbit
(RT-PCR)	AS	5′-ggc agt gca gga tgt caa agc-3′			
MMP-12	S	5′-gga gct cat gga gac tat gg-3′	460	62	Rabbit
(In situ, RT-PCR)	AS	5′-gga cac tgg ttg aac tc-3′			
MMP-13	S	5′-cct aca ccg gca aga gtc ac-3′	396	55	Rabbit
(RT-PCR)	AS	5′-tct tgg gaa tcc cag ttc ag-3′			
MMP-14	S	5′-gcg tac gag agg aag gatt gg-3′	550	55	Rabbit
(RT-PCR)	AS	5′-cca gca cca gga gta gca gc-3′			
MMP-15	S	5′-gta ctg gcg ctt caa cga g-3′	407	55	Human
(RT-PCR)	AS	5′-cca cct cct cca tct gca c-3′			

[a]S, sense; AS, antisense.
[b]Annealing temperature.
[c]Origin, primers are designed from known sequence of indicated species.

IN SITU HYBRIDIZATION

Digoxigenin-conjugated riboprobes for collagen type II and MMP-12 were synthesized using a digoxigenin RNA labeling mix kit (Roche Diagnostics Corp., Indianapolis, IN). Briefly, the cDNA fragments of MMP-12 and collagen type II were amplified by PCR using specific primers. The PCR primers and conditions are summarized in Table I. MMP-12 and collagen type II cDNA were inserted into pGEM-T easy vector (Promega). The plasmids were digested with *Nco*I or *Sal*I for linearization, and transcribed with SP6 or T7 RNA polymerase to generate antisense and sense probes. Cartilage explants were fixed with 4% paraformaldehyde, and sectioned after embedding in paraffin. Next, sections were deparaffinized in xylene, washed, and hydrated using ethanol gradients. For hybridization, the deparaffinized sections of cartilage explants were treated with 0.2 N HCl for 10 min, and permeabilized for 10 min at 37°C with 20 g/ml proteinase K. After acetylation for 10 min with 0.25% acetic anhydride, sections were incubated in hybridization buffer (40% formamide, 10% dextran sulfate, 1× Denhardt's solution, 4× SSC, 10 M dithiothreitol, 1 mg/ml yeast tRNA, and 1 mg/ml salmon sperm DNA) containing denatured sense or antisense digoxigenin-labeled riboprobes. Sections were treated for 30 min at 37°C with 10 mg/ml RNase A, and processed using an anti-digoxigenin detection assay kit (Roche Diagnostics Corp.). Hybridization signals were visualized with a solution of 4-nitro blue tetrazolium chloride and 5-bromo-4-chloro-3-iodolyl-phosphate (Roche Diagnostics Corp.).

WESTERN BLOT ANALYSIS

Whole cell lysates were prepared, as described previously [Yoon et al., 2002; Kim et al., 2007]. Proteins were size-fractionated by sodium dodecyl sulfate–polyacrylamide gel electrophoresis, transferred to nitrocellulose membrane, and the nitrocellulose sheet was blocked with 5% non-fat dry milk. The following antibodies were used to detect proteins: anti-extracellular signal-regulated kinase (ERK) (BD Biosciences, San Jose, CA), anti-phospho-ERK (New England Biolabs, Beverly, MA), anti-phospho-c-Jun N-terminal kinase (JNK), anti-JNK, anti-p38 kinase (Santa Cruz Biotechnology Inc., Santa Cruz, CA), and anti-phospho-p38 kinase (Cell Signaling Technology, Beverly, MA). Blots were developed with a peroxidase-conjugated secondary antibody and an enhanced chemiluminescence system. To evaluate MMP-12 secretion, conditioned medium (400 l) was concentrated to 20 l with a Centricon-30 miniconcentrator (Amicon, Bedford, MA), and fractionated by electrophoresis. An anti-MMP-12 antibody (R&D system, Minneapolis, MN) was employed to detect the secreted protein. The molecular weight was confirmed by running purified MMP-12 protein. The relevant band intensities were quantified by densitometric analysis.

GELATIN ZYMOGRAPHY

Conditioned medium (20 l) harvested from rabbit articular chondrocyte cultures under serum-free conditions was size-fractioned by electrophoresis on a 10% sodium dodecyl sulfate–polyacrylamide gel containing 1 mg/ml gelatin. Sodium dodecyl sulfate was removed from gels by performing two washes with 2.5% Triton X-100 for 1 h. The gel was incubated for 14 h at 37°C in buffer (50 mM Tris pH 7.5, 50 mM NaCl, and 10 mM $CaCl_2$), stained with 0.25% Coomassie brilliant blue R-250 for 30 min, and de-stained until gelatinolytic bands were clearly visible. The relevant optical densities were quantified by densitometric analysis.

REPORTER GENE ASSAY

Promoter sequences of rabbit MMP-9 and MMP-12 are not known. We, therefore, used mouse and human promoter sequences to construct reporter gene for MMP-9 and MMP-12, respectively, although gene regulation and promoter sequences may be different in different species. Briefly, the MMP-9 promoter (−1329/+19) was amplified from mouse genomic DNA. Terminal *Kpn*I and *Xho*I restriction sites were added using the primers MMP-9-5′*Kpn*I (5′-cgg ggt acc gag agt ttt gta gag agc gta tca c-3′) and MMP-9-3′*Xho*I (5′-ccg ctc gag ggt gag gac cgc agc ttc tgg-3′) from the known mouse sequence (NCBI Accession number AF403768). The MMP-12 promoter (−1433/+43) was amplified from human genomic DNA. Terminal *Xho*I and *Hin*dIII restriction enzyme sites were added using primers MMP-12-5′*Xho*I (5′-ccg ctc gag cgg agt agc ctg taa tc-3′) and MMP-12-3′*Hin*dIII (5′-ccc aag ctt ggg taa act tct aaa cgg atc-3′) from the known human sequence (NCBI Accession number NT033899). Amplified products were inserted into pGL3-Basic (Promega). After pretreatment with hyaluronidase (4 U/ml) for 6 h under serum-free conditions, chondrocytes transfected with pGL3-Basic reporter plasmid (1 g) and -galactosidase vector (0.1 g) using Metafectene™ (Biontex, Martinsried, Germany). Transfected cells were cultured for 24 h, prior to further experiments. Luciferase activity was normalized for transfection efficiency using -galactosidase.

RESULTS

IL-1β TRIGGERS MMP-12 EXPRESSION IN PRIMARY CULTURE CHONDROCYTES AND CARTILAGE EXPLANTS

Initially, we examined the effects of IL-1 on MMP expression in primary culture articular chondrocytes. RT-PCR analyses showed that IL-1 induces the expression of MMP-1, -3, -9, -12, and -13, but not MMP-2, -14, or -15 (Fig. 1A). Because MMP-12 induction by IL-1 in articular chondrocytes has not been extensively analyzed, we focused our effort on the characterization of MMP-12 expression. qRT-PCR (Fig. 1B) analyses showed that IL-1 induced MMP-12 expression in a time- and dose-dependent manner. Expression was further confirmed with the reporter gene containing the human MMP-12 promoter (encompassing positions −1433 to +43 bp from the transcription start site). IL-1 enhanced MMP-12 promoter activity in chondrocytes as early as 1 h after treatment, with the highest activity observed at 3 h (Fig. 1C). We additionally examined MMP-12 expression in cartilage explants, which mimics more in vivo-like conditions. As shown in Figure 2, IL-1 induced MMP-12 expression in chondrocytes of cartilage explants, with a concomitant decrease in collagen type II transcript levels.

IL-1β INDUCES SECRETION AND ACTIVATION OF MMP-12 VIA MAP KINASE PATHWAYS

MMPs are activated upon proteolytic cleavage of the secreted inactive proforms. MMP-12 secretion and activation were examined

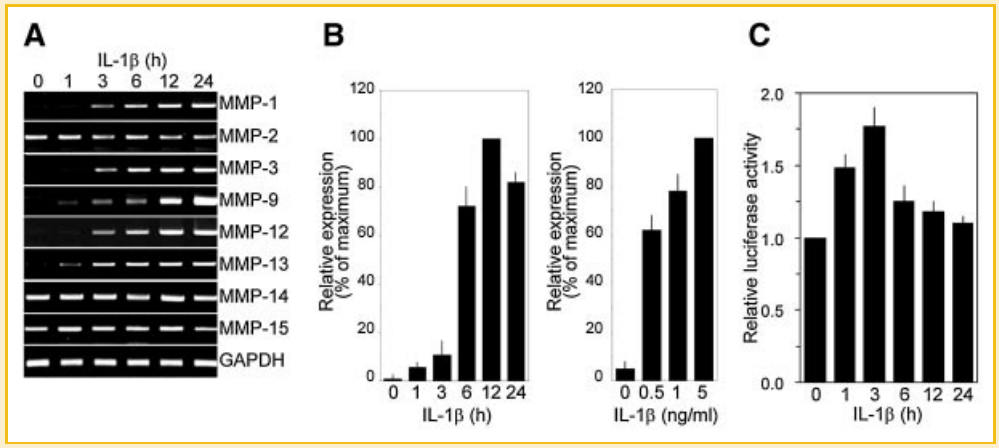

Fig. 1. IL-1 induces MMP-12 expression in primary culture articular chondrocytes. A: Rabbit articular chondrocytes were treated with 5 ng/ml of IL-1 for the indicated time period, and MMP expression was determined by RT-PCR. B: Rabbit chondrocytes were treated with 5 ng/ml of IL-1 for the indicated periods or the specified amounts of IL-1 for 24 h. The MMP-12 level was quantified with qRT-PCR (B). C: Rabbit chondrocytes were transfected with pGL3-basic reporter plasmid containing the MMP-12 reporter gene. Transfected cells were treated with 5 ng/ml of IL-1 for the indicated periods. MMP-12 promoter activity was determined with the luciferase assay. The data represent a typical result (A) and average values with standard deviation (B and C) from at least six independent experiments.

by Western blotting using conditioned media. Both pro- and active MMP-12 was visible in the culture media of IL-1-treated chondrocytes (Fig. 3A). Densitometric analyses disclosed an increase in pro-MMP-12 as early as 12 h after IL-1 treatment with a maximal peak at 36 h, whereas active MMP-12 began to appear slowly (Fig. 3B).

Since MAP kinase mediates IL-1 effects in chondrocytes, we further examined the signaling pathway of MMP-12 expression, focusing on MAP kinase. MAP kinase subtypes ERK, p38 MAP kinase, and JNK were activated by IL-1, as determined from the phosphorylation status (Fig. 4A). Specific inhibitors were employed to establish the precise involvement of the MAP kinase signaling pathway. Primary culture rabbit articular chondrocytes were pre-incubated for 30 min with PD98059 (to inhibit ERK), SB202190 (to inhibit p38 MAP kinase) or SP600125 (to inhibit JNK), and exposed to IL-1 (5 ng/ml) for 24 h for the analysis of mRNA expression or 72 h for analyzing secretion and activation. RT-PCR and Western blotting data revealed that inhibition of ERK or p38 MAP kinase blocked IL-1-induced expression, secretion, and activation of MMP-12. In contrast, blockage of JNK did not affect MMP-12 expression or secretion, but significantly inhibited MMP-12 activation (Fig. 4B). Our results clearly indicate that all three MAP kinase subtypes are involved in expression and/or activation of MMP-12 in chondrocytes.

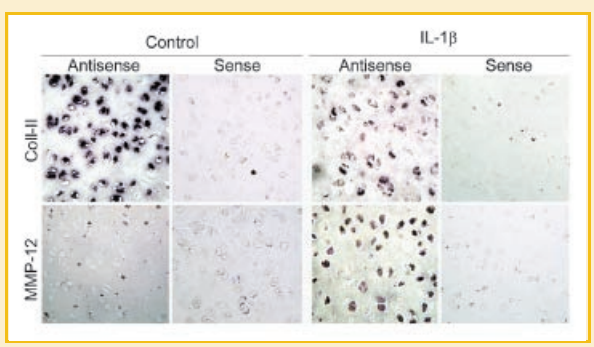

Fig. 2. IL-1 causes MMP-12 expression in cartilage explants. Rabbit cartilage explants were left untreated as a control or treated with 10 ng/ml of IL-1 for 72 h (for collagen type II) or 36 h (for MMP-12). Explants were sectioned, and transcripts of collagen type II (Col-II) and MMP-12 were detected using in situ hybridization. The data represent a typical result from three independent experiments.

MMP-12 ENHANCES MMP-9 EXPRESSION AND SECRETION IN IL-1β-TREATED CHONDROCYTES

The possibility that MMP-12 acts as a modulator of other MMP levels was examined in chondrocytes by using exogenous MMP-12. Purified MMP-12 (up to 50 ng/ml) did not affect chondrocyte morphology and viability. However, 100 ng/ml of MMP-12 caused cell shrinkage and death (data not shown). We, therefore, used low concentration of MMP-12 (below 50 ng/ml) in this study. RT-PCR analyses indicate that MMP-12 alone does not affect the expression of other MMPs. However, MMP-12 enhanced MMP-9 expression in the presence of IL-1 (Fig. 5A). qRT-PCR analyses demonstrate that MMP-12 induces MMP-9 expression in a dose-dependent manner (Fig. 5B). The stimulatory effects of MMP-12 appear not due to any possible contaminating components because MMP-12 alone did not affect MMP-9 expression. Induction of MMP-9 by MMP-12 was further confirmed by using a reporter gene containing the mouse MMP-9 promoter, including the region encompassing −1329 to +19 bp from the transcription start site. Similar to the mRNA expression pattern, purified MMP-12 alone did not affect MMP-9 promoter activity (data not shown). However, MMP-12 enhanced MMP-9 promoter activity in IL-1-treated chondrocytes in a dose-dependent manner (Fig. 5C). Using gelatin zymography, we further investigated whether MMP-12 affects the secretion and activation of MMP-9. MMP-12 enhanced MMP-9 secretion in IL-1-treated chondrocytes, but had no effects on MMP-2 secretion (Fig. 6).

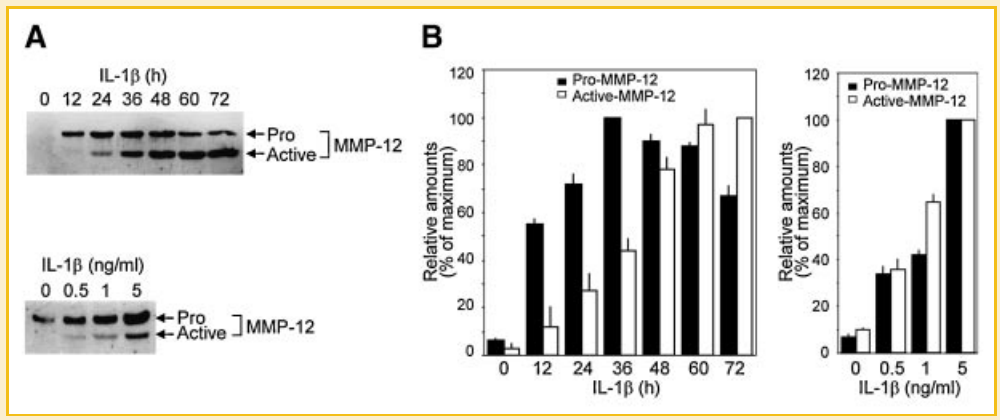

Fig. 3. Secretion and activation of MMP-12 by IL-1. A: Rabbit articular chondrocytes were treated with 5 ng/ml of IL-1 for the indicated periods or 72 h with the specified amounts of IL-1. Levels of both pro- and active MMP-12 secreted into conditioned medium were determined by Western blotting. B: The relative amounts of MMP-12 were quantified by densitometric measurement of pro- and active protein. The data represent a typical result (A) and average values with standard deviations (B) from six independent experiments.

Again, the stimulatory effects of MMP-12 on MMP-9 secretion were dose-dependent.

DISCUSSION

MMP-12, initially identified as an elastolytic metalloproteinase secreted by inflammatory macrophages [Banda and Werb, 1981; Shapiro et al., 1993], regulates a number of cellular processes, such as the lung disease emphysema [Hautamaki et al., 1997; Morris et al., 2003]. However, the expression patterns and functions of MMP-12 in chondrocytes and cartilage tissue are largely unknown. Here, we characterize the expression mechanism and function of MMP-12 in primary culture chondrocytes and cartilage explants.

IL-1 induced similar MMP-12 expression patterns in chondrocytes relative to other known MMPs, including MMP-1, -3, and -13 [Tetlow et al., 2001]. MMP-12 expression was demonstrated by PCR analysis and reporter gene assay. IL-1 also caused secretion and proteolytic cleavage of the secreted inactive form of MMP-12, as demonstrated by Western blot analysis. Although IL-1 caused dramatic increase of MMP-12 mRNA level, MMP-12 promoter reporter gene activity is increased less than twofold in our experiments. One possibility of the relative discrepancy between transcript level and promoter activity is the size of promoter. For

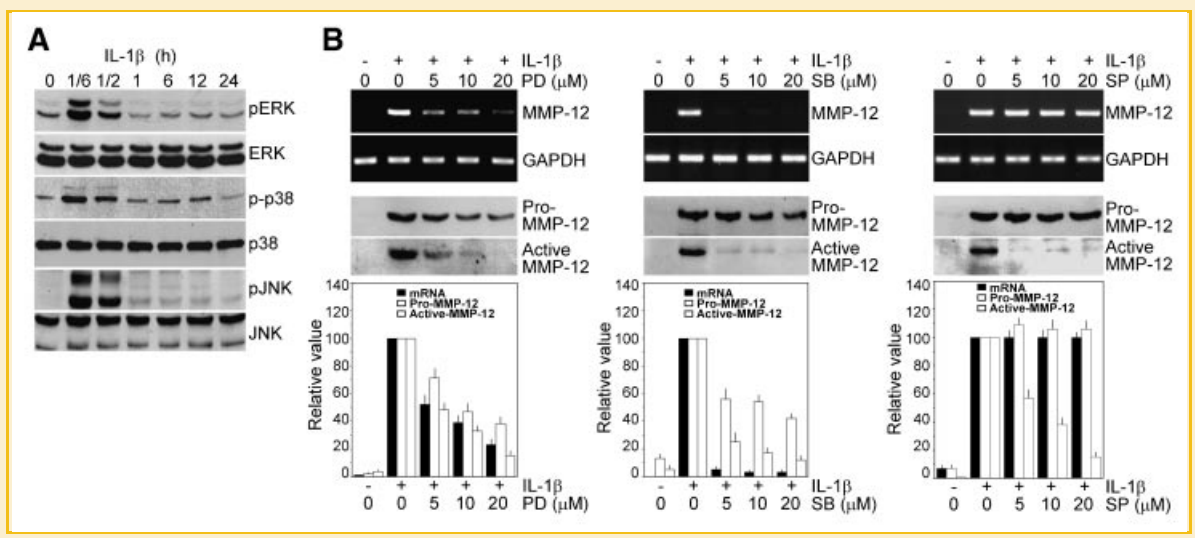

Fig. 4. MAP kinases mediate IL-1-induced expression and activation of MMP-12. A: Rabbit articular chondrocytes were treated with 5 ng/ml of IL-1 for the indicated times. Levels of phosphorylated ERK, p38 kinase, and JNK were determined by Western blotting. B: Rabbit chondrocytes were pre-treated for 30 min with vehicle alone or the specified concentrations of PD98059 (PD) to inhibit ERK, SB202190 (SB) to inhibit p38 kinase, or SP600125 (SP) to inhibit JNK. Cells were exposed to 5 ng/ml of IL-1 for 24 h (for RT-PCR) or 72 h (for Western blotting) in the presence of the inhibitors. MMP-12 mRNA was detected by RT-PCR. Levels of both pro- and active MMP-12 were determined by Western blotting. Relative levels of MMP-12 mRNA and protein (pro- and active forms) were quantified with qRT-PCR and densitometry analyses, respectively. The data represent typical results or average values with standard deviations from six independent experiments.

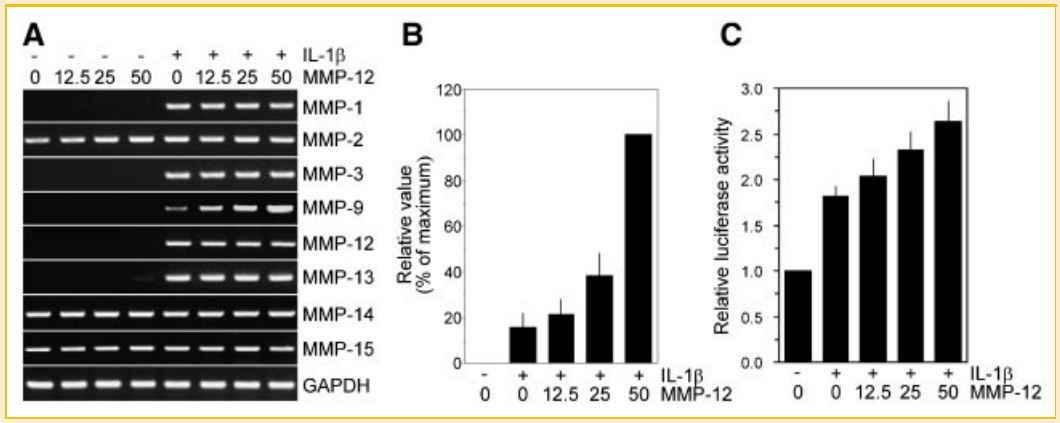

Fig. 5. MMP-12 regulates MMP-9 expression in articular chondrocytes. A,B: Rabbit articular chondrocytes were treated with the indicated amounts of exogenous MMP-12 protein for 36 h with or without 5 ng/ml of IL-1 in serum-free condition. Expression of the indicated MMPs was determined with RT-PCR (A). MMP-9 mRNA levels were quantified using qRT-PCR (B). C: Mouse rib chondrocytes were transfected with pGL3-basic reporter plasmids containing the MMP-9 reporter gene. Transfected cells were treated with the indicated amounts of exogenous MMP-12 for 36 h in the absence or presence of 5 ng/ml of IL-1. MMP-9 promoter activity was determined with the luciferase assay. The data represent a typical result (A) and average values with standard deviations (B and C) from six independent experiments.

instance, the human MMP-12 promoter we used (−1433/+43) may not sufficient to contain all cis-elements, which respond to IL-1. Indeed, IL-1 did not induce human MMP-12 promoter (−1047/+42) activity in the U937 cells [Wu et al., 2001]. The other possibility is that IL-1 causes the increase MMP-12 mRNA stability, in addition to the increase of promoter activity. However, no reports are available to indicate the stabilization of MMP-12 mRNA by IL-1.

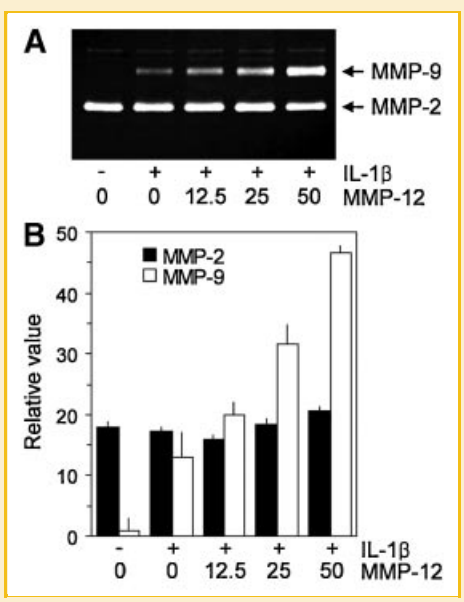

Fig. 6. MMP-12 regulates MMP-9 secretion. A: Rabbit articular chondrocytes were treated with the indicated amounts of purified MMP-12 protein for 36 h with or without 5 ng/ml of IL-1 in serum-free condition. MMP-9 secretion was analyzed using gelatin zymography. The data represent a typical result from four independent experiments. B: Relative activities of MMP-2 and -9 were quantified using densitometric analysis. The data represent average values with standard deviations from four independent experiments.

We also found that the MAP kinase subtypes differentially regulate the expression and activation of MMP-12. Among these, both ERK and p38 kinase regulate MMP-12 expression. The levels of secreted and active MMP-12 were additionally decreased upon inhibition of ERK and p38 kinase. However, it is unclear whether this is due to direct inhibition of secretion and activation or indirectly due to suppression of expression. In contrast, JNK regulated MMP-12 activation, but had no effects on expression and secretion. Human MMP-12 promoter contains AP-1 binding site [Karin et al., 1997]. AP-1 is composed of a homodimer of Jun isoforms (c-Jun, JunB, or JunD) or a heterodimer of Jun/Fos isoforms (FosB, c-Fos, Fra-1, or Fra-2). The dimeric complex composed of JunD is important regulatory molecules for MMP-12 promoter activity [Wu et al., 2001, 2003]. Indeed, granulocyte macrophage colony-stimulating factor-induced MMP-12 expression in U937 monocytic cells is mediated by AP-1 binding activity [Wu et al., 2001]. However, IL-1 failed to induce promoter activity and expression of MMP-12 in the same cells although it increased the AP-1 binding of MMP-12 promoter [Wu et al., 2001]. As JNK is a cytosolic kinase and MMP-12 activation occurs after secretion [Shapiro et al., 1993], it is unlikely that JNK directly modulates proteolytic activation of MMP-12. One possibility is that JNK directly phosphorylates pro-MMP-12 prior to its secretion, thereby regulating proteolytic cleavage for activation after secretion. Regulation of MMP-12 expression and activation by MAP kinases are similar to the regulatory mechanisms observed in other cell types, although the specific pathways are different. For instance, MMP-12 expression and activation in airway smooth muscle cells are mediated by ERK and JNK, but not p38 kinase [Xie et al., 2005].

In addition to its role in the degradation of matrix molecules, MMP-12 enhances IL-1-induced MMP-9 expression and secretion in chondrocytes, as evident from transcript levels and the reporter gene assay. Although the regulatory mechanism was not investigated in

detail, our data are consistent with the observation that MMPs have other functions in addition to degradation of ECM components. For example, MMP-1, -2, -3, -7, -9, and -11 degrade non-matrix substrates, such as growth factors, growth factor-binding proteins, pro-IL-1, pro-tumor necrosis factor, and other MMPs [McCawley and Matrisian, 2001]. Furthermore, MMP-2 is translocated into the cell nucleus and degrades nuclear matrix protein poly (ADP-ribose) polymerase [Kwan et al., 2004]. MMP-3 localizes to the cell nucleus and induces apoptosis via catalytic activity [Si-Tayeb et al., 2006]. Recently, Eguchi et al. [2008] reported a transcription factor-like function of MMP-3. Specifically, MMP-3 regulates connective tissue growth factor expression in the nucleus in human chondrosarcoma HCS-2/8 cells. MMP-12 appears to modulate MMP-9 expression in the nucleus. Interestingly, unlike MMP-2 and -3, MMP-12 does not contain a nuclear localization sequence [Si-Tayeb et al., 2006], although it is possible that the protein is localized in the nucleus in association with other nuclear proteins. Since we used exogenous MMP-12 protein, it is unlikely that MMP-12 directly regulates MMP-9 expression in the nucleus. In addition, purified MMP-12 enhanced MMP-9 expression only in the presence of IL-1. Although the stimulatory mechanisms of MMP-12 were not determined in this study, our current hypothesis is that cellular factors modulated by both exogenous IL-1 and MMP-12 cause transcriptional stimulation of MMP-9 in chondrocytes. Further studies are necessary to clarify the detailed mechanism of MMP-9 induction by MMP-12.

The role of MMP-12 in cartilage destruction is not yet clear. It has been reported that MMP-12 is highly expressed in osteoarthritis and normal cartilage with trends toward decreased expression in osteoarthritic cartilage [Kevorkian et al., 2004; Davidson et al., 2006]. However, many evidences indicate association of MMP-12 with cartilage destruction in arthritis. For instance, MMP-12 degrades several ECM components, including elastin, collagen type IV, fibronectin, gelatin, vitronectin, entactin, PGs, heparan and chondroitin sulfate, and plasminogen [Chandler et al., 1996; Gronski et al., 1997; Hiller et al., 2000]. Degradation of these substrates is associated with arthritic disease [Aigner and McKenna, 2002]. In addition, inflammatory arthritis was enhanced in transgenic rabbit expressing human MMP-12 in the macrophage lineage by increasing macrophage infiltration into synovial tissue [Wang et al., 2004]. Analysis of gene expression profile by microarrays indicated that MMP-12 expression was induced in chondrocytes stimulated by supernatant of synovial fibroblasts derived from a patient with rheumatoid arthritis [Andreas et al., 2008].

In addition to MMP-12, MMP-9 acts on a variety of ECM substrates, such as denatured collagen generated by native collagen types I, II, IV, V, and XI after specific cleavage by collagenase, as well as other substrates, such as aggrecan, elastin, and fibronectin [McCawley and Matrisian, 2001; Van den Steen et al., 2002]. MMP-9 is expressed in human osteoarthritic cartilage [Mohtai et al., 1993; Tsuchiya et al., 1997; Soder et al., 2006], implying possible involvement in arthritis. Thus, our observations suggest a possibility that MMP-12 induction by IL-1 is associated with the regulation of cartilage ECM homeostasis due to the actions of both MMP-12 and MMP-9, which is upregulated by MMP-12.

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