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ORIGINAL ARTICLE

Hyaluronan inhibits IL-1β-stimulated collagenase production via down-regulation of phosphorylated p38 in SW-1353 human chondrosarcoma cells

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Abstract We investigated the intracellular mechanism for the inhibitory effects of hyaluronan (HA) on interleukin-1β (IL-1β)-stimulated collagenase-1 and -3 (matrix metalloproteinases (MMPs)-1 and -13) production in a human chondrosarcoma cell line, SW-1353. MMPs-1 and -13 were induced by IL-1β at 2 ng/ml in SW-1353 cells for 48 h. HA of 800 kDa, which is used clinically, significantly suppressed IL-1β-stimulated production of MMPs-1 and -13 by immunoblotting. SW-1353 cells express the standard form of CD44 (CD44H), and immunofluorescent cytochemistry demonstrated the association of HA with CD44 on SW-1353 cells. Phosphorylated p38 (Phos-p38) mitogen-activated protein kinase was stimulated in SW-1353 cells by IL-1β but not by HA alone. SB203580, a p38 MAPK inhibitor, partially blocked the MMP-1 and -13 production stimulated by IL-1β. 800-kDa HA suppressed IL-1β-activated Phos-p38 in a dose-dependent manner. CD44 blocking significantly reversed the inhibitory effects of HA on IL-1β-activated Phos-p38 production. The present study clearly suggests that HA binds CD44 and inhibits IL-1β-induced MMP-1 and -13 expression via down-regulation of Phos-p38 in SW-1353 cells.

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Introduction

Hyaluronan (HA) is a major component of synovial fluid and the cartilage matrix and plays a central role in joint lubrication. Intraarticular injection of HA exerts a positive effect in reducing the progression of joint destruction in osteoarthritis (OA) [1]. However, the level at which the drug acts remains unclear. Our recent study showed that HA can penetrate human articular cartilage and can inhibit interleukin-1β (IL-1β)-stimulated matrix metalloproteinase (MMP) production in vitro through the interaction between HA and CD44 [2], the principal cell surface receptor for HA [3]. At present, the downstream effects of HA on IL-1β-stimulated MMPs in chondrocytes remain to be identified.

MMPs consist of a family of zinc-dependent enzymes that mediate the turnover of extracellular matrix proteins. Up-regulation of MMPs has been implicated in numerous pathologic processes, including OA and rheumatoid arthritis (RA). Among the MMPs, collagenases are particularly important because of their ability to cleave fibrillar collagen, which is the most abundant component of the extracellular matrix [4]. MMP-1 (collagenase 1) is expressed ubiquitously and is found in various cells, including chondrocytes [5], while MMP-8 (collagenase 2) is expressed mainly in neutrophils and may be expressed in OA chondrocytes [5]. MMP-13 (collagenase 3) exhibits the highest activity against type II collagen, which is the predominant form of collagen in cartilages [6]. IL-1β contributes to the elevated expression of MMPs by chondrocytes in vitro [2] and in vivo [7].

IL-1β is known to activate the mitogen-activated protein kinase (MAPK) family including p38 MAPK (p38), c-Jun N-terminal kinase (JNK), and the extracellular signal-regulated kinase (ERK) in human chondrocytes [8, 9]. The most relevant MAPKs for the expression of IL-1β-induced catabolic mediators in arthritis appear to be p38 and JNK because these kinases are involved in the activation of activator protein-1 (AP-1) [10–12], which plays a central role in the regulation of gene expression for MMP-1 [13] and MMP-13 [14].

In response to IL-1β, a human chondrosarcoma cell line, SW-1353, has been demonstrated to serve as a model that is compatible with primary chondrocytes in OA [10]. Previous studies have shown that the three MAPKs—p38, ERK, and JNK—individually play a role in collagenase induction by IL-1β-stimulated SW-1353 cells. Of the MAPKs, p38 is reportedly the only kinase that contributes to the induction of both MMP-1 and MMP-13 [10]. This study aimed to clarify intracellular signaling effects through the interaction between HA and CD44 on collagenase production in IL-1β-stimulated SW-1353 cells. We showed here that HA inhibition of IL-1β-induced MMPs-1 and -13 involve p38 down-regulation via CD44.

Materials and methods

Reagents

Hyaluronan (HA) of 800 kDa, the form clinically used for the treatment of OA in Japan, 5-aminofluorescein-labeled HA of 900 kDa (5-AF-HA), and fluorescein isothiocyanate (FITC)-conjugated OS/37 anti-CD44 antibody were obtained from Seikagaku Co. (Tokyo, Japan). Recombinant human IL-1β was purchased from R&D Systems (Minneapolis, MN, USA). Anti-human MMP-1 that reacts with the 53 and 51 kDa proenzyme (M4177) and anti-human MMP-13 that recognizes the latent proenzyme (60 kDa) (M4052) were obtained from Sigma (St. Louis, MO, USA). Anti-human p38 MAPK antibody (#9212) and anti-human phospho-p38 MAPK antibody (#9211) were purchased from Cell Signaling Technology (Beverly, MA, USA). Alkaline phosphatase (ALP)-conjugated goat anti-rabbit IgG was purchased from Southern Biotechnology Associates, Inc. (Birmingham, AL, USA). SB203580 was purchased from Calbiochem (Darmstadt, Germany). Anti-CD44 antibody IM7 (no azide/low endotoxin) (#553130) and isotype-matched control Rat IgG2b (#559478) were obtained from Fujisawa Pharmaceuticals (Tokyo, Japan).

Cell culture

Human SW-1353 chondrosarcoma cells (American Type Culture Collection, Rockville, MD, USA) were cultured in Dulbecco's modified Eagle's medium (DMEM) containing 100 units/ml penicillin, 100 μg/ml streptomycin, 10 mM HEPES (all from GIBCO BRL, Grand Island, NY, USA), 3.7 g/l $NaHCO_3$ (Wako Pure Chemical Industries, Ltd, Osaka, Japan) and 10% fetal bovine serum (FBS; ICN, Aurora, OH, USA) in six-well plates (Corning, NY, USA) at 37 °C in a humidified 5% CO_2 atmosphere. At confluence, cells were washed with phosphate-buffered saline (PBS; pH 7.4) twice and pre-cultured for 24 h in 2 ml of serum-free DMEM and then washed with PBS twice. Confluent cells were treated with or without IL-1β at 2 ng/ml in the absence or presence of increasing concentrations of 800-kDa HA (0.1, 1, 2, or 4 mg/ml) for 48 h or 30 min. In another set of experiments, the cells were stimulated with IL-1β at 2 ng/ml for 0, 5, 15, 30, 45, 60 or 120 min. In some experiments, after preincubation with SB203580 and/or 800-kDa HA for 1 h, the cells were incubated with IL-1β at 2 ng/ml for 48 h or 30 min. In other experiments, cells were preincubated with anti-CD44 antibody or nonspecific control IgG for 1 h before the treatment with 800-kDa HA for 1 h and/or IL-1β for 30 min. While conditioned media were collected, cell extracts were prepared either by adding 200 μl of 2 × SDS loading buffer to each well of a confluent six-well plate [10], or were prepared as previously described [15]. The samples were stored at −80 °C until further experiments.

Immunoblot analysis

Immunoblot analyses were performed as described previously [2]. Briefly, conditioned media or cell lysates were heated with SDS-PAGE sample buffer (0.125 M Tris-HCl, pH 6.8, 10% 2-mercaptoethanol, 4% SDS, 10% sucrose, 0.004% bromophenol blue) at 80 °C for 20 min under reducing conditions. To analyze CD44 levels, cell lysates were heated with SDS-PAGE sample buffer under nonreducing conditions. The loaded amounts of conditioned media or cell lysates were standardized, based on DNA content and protein content, respectively. Proteins were separated by 10% SDS-PAGE and then transferred onto nitrocellulose membranes (Schleicher & Schuell, Dassel, Germany). Some of the membranes were blocked in PBS or Tris-buffered saline containing 5% non-fat dry milk and 0.1% Tween 20 at room temperature for 1–2 h, followed by incubation with the first antibody at 4 °C for overnight. After incubation with ALP-conjugated second antibody (dilution 1:1,000) at room temperature for 2 h, immuno-

reactive bands were visualized with 5-bromo-4-chloro-3-indolyl phosphate and nitroblue tetrazolium.

Evaluation of HA binding to CD44 by immunofluorescence cytochemistry

HA binding to CD44 was evaluated by immunofluorescence cytochemistry as described previously [16]. Briefly, SW-1353 cells were cultured on collagen-coated cover glass slips (Asahi Techno Glass, Chiba, Japan) in 24-well plates (Corning) with DMEM containing 10% FBS overnight at 37 °C. After blocking with 1% bovine serum albumin (BSA) in PBS for 1 h, the cells were treated with 2.5 μg/ml of FITC-anti-CD44 antibody or 2.5 μg/ml of subclass-matched FITC-conjugated mouse IgG1 (R&D Systems) overnight at 37 °C to investigate the expression of CD44 in SW-1353 cells. In another set of experiments, after extensive washing with PBS, the cells were treated with 12.5 μg/ml of 5-AF-HA in DMEM overnight at 37 °C, with or without pretreatment with 100 μg/ml of 800-kDa HA, 2.5 μg/ml anti-CD44 antibody and/or a subclass-matched mouse IgG1 (ICN Biomedicals, Inc.) for 1 h at 37 °C. The cells were fixed with 4% paraformaldehyde in PBS for 30 min. After washing with PBS, the cells were counterstained with propidium iodide (KPL, Gaithersburg, MD, USA). All cover glass slips were mounted in Dako Glycergel on slides, and the signals were evaluated by a confocal microscope (Fluoview, Olympus, Tokyo, Japan).

Assay for DNA

After collection of conditioned media, cultured cells were digested with 0.5 mg/ml proteinase K in 50 mM Tris-HCl (pH 7.5) for 6 h at 37 °C. The DNA content of proteinase K digests was determined as described previously [17]. We found no significant differences in DNA content in SW-1353 cells between any of the treatment groups (data not shown).

Densitometric analysis

The immunoreactive band intensities were measured using the National Institutes of Health Image 1.62 software and were subjected to statistical analysis.

Statistical analysis

All data are expressed as mean ± SD. Data were compared using two factor analysis of variance (ANOVA) and Student's t test. Significant differences were set at $P < 0.05$.

Results

Suppression of collagenase production in IL-1β-stimulated SW-1353 cells by HA

Incubation of SW-1353 cells with 2 ng/ml IL-1β for 48 h resulted in enhanced secretion of MMPs-1 and -13 into the conditioned media, as determined by immunoblot analysis (Fig. 1). When the SW-1353 cells were co-incubated with increasing concentrations of HA for 48 h (0.1, 1 or 2 mg/ml), which is within the range of HA concentration (2–4 mg/ml) in normal synovial fluid [18], collagenase production was inhibited in a dose-dependent manner (Fig. 1).

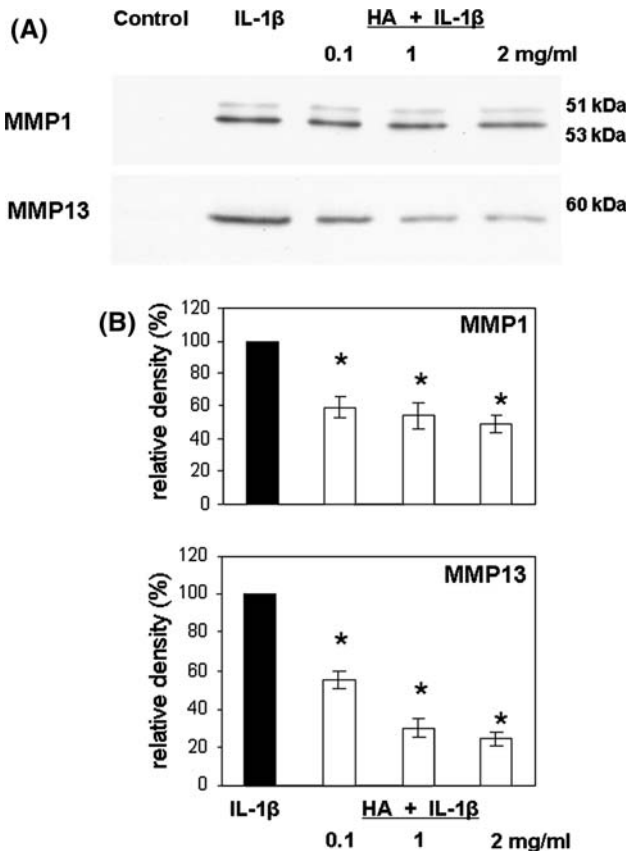

Fig. 1 Inhibitory effects of hyaluronan (HA) on interleukin-1β (IL-1β)-stimulated collagenase production in SW-1353 cell culture. **a** SW-1353 cells were incubated with or without 2 ng/ml IL-1β in the presence or absence of 800-kDa HA at 0.1, 1.0, or 2.0 mg/ml for 48 h. Secreted levels of MMP-1 and MMP-13 in the conditioned media were detected by immunoblotting using specific antibodies. The amount of the sample applied was determined based on the DNA content of the cultured cells. Control cultures contained no additives. **b** Densitometric analysis of four separate experiments in SW-1353 cells. The band intensity of the protein from IL-1β-stimulated SW-1353 cells is defined as 100%. Values are the mean and standard deviation (SD) of four separate experiments. Two-factor analysis of variance (ANOVA) confirmed the significant effects of HA concentrations on IL-1β-stimulated MMP-1 and MMP-13 levels compared with solely IL-1β-treated cultures. * = $P < 0.05$ versus IL-1β-stimulated SW-1353-cells; by Student's t test

HA at 2 mg/ml alone had no effect on MMP levels (data not shown). Compared with IL-1β-induced levels in the absence of HA (calculated as 100%), the levels of MMPs-1 and -13 in IL-1β-treated SW-1353 cell cultures in the presence of 0.1, 1 or 2 mg/ml of HA were 59.3 $\pm$ 6.38 ($P < 0.05$), 53.7 $\pm$ 8.17 ($P < 0.05$), 49.3 $\pm$ 5.08 ($P < 0.05$) and 54.8 $\pm$ 4.51 ($P < 0.05$), 30.1 $\pm$ 5.13 ($P < 0.05$), 24.2 $\pm$ 3.80 ($P < 0.05$), respectively (mean $\pm$ SD; $n = 4$) (Fig. 1b).

Association of HA with SW-1353 cells via CD44

We previously showed that the inhibitory effects of HA on MMP production in IL-1β-stimulated human articular cartilage explant cultures are mediated by CD44 [2], the principal cell surface receptor for HA [3]. Immunofluorescent cytochemistry using FITC-conjugated anti-CD44 antibody revealed that SW-1353 cells constitutively expressed CD44 (Fig. 2a), in contrast to isotype-matched IgG control (Fig. 2b). Preincubation with anti-CD44 antibody partially blocked the association of 5-AF-HA with CD44 (Fig. 2c), in contrast to nonspecific IgG control (Fig. 2d). Furthermore, preincubation with nonconjugated 800-kDa HA at 100 µg/ml canceled the labeling of 12.5 µg/ml 5-AF-HA (Fig. 2e) compared to no preincubation (Fig. 2f). Taken together, these results show that CD44 can mediate the association between HA and SW-1353 cells.

No alteration in CD44 levels by HA

A possible mechanism by which HA inhibits IL-1β-induced collagenase production is through the alteration of CD44 levels during treatment with HA. Immunoblotting of CD44 was carried out using an IM7 anti-CD44 antibody, and a doublet band at approximately 80 kDa was detected (Fig. 3, lane 1), consistent with the major isoform being the standard form of CD44 (CD44H). CD44 levels in IL-1β-treated SW-1353 cells were up-regulated (Fig. 3, lane 3) compared with those in untreated cells. HA alone had no effect on CD44 levels (Fig. 3, lane 2). Based on Figs. 2 and 3 we concluded that SW-1353 cells express the standard

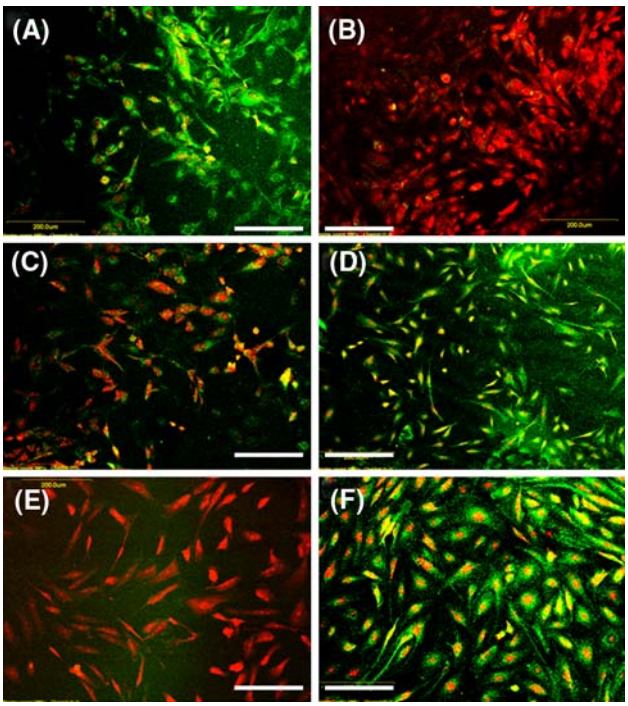

Fig. 2 Association of HA with SW-1353 cells via CD44, as demonstrated by immunofluorescence cytochemistry. Following blocking with 1% bovine serum albumin (BSA) in PBS, SW-1353 cells were incubated with FITC-conjugated anti-CD44 antibody (**a**) or FITC-conjugated nonspecific IgG (**b**). Following pretreatment with anti-CD44 antibody (**c**) or nonspecific IgG (**d**) SW-1353 cells were treated with 5-AF-HA. Alternatively, following preincubation with (**e**) or without (**f**) nonconjugated HA, SW-1353 cells were treated with 5-AF-HA. Results are representative of three separate experiments, all of which yielded similar results. *Bars* 100 µm

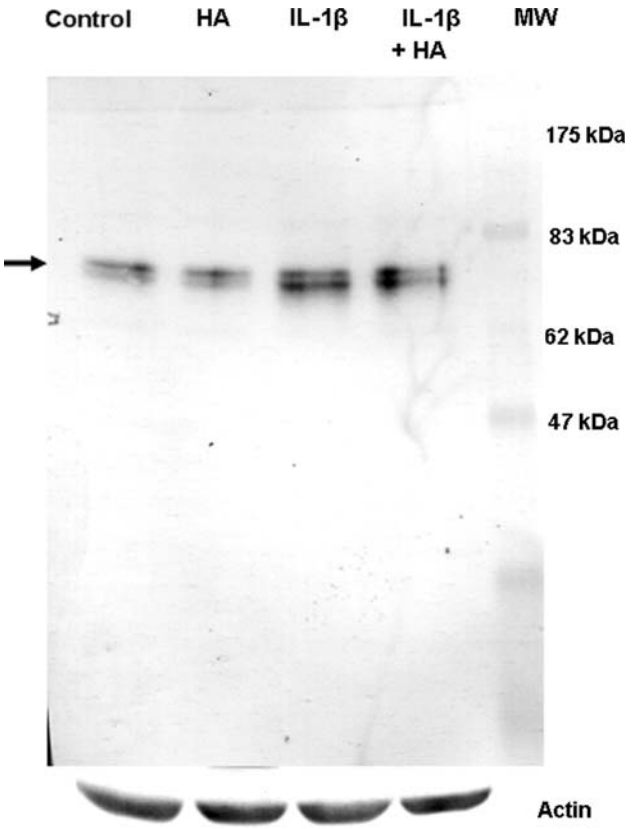

Fig. 3 Effects of HA on IL-1β-stimulated CD44 levels. After serum starvation SW-1353 cells were incubated with or without 2 ng/ml IL-1β in the presence or absence of 1 mg/ml 800-kDa HA for 48 h. Control cultures had no additives. Immunoblot analysis shows that IL-1β stimulated CD44 production, while HA did not have any effect on IL-1β-stimulated CD44 expression

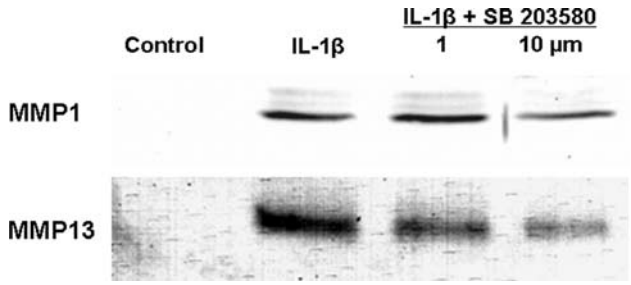

Fig. 4 Requirement of p38 for collagenase production in SW-1353 cell culture. After pretreatment with a p38 inhibitor (SB203580) for 1 h, SW-1353 cells were incubated with 2 ng/ml IL-1β for 48 h. Control cultures had no additives. Secreted levels of MMPs-1 and -13 in conditioned media were analyzed by immunoblotting using specific antibodies. The amount of sample applied was determined based on the DNA content of the cultured cells. Three separate experiments showed similar results

form of CD44 which bind to HA, but 800-kDa HA failed to affect the IL-1β -induced levels of CD44 (Fig. 3, lane 4).

Involvement of p38 down-regulation in HA action on collagenase production by IL-1β

Another possible potent mechanism is an alteration in IL-1β-activated intracellular pathway(s) by HA. IL-1β has been shown to activate MAPKs (ERK, p38, and JNK) and nuclear factor (NF)-κB during collagenase production in SW-1353 cells [8–12]. While MMP-1 induction requires the activation of p38 and ERK, MMP-13 induction requires the activation of p38, JNK, and NF-κB [10]. Thus, p38 contributes to the induction of both collagenases in IL-1β-stimulated SW-1353 cells. This was compatible with the results of our inhibition studies that showed that the p38 inhibitor (SB203580) suppressed the IL-1β-stimulated production of MMPs-1 and -13 (Fig. 4).

In order to examine whether HA affects p38 activation in IL-1β-stimulated SW-1353 cells, the phosphorylation levels of p38 (Phos-p38) were compared between the treatments with IL-1β in the presence or absence of HA. As shown in Fig. 5, IL-1β at 2 ng/ml stimulated Phos-p38 level in a time-dependent manner. The stimulation of Phos-p38 level was induced by 5 min and peaked at 30 min. Thereafter we used 30 min for the induction of phos-p38 for subsequent experiments.

Figure 5 shows that the basal level of Phos-p38 was detectable in SW-1353 cell cultures without IL-1β treatment (Fig. 5). When the SW-1353 cells were pre-incubated with increasing concentrations of 800-kDa HA (1, 2, or 4 mg/ml) for 1 h, followed by stimulation with 2 ng/ml IL-1β for 30 min, the Phos-p38 level was inhibited in a dose-dependent manner (Fig. 6). 800-kDa HA at 1 mg/ml alone had no effect on Phos-p38 levels (Fig. 7). HA at 1 mg/ml was used for subsequent experiments.

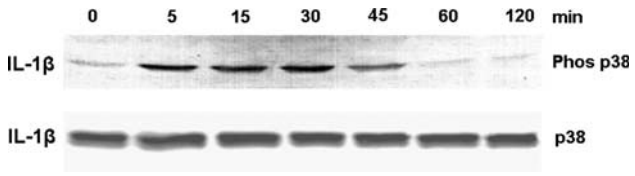

Fig. 5 Phosphorylation of p38 mitogen activated protein kinase (MAPK) in IL-1β-stimulated SW-1353 cells. Confluent cells starved of serum for 24 h were washed with PBS twice and the medium was exchanged for fresh medium containing IL-1β (2 ng/ml). The cells were harvested at the indicated times and lysed with 2 × SDS loading buffer. Aliquots of cell lysates were subjected to immunoblotting using the antibodies that recognize phosphorylated p38 (Phos-p38) or total p38 (p38). Three separate experiments showed similar results

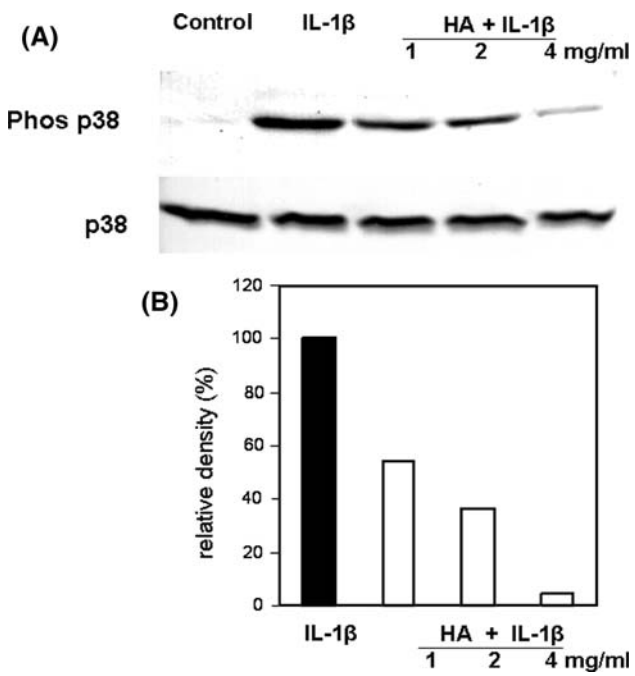

Fig. 6 Dose-dependent effect of HA on IL-1β-stimulated Phos-p38 in SW-1353 cells. **a** Following serum starvation, SW-1353 cells were pretreated with or without 800-kDa HA at increasing concentrations (1, 2 or 4 mg/ml) for 1 h, and the cells were stimulated with or without IL-1β (2 ng/ml) for 30 min. Control cultures contained no additives. Samples of cell lysates were run on SDS-PAGE gels and transferred to membranes. Antibodies to Phos-p38 and p38 were used to visualize those proteins. **b** The graph represents the mean of the band intensity of two separate experiments

Role of HA ligation with CD44 in the inhibition of IL-1β-stimulated p38 phosphorylation in SW-1353 cells

In order to investigate whether the mechanism of HA is biologically mediated by CD44 on SW-1353 cells, the anti-CD44 antibody IM7 was used to block HA binding to SW-1353 cells in monolayer cultures. Pretreatment with 2.5 μg/ml of IM7 for 1 h blocked the inhibitory effect of 1 mg/ml of 800-kDa HA on the IL-1β-stimulated Phos-p38 level, in contrast to the nonspecific IgG control (Fig. 7a).

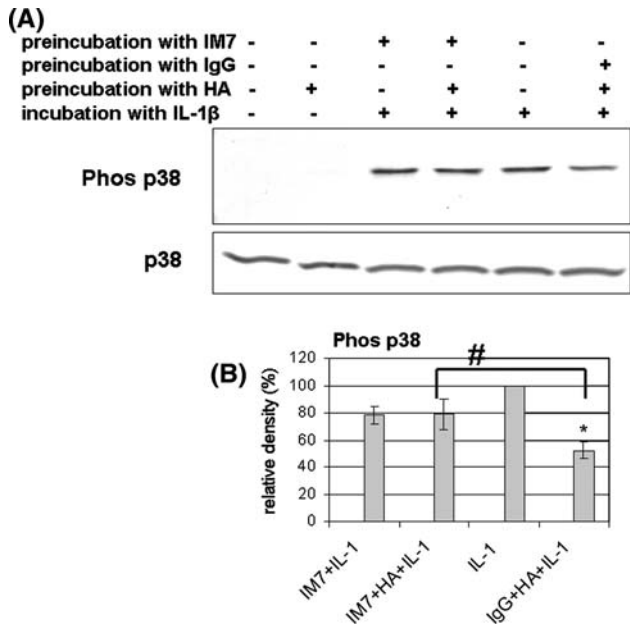

Fig. 7 Effects of the anti-CD44 antibody IM7 on the action of 800-kD HA in IL-1β-stimulated Phos-p38 in SW-1353 cells. **a** Following serum starvation, SW-1353 cells were preincubated with anti-CD44 antibody IM7 (2.5 μg/ml) and isotype-matched IgG (2.5 μg/ml) for 1 h, then treated in the presence and absence of HA (1 mg/ml) for 1 h. After that the cells were stimulated with or without IL-1β at 2 ng/ml for another 30 min. Samples of cell lysates were run on SDS-PAGE gels and transferred to membranes. Antibodies to Phos-p38 and p38 were used to visualize these proteins. **b** Densitometric analysis of four separate experiments in SW-1353 cells. The band intensity of the protein from IL-1β-stimulated SW-1353 cells is defined as 100%. Values are the mean and SD of four separate experiments. * = $P < 0.05$ versus IL-1β-stimulated SW-1353 cells; # = $P < 0.05$, by Student's t test and two-factor ANOVA

A densitometric analysis confirmed that preincubation of IM7 significantly reversed the effects of 800-kDa HA on the Phos-p38 level induced by IL-1β, in contrast to the isotype-matched nonspecific IgG control (Fig. 7b). Compared with 1 mg/ml of 800-kDa HA, 2.5 μg/ml of IM7 caused a weak but not significant suppression of IL-1β-stimulated Phos-p38 in SW-1353 cells (Fig. 7b). Preincubation with nonspecific IgG alone did not cause any effect on the IL-1β-stimulated Phos-p38 level (data not shown).

Discussion

In OA and RA cartilages, proteoglycan loss results in a reduction in cartilage stiffness [19, 20], whereas degradation and loss of type II collagen that involves collagenases (MMPs-1 and -13) result in an irreversible loss of tensile properties and structural integrity [20]. IL-1β and collagenase localize to arthritic joints and are recognized as key mediators of cartilage degradation in arthritis. Thus, HA inhibition of IL-1β-induced collagenases by chondrocytes, which was shown in our previous study [2], decelerates cartilage damage progression in arthritis. This study extends our previous results [2] and demonstrates that 0.1 mg/ml of exogenous 800-kDa HA, which is well below the range of physiologic concentrations (2–4 mg/ml) in synovial fluids [18], can block the IL-1β-stimulated production of MMPs-1 and -13 in SW-1353 cells (Fig. 1). Furthermore, in addition to type II collagen, MMP-13 degrades aggrecan [21], and blocking aggrecanase cleavage in the aggrecan interglobular domain promotes cartilage repair [22]. Thus, HA inhibition of IL-1β-induced collagenases by chondrocytes may potentiate cartilage repair, but this remains to be determined.

The regulation of MMP mRNA expression by IL-1β involves distinct MAPK pathways that lead to the activation of transcription factors including AP-1, with some differences among various MMP genes in chondrocytes [10]. In SW-1353 cells, IL-1β activates all three MAPKs: p38, ERK, and JNK [11]. Of those, p38 regulates both MMP-1 and MMP-13 in response to IL-1β. The inhibitory effects of HA on particular MAPK pathways have never been examined in chondrocytes. This is the first study to show that HA down-regulates IL-1β-stimulated p38 in a dose-dependent manner (Fig. 6). Activation of p38 is required for the expression of a variety of inflammatory genes. P38 has been implicated as contributing to the pathogenesis of arthritis. A previous study shows that suppression of p38 with the specific inhibitor (SB203580) blocks IL-1-mediated collagen breakdown in bovine cartilage explants [23]. In collagen-induced arthritis, the p38 inhibitor suppresses the production of TNFα and IL-6, reduces paw inflammation, and inhibits the formation of joint lesions [24]. Therefore, p38 is one of the main therapeutic targets for arthritis. From the present results, HA introduced exogenously by intraarticular injection may suppress p38 activation in arthritic joints.

The present results using the p38 inhibitor indicate a differential regulation in the production of individual MMPs by IL-1β in SW-1353 cells. Concentrations as high as 10 μM of p38 inhibitor, SB 203580, are required to partially block MMP-1. In contrast, 1 μM of SB 203580 is sufficient to block MMP-13 production by IL-1β in SW-1353 cells (Fig. 4). Consistent with this result, a recent report found that p38 kinase inhibitors strongly inhibited IL-1β-induced MMP-13 expression in a dose-dependent fashion while having a somewhat weaker inhibitory effect on MMP-1 expression in SW-1353 cells [25], suggesting the strong participation of p38 in the induction of MMP-13 by IL-1β-stimulated SW-1353 cells. HA inhibition of IL-1β induced MMP-13 was more potent in contrast to MMP-1 in SW-1353 cells. Based on the findings shown in Fig. 6, dose-dependent decreases in IL-1β-induced Phos-p38 in

response to HA could result in the reduction of the cytokine-stimulated production of collagenases (Fig. 1).

IM7, a monoclonal anti-CD44 antibody, partially blocked the association of HA with SW-1353 cells (Fig. 2b), which express the standard form of CD44 (Fig. 3), indicating that HA associates with SW-1353 cells at least in part via CD44. IL-1β strongly induces CD44 in chondrocytes, as shown in this study (Fig. 3) and others [26]. Further, anti-CD44 treatment reduces joint swelling and leukocyte infiltration in a murine model of arthritis [27]. These data suggest that CD44 may mediate inflammatory processes and joint destruction. However, in this study HA failed to reduce the IL-1β-induced CD44 level in SW-1353 cells (Fig. 3). Another possible mechanism is that the alteration in intracellular signaling through CD44 may involve HA inhibition of IL-1β action. This notion may be supported by recent reports [28, 29], in line with our study [2], suggesting that HA acts as a signaling molecule in the down-regulation of MMPs.

In order to investigate whether the mechanism of HA action is biologically mediated by CD44 in SW-1353 cells, we performed HA-binding inhibition studies using the anti-CD44 antibody. Treatment with IM7 resulted in a significant reduction in the inhibitory effects of HA on Phos-p38 production in IL-1β-stimulated SW-1353 cells (Fig. 7). The interaction between HA and CD44 has been shown to reduce IL-1β-induced MMP-1, MMP-3 and MMP-13 production in normal and OA explant cartilage cultures [2] and MMP-1 in chondrocyte monolayer cultures [30], all of which indicate the direct involvement of CD44 in the mechanism of HA action in chondrocytes. In addition to chondrocytes, HA down-regulates both IL-1β- and TNFα-induced MMP-1 in rheumatoid synovial fibroblast [16] and also IL-1β-induced aggrecanase, TNFα, IL-8 and iNOS in human fibroblast-like synoviocytes (FLS) [31] via CD44, which indicate prevailing beneficial effects of HA in arthritis.

On the other hand, degradation products of the extracellular matrices including fibronectin fragments are of interest as amplifiers or catalysts in diseased joints (recently reviewed by Yasuda [32]). Native HA exists as a high molecular weight (HMW) polymer, but during inflammation, lower molecular weight (LMW) fragments accumulate which have biological activities. HA fragments can induce MMP-13 in articular chondrocytes by the activation of NF-κB and p38 through both CD44-dependent and -independent pathways [29], whereas HMW-HA did not (Ohno [29] and Figs. 6, 7). Emerging data suggest that HMW-HA has the potential to suppress the signaling activated by LMW-HA fragments in chondrocytes [28]. Further, a recent study shows that HA suppresses prostaglandin E2, IL-6 and MMP-3 in lipopolysaccharide-challenged equine FLS regardless of the molecular weight of HA [33], which shows the therapeutic benefits of treatment with HA regardless of molecular weight.

A decrease in the association of HA with SW-1353 cells by anti-CD44 antibody treatment (Fig. 2) indicates that HA associated with SW-1353 cells via CD44, and that ligation of CD44 with HA could stimulate some intracellular pathways that suppress p38 activation. Overall, the present study suggests the specific role of CD44 and p38 on chondrocytic cells in the inhibitory action of HA on IL-1β-induced collagenase production. A thorough understanding of HA-induced intracellular events that affect p38 or another pathway requires further study and may be helpful for the prevention of cartilage degradation.

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