Mod Rheumatol (2007) 17:391–397
DOI 10.1007/s10165-007-0606-7

ORIGINAL ARTICLE

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Inhibition of nuclear factor-κB by hyaluronan in rheumatoid chondrocytes stimulated with COOH-terminal heparin-binding fibronectin fragment

Received: March 6, 2007 / Accepted: June 1, 2007

Abstract The aim of this study was to examine the inhibitory effect of high molecular weight hyaluronan (HA) on nuclear factor (NF)-κB activation by COOH-terminal heparin-binding fibronectin fragment (HBFN-f) in rheumatoid arthritis (RA) chondrocytes. When RA chondrocytes in monolayer or cartilage explants were cultured with HBFN-f, the fragment stimulated the phosphorylation and nuclear translocation of NF-κB, leading to nitric oxide (NO) production in association with inducible form of NO synthase (iNOS) up-regulation. Inhibition studies with NF-κB inhibitors indicated the requirement of NF-κB for HBFN-f-induced NO production. Pretreatment with 2700 kDa HA resulted in significant suppression of NF-κB activation by HBFN-f. HA also inhibited HBFN-f-stimulated NO production with down-regulation of iNOS. The present study clearly demonstrated that high molecular weight HA suppressed HBFN-f-activated NF-κB in RA chondrocytes. HA could down-regulate the catabolic action of fibronectin fragments like HBFN-f in RA joints as a potent NF-κB inhibitor.

Key words Fibronectin fragment · Hyaluronan · NF-κB · Nitric oxide · Rheumatoid arthritis

Introduction

Elevated levels of fibronectin (FN) are found in osteoarthritis (OA) cartilage[1] and in both synovial fluid and plasma of OA and rheumatoid arthritis (RA).[2,3] Fibronectin fragments (FN-fs) are generated by proteolysis of native FN.[1] Increased FN-fs are thought to be involved in cartilage destruction in OA and RA through their catabolic activities. Of FN-fs, we have previously shown that 40 kDa carboxyl (COOH)-terminal heparin-binding fibronectin fragment (HBFN-f) containing both the III12-14 and IIICS domains can stimulate nitric oxide (NO) production in RA articular cartilage explant culture.[4]

Nitric oxide is a short-lived free radical that is synthesized enzymatically from L-arginine by a family of NO synthase (NOS) isoenzymes.[5] NO is produced by a variety of cells including chondrocytes. Inducible NOS (iNOS) is expressed in response to bacterial endotoxin and proinflammatory cytokines such as interleukin-1 (IL-1). Once synthesized, iNOS generates large amounts of NO. iNOS is strongly expressed in synovium and cartilage of patients with inflammatory joint diseases.[6] The promoter for human iNOS contains activator protein-1 (AP-1) recognition sites and nuclear factor (NF)-κB response elements.[7] There is evidence that AP-1 and NF-κB are both involved in cytokine-mediated induction of the human iNOS gene.[8]

AP-1 can be activated by protein kinases that phosphorylate specific amino acid residues, especially by mitogen-activated protein kinases (MAPKs).[9] Three major MAPK families have been identified: extracellular signal-regulated kinase (ERK), p38 MAPK, and c-Jun amino (NH_2)-terminal kinase (JNK). NH_2-terminal heparin-binding and central cell-binding FN-fs can stimulate the activation of ERK, p38, and JNK in human chondrocyte monolayer cultures.[10,11] In addition, our recent studies have demonstrated that HBFN-f activates all the three MAPK pathways in human articular chondrocytes, leading to up-regulation of collagenases in association with type II collagen degradation.[12]

The prototypical NF-κB complex consists of a p65-p50 heterodimer. The activity of NF-κB is regulated by the phosphorylation and degradation of IκB, an endogenous inhibitor that binds to NF-κB in the cytoplasm. Phosphorylation of p65/RelA is also required to activate NF-κB-dependent transcription. The released NF-κB translocates

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to the nucleus where it binds to specific NF-κB DNA binding sites and initiates gene expression. Recently, the central cell-binding FN-f has been shown to stimulate NF-κB activation in human normal ankle chondrocytes.[13] At present, however, whether other FN-f like HBFN-f can activate NF-κB in articular chondrocytes remains unknown. Of importance, chondrocyte response to FN-f may differ between normal and RA cartilages because RA cartilage produces higher amounts of NO than normal cartilage in response to HBFN-f.[4] Thus, it is necessary to confirm FN-f action on RA cartilage.

There is an increasing body of evidence that the mechanisms whereby FN-fs exert their activities involve the cell surface receptors. Matrix metalloproteinase (MMP) production by the central cell-binding FN-f is probably mediated by α5β1 integrin because anti-α5β1 integrin antibody and RGD-containing peptide induce MMP-1 and gelatinase in rabbit synovial fibroblasts.[14] Recent studies using antisense oligonucleotides to α5 integrin subunit have also shown the involvement of α5 integrin in bovine cartilage chondrolysis induced by 29 kDa NH_2-terminal heparin-binding and 50 kDa NH_2-terminal gelatin-binding FN-fs in addition to the cell-binding FN-f.[15] Furthermore, CD44 is responsible for HBFN-f-stimulated induction of MMP[16] and NO[4] in cartilage.

Hyaluronan (HA) of high molecular weight is widely used in the treatment of OA and RA by intra-articular injection. Our recent studies have demonstrated that HA action is mediated through its cell surface receptors.[17,18] HA can associate with its principal cell surface receptor CD44 on chondrocytes in cartilage explants.[18] We have also demonstrated that HA suppresses IL-1β-stimulated phosphorylation of p65 NF-κB and p38 MAPK in RA synovial fibroblasts.[19] Although HA can inhibit proteoglycan depletion by NH_2-terminal heparin-binding FN-f in human knee cartilage explant culture,[20] HA effect on FN-f-activated intracellular pathways remains to be clarified. Effective suppression of HBFN-f stimulation by anti-CD44 treatment[4] indicates possible inhibition by HA of HBFN-f action. This led us to investigate HA effect on NF-κB activation in RA chondrocytes stimulated with HBFN-f.

Materials and methods

Antibodies and reagents

Human plasma FN and HBFN-f generated with α-chymotrypsin digestion of human plasma FN were purchased from GIBCO BRL (Rockville, MD, USA). The purity of the protein preparation was confirmed using the same method as used in our previous studies.[21] The FN and HBFN-f were tested for endotoxin levels with the endotoxin assay kit (Sigma, St. Louis, MO, USA) prior to use and found to be free of detec-table endotoxin. Ammonium pyrrolidinedithiocarbamate (APDC) and BAY11-7085 were purchased from Wako Pure Chemical Industries (Osaka, Japan). Anti-iNOS antibody was obtained from Transduction Laboratories (Lexington, KY, USA). Anti-human NF-κB p65 antibody (#3034) and anti-human phospho-NF-κB p65 antibody (#3031) were purchased from Cell Signaling Technology (Beverly, MA, USA). Anti-human MMP-1 (M4177) and MMP-13 (M4052) antibodies were obtained from Sigma. Anti-β-actin antibody and alkaline phosphatase-conjugated goat anti-mouse and rabbit IgG were purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA). HA of 2700 kDa (weight average) was a gift from Denkikagakukogyo (Tokyo, Japan).

Articular cartilage explant culture

Rheumatoid arthritis cartilage specimens were obtained from non-weight-bearing regions of the distal femur from four female patients undergoing total knee replacement surgery who were diagnosed as having RA on the basis of the American College of Rheumatology 1987 revised criteria. All the samples were obtained under each patient's consensus according to the declaration of Helsinki, and the experimental design was approved by the institution's ethical committee. The cartilage was assigned to 24-well plate (ca. 80 mg/well) and kept in 1.5 ml serum-free Dulbecco's-modified Eagle's medium containing 100 μg/ml penicillin, 100 units/ml streptomycin, and 10 mM 2-[4-(2-hydroxyethyl)-1-piperadinyl] ethansulfonic acid (HEPES) (DMEM, all from GIBCO BRL) in a humidified 5% CO_2 atmosphere at 37°C. The cartilage was precultured for 2 days and medium was changed at day 0. Cartilage was incubated with HBFN-f or FN for 72 h from day 0. In some experiments, following preincubation with one of APDC, BAY11-7085, and HA for 1 h, articular cartilage was coincubated with HBFN-f from day 0. Control cultures had no additives. The cartilage explant and conditioned media were harvested at day 3, and stored at –20°C.

Articular chondrocyte monolayer culture

Articular chondrocytes were separated by collagenase digestion from articular cartilage obtained from RA knee joint as described above. The cells were kept in DMEM containing 10% fetal bovine serum, grown to confluence in 6-well plate, washed with phosphate buffured saline (PBS), and precultured for 2 days in 2 ml serum-free DMEM. Confluent primary chondrocytes were incubated with HBFN-f or FN under serum-free conditions for 72 h. In another set of experiments, following preculture in serum-free DMEM for 2 days, cells were preincubated with one of APDC, BAY11-7085, and HA for 1 h, thereafter coincubated with or without HBFN-f under serum-free conditions for 72 h. Control cultures had no additives.

Total cell lysate and nuclear extract preparation

After serum starvation for 24 h, cells were preincubated with or without HA for 1 h, followed by incubation with or without HBFN-f under serum-free conditions for various

periods of time at 37°C. Thereafter, cells were washed twice with cold PBS and then lysed in a lysis buffer containing 50 mM Tris (pH 7.5), 150 mM NaCl, 5 mM ethylenediaminetetraacetic acid, 10 mM NaF, 2 mM Na_3VO_4, 1 mM phenylmethylsulfonyl fluoride, 5 µg/ml aprotinin, 2 mM N-ethylmaleimide and 1% Triton X-100 at 4°C. Total cell lysates were cleared by centrifugation at 16 000 *g* for 10 min at 4°C.

The nuclear pellets were prepared as previously described[22] by resuspending cells in 400 µl of the cold buffer containing 10 mM HEPES, pH 7.8/10 mM KCl/0.1 mM ethylenediamine tetraacetic acid (EDTA)/0.5 mM phenylmethylsulfonyl fluoride/1 µg/ml pepstatin A/10 µg/ml leupeptin/10 µg/ml aprotinin) on ice for 15 min in the presence of 25 µl of 1% Nonidet P-40. Then, samples were vortexed and centrifuged at 10 000×*g* and the pellet was resuspended in 100 µl of the buffer with 20 mM HEPES (pH 7.8)/400 mM NaCl/1 mM EDTA/0.5 mM phenylmethylsulfonyl fluoride/1 µg/ml pepstatin A/10 µg/ml leupeptin/10 µg/ml aprotinin, followed by centrifugation at 10 000×*g*.

Immunoblot analysis

Total cell lysates, nuclear extracts, and conditioned media 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. Proteins were separated by SDS-PAGE under reducing conditions, and thereafter transferred onto nitrocellulose membranes (Bio-Rad Laboratories, Hercules, CA, USA). Gel loading was standardized on the basis of the DNA contents in chondrocyte cell layers. Immunoblot analysis for β-actin verified the equal loading of each sample on the basis of the DNA content. Membranes were blocked in tris buffered saline (TBS) containing 5% non-fat dry milk and 0.1% Tween 20 and incubated with the first antibody (concentration 1/1000) overnight at 4°C. After incubation with alkaline phosphatase-conjugated second antibody (concentration 1/1000) for 3 h at room temperature, immunoreactive bands were visualized using 5-bromo-4-chloro-3-indolyl phosphate and nitroblue tetrazolium. The protein band intensity was evaluated by densitometry using National Institute of Health image 1.62 software (Bethesda, MD, USA).

Analysis of NO release

NO release was measured by estimating the stable NO metabolite, nitrite, in conditioned media using a spectrophotometric method on the basis of the Griess reaction as described previously.[4] Nitrite concentrations were determined by measuring absorbance at 550 nm.

Assay for DNA

DNA content was measured with the proteinase K digests of articular cartilage explants and chondrocyte monolayers as described earlier.[4,12]

Statistical analysis

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

Results

Requirement of NF-κB for HBFN-f-stimulated NO production

Initially, the involvement of NF-κB in HBFN-f stimulation was investigated. As shown in our previous studies,[4] HBFN-f stimulated NO production in a dosage-dependent manner (data not shown) and 100 nM of HBFN-f significantly enhanced NO production in RA cartilage explant culture (Fig. 1). Similarly, HBFN-f at 100 nM effectively stimulated

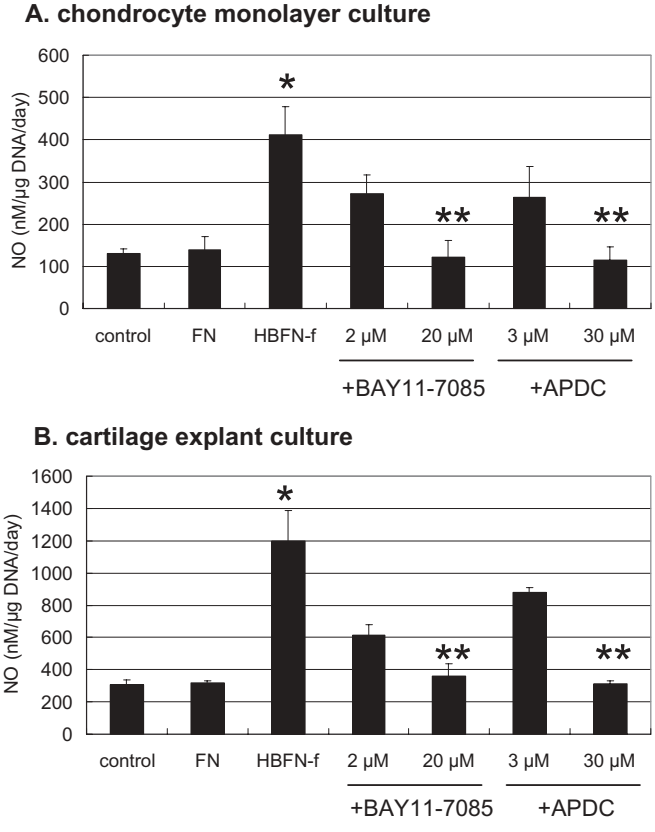

Fig. 1. Involvement of nuclear factor (NF)-κB in increased nitric oxide (NO) production by COOH-terminal heparin-binding fibronectin fragment (HBFN-f). Rheumatoid arthritis (RA) articular chondrocytes in monolayer culture (**A**) and RA cartilage slices in explant culture (**B**) were incubated with 100 nM HBFN-f or intact fibronectin (FN) for 72 h under serum-free conditions. Some cultures of RA chondrocytes and cartilage slices were incubated with 100 nM HBFN-f in the presence of BAY11-7085 at 2 µM and 20 µM or ammonium pyrrolidinedithiocarbamate (APDC) at 3 µM and 30 µM. Control cultures had no additives. Nitrite levels in conditioned media were determined as described in "Materials and methods" section. Values are the mean ± SD for four determinations. One-way analysis of variance (ANOVA) confirmed a significant effect of APDC concentration on resultant NO levels compared with those in HBFN-f-treated cultures ($P < 0.05$). *$P < 0.05$ vs. control cultures, and **$P < 0.05$ vs. HBFN-f-treated cultures, by *t* test. Three separate experiments were preformed with similar results

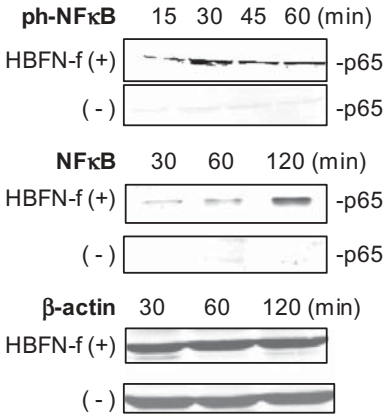

Fig. 2. Activation of NF-κB by HBFN-f. After incubation with or without 100 nM HBFN-f for the period indicated, the nuclear extracts and total cell lysates of RA chondrocytes in monolayer were subjected for immunoblotting for p65 NF-κB and phospho (ph)-NF-κB, respectively. Gel loading was standardized on the basis of the DNA contents in chondrocyte cell layers. Immunoblot analysis for β-actin verified the equal loading of each sample. The result shown is the representative of three separate experiments with similar results

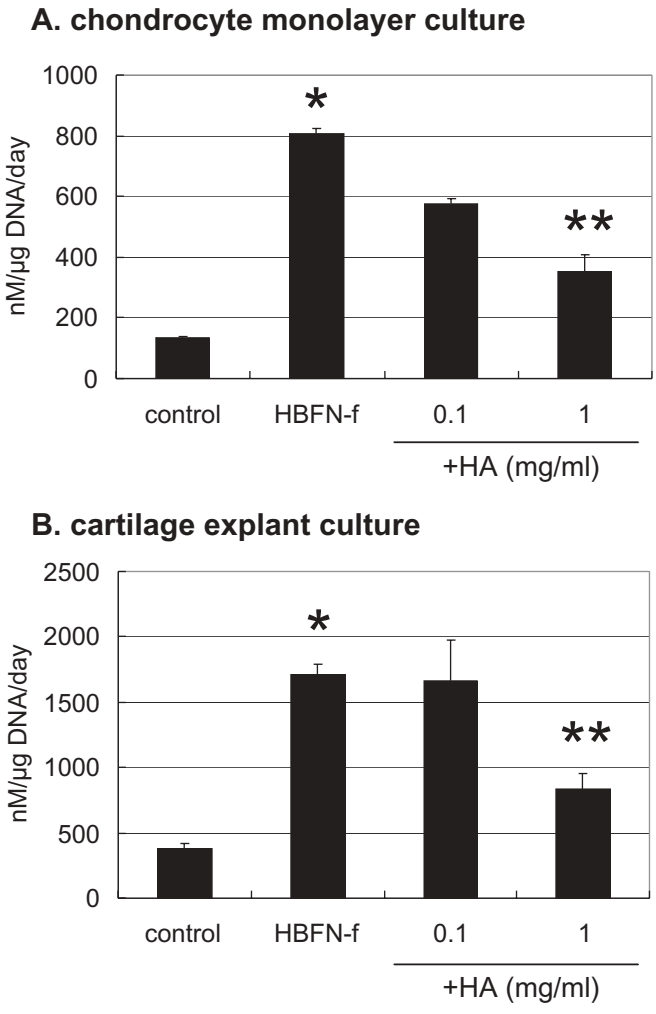

Fig. 3. Inhibition of HBFN-f-stimulated NO production by hyaluronan (HA). Following preincubation with or without 2700 kDa HA at 0.1 mg/ml or 1 mg/ml for 1 h, RA chondrocytes in monolayer (**A**) and cartilage explants (**B**) were incubated with 100 nM HBFN-f for 72 h under serum-free conditions. Control cultures had no additives. Nitrite levels in conditioned media were determined as described in "Materials and methods" section. Values are the mean ± SD for four determinations. One-way ANOVA confirmed a significant effect of HA concentration on resultant NO levels compared with those in HBFN-f-treated cultures ($P < 0.05$). *$P < 0.05$ vs. control cultures, and **$P < 0.05$ vs. HBFN-f-treated cultures, by t test. Three separate experiments were preformed with similar results

NO production in RA chondrocyte monolayer culture (Fig. 1). In contrast to HBFN-f, native FN at 100 nM had no effect on NO production. When RA chondrocytes or cartilage explants were pretreated with the NF-κB inhibitor BAY11–7085, NO production enhanced by HBFN-f was significantly suppressed in a dosage-dependent manner (Fig. 1). Another NF-κB inhibitor, APDC, also reduced HBFN-f-stimulated NO levels (Fig. 1). In association with NO production, 100 nM HBFN-f has been shown to up-regulate iNOS in RA chondrocytes.[4]

Next, the activation of p65 NF-κB by HBFN-f was investigated. The cell lysates of RA chondrocyte monolayer culture in the presence or absence of 100 nM HBFN-f were subjected to immunoblot analysis for phosphorylated p65 NF-κB. We found that HBFN-f caused significant phosphorylation of p65 NF-κB (Fig. 2). Immunoblot analysis using nuclear protein extracts revealed that HBFN-f-induced activation of NF-κB resulted in enhanced NF-κB translocation to the nucleus in RA chondrocytes (Fig. 2). Pretreatment with 2700 kDa HA at 1 mg/ml for 1 h significantly reduced the nuclear accumulation of NF-κB by HBFN-f (Fig. 2). Immunoblot analysis for β-actin verified the equal loading of each sample on the basis of the DNA content (Fig. 2).

Thus, HBFN-f-induced NO production was considered to require NF-κB activation.

Inhibition by HA of HBFN-f action on NO and NF-κB

When RA chondrocytes in monolayer were preincubated with 2700 kDa HA at 0.1 and 1 mg/ml for 1 h, 1 mg/ml of HA significantly blocked HBFN-f-stimulated NO production (Fig. 3). Similar inhibition by HA was also found in RA cartilage explant culture (Fig. 3). Immunoblot analysis revealed that pretreatment with 2700 kDa HA at 1 mg/ml resulted in a significant decrease in HBFN-f-enhanced levels of p65 NF-κB phosphorylation in association with a reduction of NF-κB nuclear translocation, leading to down-regulation of iNOS induced by HBFN-f (Fig. 4). Incubation of cartilage with 1 mg/ml HA caused no clear effect. The molecular weight (2700 kDa) and the maximal concentration (1 mg/ml) of HA used in the experiment are within a range of physiological molecular weight (2150–4960 kDa) and concentration (<4 mg/ml) of HA in synovial fluid,[23] respectively.

Inhibition by HA of HBFN-f action on MMP

As previously shown in human normal cartilage,[12,16] treatment with 100 nM HBFN-f resulted in enhanced levels of

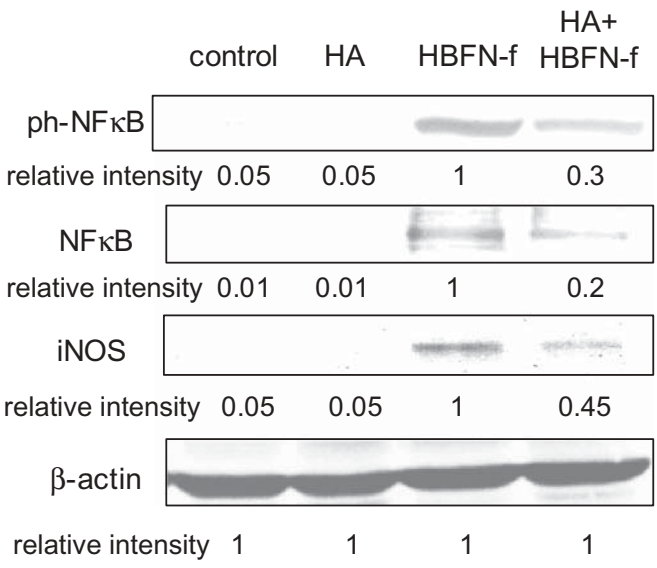

Fig. 4. Inhibition by HA of HBFN-f action on NF-κB and inducible form of NO synthase (iNOS). After preincubation with or without 2700 kDa HA at 1 mg/ml for 60 min, RA chondrocytes were stimulated with 100 nM HBFN-f. The total cell lysates of chondrocyte layers after exposure to HBFN-f for 60 min were subjected for immunoblotting for ph-NF-κB, iNOS, and β-actin. The nuclear extracts of chondrocytes after treatment with HBFN-f for 120 min were subjected to immunoblotting for NF-κB. Control cultures were without any additives. Gel loading was standardized on the basis of the DNA contents in chondrocyte cell layers. Immunoblot analysis for β-actin verified the equal loading of each sample on the basis of the DNA content. Densitometry of the membrane blot is shown as relative intensity compared with the density of HBFN-f-treated cell lysate (indicated as *1*). The result shown is the representative of three separate experiments with similar results

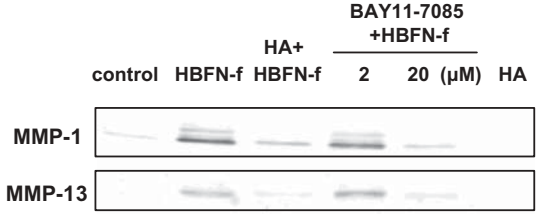

Fig. 5. Suppression of HBFN-f-stimulated production of MMP-1 and MMP-13 by HA and NF-κB inhibitor in RA cartilage explant culture. After preincubation with or without 2700 kDa HA at 1 mg/ml or BAY11-7085 at 2 and 20 μM for 60 min, RA cartilage slices were stimulated with 100 nM HBFN-f. Control cultures were without any additives. Conditioned media were collected at day 3. Levels of MMP-1 and MMP-13 in conditioned media were analyzed by immunoblotting. Two separate experiments were performed with similar results

MMP-1 and MMP-13 in conditioned media in RA cartilage explant cultures (Fig. 5). The inhibition by BAY11–7085 of HBFN-f-induced MMPs (Fig. 5) supports that NF-κB is a key regulator for the collagenases.[24,25] When RA cartilage slices were preincubated with 2700 kDa HA at 1 mg/ml for 1 h, HA significantly suppressed HBFN-f-stimulated MMP production (Fig. 5). Incubation of cartilage with 1 mg/ml HA caused no clear effect in MMP levels.

Discussion

The transcription factor NF-κB is one of primary target molecules for RA treatment. NF-κB is important for initiating and sustaining inflammatory reactions. NF-κB regulates many genes including cytokines, chemokines, and adhesion molecules that participate in the pathophysiology of synovial inflammation and bone and cartilage degradation. Actually, NF-κB mediates up-regulation of cytokines and chemokines in human articular chondrocytes in response to the central cell-binding FN-f.[13] The present study extended the previous finding and has shown for the first time that HBFN-f can activate NF-κB, leading to NO production in association with iNOS up-regulation in RA chondrocytes. Although this study provided no data that HBFN-f induced NF-κB activation in RA cartilage explant culture, significant suppression by NF-κB inhibitors of HBFN-f-stimulated NO production in RA cartilage explants (Fig. 1) suggests that HBFN-f could activate NF-κB in RA cartilage explants, resulting in the increased NO production.

In synovial tissue from patients with RA, NF-κB has been shown to be present in the nuclei in the synovial macrophages and fibroblasts.[26] In RA synovium NF-κB expression increases especially at the site of cartilage–pannus junction.[27] FN isoforms with III12-14 and IIICS are present in human cartilage.[28] Although the presence of HBFN-f used in the present study in RA cartilage remains unclear, it seems likely that FN-fs with the COOH-terminal heparin-binding region like HBFN-f could be generated by enhanced proteolysis in RA joints. In OA synovial fluids approximately 1 μM of 100 to 200 kDa FN-fs have been found.[2] Because the levels of FN-fs in RA cartilage have been suggested to be similar to those in RA synovial fluids,[1] contents of FN-fs may reach 100 nM in RA cartilage, comparable with the concentration used in the present study. In in vivo RA cartilage, therefore, some FN-fs-like HBFN-f may contribute to NF-κB activation.

Recently, high molecular weight HA is employed for the treatment of RA. Because HA is an intrinsic glycosaminoglycan in the body, it has theoretical advantage over synthesized molecules in terms of biophysiologic effects as well as possible side effects. HA can inhibit IL-1β-activated phosphorylation of p65 NF-κB, which results in a reduction of the cytokine-stimulated MMP production in RA synovial fibroblasts.[19] This study is the first to show that HA can inhibit HBFN-f-induced activation of NF-κB in association with a decrease in NO and MMP production by HBFN-f in RA chondrocytes. When HA is therapeutically introduced in RA joints, therefore, HA may be beneficial to the suppression of proinflammatory cytokines and FN-fs as a potent NF-κB inhibitor.

AP-1 is another pivotal transcriptional factor that regulates the catabolic events like NO[7,8] and MMP[29] production. AP-1 can be activated by MAPKs.[9] HBFN-f has been shown to activate MAPKs in chondrocytes.[12] Because HA suppresses IL-1β-activated p38 in RA synovial fibroblasts,[19] HA could inhibit MAPK activation in addition to NF-κB in HBFN-f-stimulated chondrocytes. HA effect on MAPK

pathways activated by HBFN-f will be investigated in our future studies.

The principal HA receptor CD44 is up-regulated in RA cartilage and the proportion of CD44-positive chondrocytes is significantly higher than that in normal cartilage.[4] Increased proinflammatory cytokines could cause CD44 up-regulation in RA cartilage because TNFα and IL-1β can enhance the HA receptor levels.[17] Those findings implicate the role of CD44 in the pathogenesis of inflammation. Indeed, anti-CD44 treatment reduces joint swelling and leukocyte infiltration in murine arthritis model[30] and cartilage destruction by RA synovial fibroblasts.[31]

Hyaluronan action could be mediated through its cell surface receptors.[17–19] HBFN-f is known to bind CD44.[32] We have already demonstrated the involvement of CD44 in NO[4] and MMP[16] induction stimulated by HBFN-f in articular cartilage using the anti-CD44 antibody that blocks CD44 binding to its ligand. Fluorescein microscopic analysis has revealed that FITC-conjugated anti-CD44 antibody localizes CD44 in association with chondrocytes,[16] indicating that occupancy of CD44 by the antibody on chondrocytes can block the HBFN-f-induced production of NO and MMPs. Similar to anti-CD44 antibody, HA, the principal ligand for CD44, suppressed HBFN-f-induced NO and MMPs in the present study. HA has been shown to bind CD44 on chondrocytes after penetration into cartilage explants.[18] Thus, HA is likely to block HBFN-f action via CD44 on the cells. The observation that ligation of CD44 with HA fails to block the binding of anti-vascular cell adhesion molecule (VCAM)-1 antibody to VCAM-1 on RA synovial fibroblasts[17] suggests that the mechanism of HA action is not a barrier effect. However, it is not totally denied that HA may block catabolic substances as a barrier at the surface of cartilage when HA is used in the treatment of RA by intraarticular injection.

Alteration in intracellular signaling through CD44 may involve HA inhibition of HBFN-f action. It has been demonstrated that CD44 functions as a signaling receptor in various types of cells. Cell stimulation by anti-CD44 antibodies or natural CD44 ligands transmits the signal into the cells, leading to activation of T cells and release of cytokine or chemokine from monocytes and RA synovial fibroblasts.[33,34] In addition, there is evidence that the anti-CD44 antibody IM7 causes the induction of fibroblast apoptosis via CD44.[35] Thus, anti-CD44 treatment could activate some intracellular signaling pathways that block catabolic actions on chondrocytes, which remain to be determined.

Acknowledgments This work was partly supported by a grant from Chugai Pharmaceutical Company.

References

1. Homandberg GA, Wen C, Hui F. Cartilage damaging activities of fibronectin fragments derived from cartilage and synovial fluid. Osteoarthritis Cartilage 1998;6:231–44.
2. Xie DL, Meyers R, Homandberg GA. Fibronectin fragments in osteoarthritic synovial fluid. J Rheumatol 1992;19:1448–52.
3. Scott DL, Wainwright AC, Walton KW, Williamson N. Significance of fibronectin in rheumatoid arthritis and osteoarthrosis. Ann Rheum Dis 1981;40:142–53.
4. Yasuda T, Kakinuma T, Julovi SM, Yoshida M, Hiramitsu T, Akiyoshi M, et al. COOH-terminal heparin-binding fibronectin fragment induces nitric oxide production in rheumatoid cartilage through CD44. Rheumatology (Oxford) 2004;43:1116–20.
5. Moncada S, Higgs A. The L-arginine-nitric oxide pathway. N Engl J Med 1993;329:2002–12.
6. Sakurai H, Kohsaka H, Liu MF, Higashiyama H, Hirata Y, Kanno K, et al. Nitric oxide production and inducible nitric oxide synthase expression in inflammatory arthritides. J Clin Invest 1995;96: 2357–63.
7. Chu SC, Marks-Konczalik J, Wu HP, Banks TC, Moss J. Analysis of the cytokine-stimulated human inducible nitric oxide synthase (iNOS) gene: characterization of differences between human and mouse iNOS promoters. Biochem Biophys Res Commun 1998; 248:871–8.
8. Marks-Konczalik J, Chu SC, Moss J. Cytokine-mediated transcriptional induction of the human inducible nitric oxide synthase gene requires both activator protein 1 and nuclear factor κB-binding sites. J Biol Chem 1998;273:22201–8.
9. Karin M, Liu ZG, Zandi E. AP-1 function and regulation. Curr Opin Cell Biol 1997;9:240–6.
10. Gemba T, Valbracht J, Alsalameh S, Lotz M. Focal adhesion kinase and mitogen-activated protein kinases are involved in chondrocyte activation by the 29-kDa amino-terminal fibronectin fragment. J Biol Chem 2002;277:907–11.
11. Forsyth CB, Pulai J, Loeser RF. Fibronectin fragments and blocking antibodies to α2β1 and α5β1 integrins stimulate mitogen-activated protein kinase signaling and increase collagenase 3 (matrix metalloproteinase 13) production by human articular chondrocytes. Arthritis Rheum 2002;46:2368–76.
12. Yasuda T, Julovi SM, Hiramitsu T, Yoshida M, Nakamura T. Requirement of mitogen-activated protein kinase for collagenase production by the fibronectin fragment in human articular chondrocytes in culture. Mod Rheumatol 2004;14:54–60.
13. Pulai JI, Chen H, Im H-J, Kumar S, Hanning C, Hedge PS, et al. NF-κB mediates the stimulation of cytokine and chemokine expression by human articular chondrocytes in response to fibronectin fragments. J Immunol 2005;174:5781–88.
14. Werb Z, Tremble PM, Behrendtsen O, Crowley E, Damsky CH. Signal transduction through the fibronectin receptor induces collagenase and stromelysin gene expression. J Cell Biol 1989;109: 877–89.
15. Homandberg GA, Costa V, Ummadi V, Pichika R. Antisense oligonucleotides to the integrin receptor subunit α5 decrease fibronectin fragment mediated cartilage chondrolysis. Osteoarthritis Cartilage 2002;10:381–93.
16. Yasuda T, Poole AR, Shimizu M, Nakagawa T, Julovi SM, Tamamura H, et al. Involvement of CD44 in induction of matrix metalloproteinases by a COOH-terminal heparin-binding fragment of fibronectin in human articular cartilage in culture. Arthritis Rheum 2003;48:1271–80.
17. Shimizu M, Yasuda T, Nakagawa T, Yamashita E, Julovi SM, Hiramitsu T, et al. Hyaluronan inhibits matrix metalloproteinase-1 production by rheumatoid synovial fibroblasts stimulated by proinflammatory cytokines. J Rheumatol 2003;30:1164–72.
18. Julovi SM, Yasuda T, Shimizu M, Hiramitsu T, Nakamura T. Inhibition of interleukin-1β-stimulated production of matrix metalloproteinases by hyaluronan via CD44 in human articular cartilage. Arthritis Rheum 2004;50:516–25.
19. Hiramitsu T, Yasuda T, Ito H, Shimizu M, Julovi SM, Kakinuma T, et al. Intercellular adhesion molecule-1 mediates the inhibitory effects of hyaluronan on interleukin-1β-induced matrix metalloproteinase production in rheumatoid synovial fibroblasts via down-regulation of NF-κB and p38. Rheumatology (Oxford) 2006;45: 824–32.
20. Kang Y, Eger W, Koepp H, Williams JM, Kuettner KE, Homandberg GA. Hyaluronan suppresses fibronectin fragment-mediated damage to human cartilage explant cultures by enhancing proteoglycan synthesis. J Orthop Res 1999;17:858–69.
21. Yasuda T, Poole AR. A fibronectin fragment induces type II collagen degradation by collagenase through an interleukin-1-mediated pathway. Arthritis Rheum 2002;46:138–48.

22. Tang X, Metzger D, Leeman S, Amar S. LPS-induced TNF-α factor (LITAF)-deficient mice express reduced LPS-induced cytokine: evidence for LITAF-dependent LPS signaling pathways. Proc Natl Acad Sci USA 2006;103:13777–82.
23. Dahl LB, Dahl IM, Laurent AE, Granath K. Concentration and molecular weight of sodium hyaluronate in synovial fluid from patients with rheumatoid arthritis and other arthropathies. Ann Rheum Dis 1985;44:817–22.
24. Vincenti MP, Coon CI, Brinckerhoff CE. Nuclear factor κB/p50 activates an element in the distal matrix metalloproteinase 1 promoter in interleukin-1β-stimulated synovial fibroblasts. Arthritis Rheum 1998;41:1987–94.
25. Mengshol JA, Vincenti MP, Coon CI, Barchowsky A, Brinckerhoff CE. Interleukin-1 induction of collagenase-3 (matrix metalloproteinase 13) gene expression in chondrocytes requires p38, c-Jun N-terminal kinase, and nuclear factor κB: differential regulation of collagenase 1 and collagenase 3. Arthritis Rheum 2000;43:801–11.
26. Marok R, Winyard PG, Coumbe A, Kus ML, Gaffney K, Blades S, et al. Activation of the transcription factor nuclear factor-κB in human inflamed synovial tissue. Arthritis Rheum 1996;39:583–91.
27. Benito MJ, Murphy E, Murphy EP, van den Berg WB, FitzGelard O, Bresnihan B. Increased synovial tissue NF-κB1 expression at sites adjacent to the cartilage-pannus junction in rheumatoid arthritis. Arthritis Rheum 2004;50:1781–7.
28. Parker AE, Boutell J, Carr A, Maciewicz RA. Novel cartilage-specific splice variants of fibronectin. Osteoarthritis Cartilage 2002; 10:528–34.
29. Rutter JL, Benbow U, Coon CI, Brinckerhoff CE. Cell-type specific regulation of human interstitial collagense-1 gene expression by interleukin-1β (IL-1β) in human fibroblasts and BC-8701 breast cancer cells. J Cell Biochem 1997;66:322–36.
30. Mikecz K, Dennis K, Shi M, Kim JH. Modulation of hyaluronan receptor (CD44) function in vivo in a murine model of rheumatoid arthritis. Arthritis Rheum 1999;42:659–68.
31. Neidhart M, Gay RE, Gay S. Anti-interleukin-1 and anti-CD44 interventions producing significant inhibition of cartilage destruction in an in vitro model of cartilage invasion by rheumatoid arthritis synovial fibroblasts. Arthritis Rheum 2000;43:1719–28.
32. Jalkanen S, Jalkanen M. Lymphocyte CD44 binds the COOH-terminal heparin-binding domain of fibronectin. J Cell Biol 1992; 116:817–25.
33. McKee CM, Penno MB, Cowman M, Burdick MD, Strieter RM, Bao C, et al. Hyaluronan fragments induce chemokine gene expression in alveolar macrophages. J Clin Invest 1996;98: 2403–13.
34. Fujii K, Tanaka Y, Hübscher S, Saito K, Ota T, Eto S. Crosslinking of CD44 on rheumatoid synovial cells augments interleukin 6 production. Lab Invest 1999;79:1439–46.
35. Henke C, Bitterman P, Roongta U, Ingbar D, Polunovsky V. Induction of fibroblast apoptosis by anti-CD44 antibody: implications for the treatment of fibroproliferative lung disease. Am J Pathol 1996;149:1639–50.