

Gene Expression and Localization of High-mobility Group Box Chromosomal Protein-1 (HMGB-1) in Human Osteoarthritic Cartilage

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We investigated the expression and localization of high-mobility group box chromosomal protein-1 (HMGB-1) in human osteoarthritic (OA) cartilage in relation to the histopathological grade of cartilage destruction, and examined the role of HMGB-1 in the regulation of proinflammatory cytokine expression in chondrocytes. An immunohistochemical study demonstrated that total HMGB-1-positive cell ratios increase as the Osteoarthritis Research Society International (OARSI) histological grade increased. The population of cytoplasmic HMGB-1-positive chondrocytes was especially increased in the deep layers of higher-grade cartilage. The ratios and localization of receptors for advanced glycation end products (RAGE) expression by chondrocytes in Grade 2, 3, and 4 were significantly higher than those in Grade 1. *In vitro* stimulation with IL-1 β , but not TNF α , significantly upregulated the expression of HMGB-1 mRNA by human OA chondrocytes. Both IL-1 β and TNF α promoted the translocation of HMGB-1 from nuclei to cytoplasm. IL-1 β and TNF α secretions were stimulated at higher levels of HMGB-1. The results of our study suggest the involvement of HMGB-1 in the pathogenesis of cartilage destruction in OA.

Key words: HMGB-1, RAGE, chondrocyte, osteoarthritis, cartilage

Osteoarthritis (OA) is a proliferative joint disease characterized by articular cartilage degeneration, osteophyte formation, subchondral bone sclerosis, and secondary induced synovitis [1]. Although numerous studies have revealed the contribution of genetic factors, growth factors and cytokines, mechanical stress, proteinase, or altered responses of chondrocytes in the progression of OA,

the pathogenesis of OA has not been fully understood.

High-mobility group box chromosomal protein (HMGB-1), previously called HMG-1 or amphoterin, named for its rapid mobility on electrophoresis gels, is a ubiquitous, approximately 27-kDa nonhistone protein with a highly conserved amino acid sequence identity between rodents and humans [2-4]. Nuclear HMGB-1 has been widely studied as a deoxyribonucleic acid (DNA)-binding protein. It participates in the maintenance of nucleosomal structure and stability and facilitates the binding of transcription factors to their cognate DNA sequences [5]. HMGB-1 also has func-

tions in DNA transcription, recombination [6, 7], repair, cell replication, cell migration, and tumor growth [8, 9].

In contrast to its intranuclear role, HMGB-1 plays a critical role outside the cell as a proinflammatory cytokine mediating delayed endotoxin lethality as well as acute lung injury in mice [10, 11]. Moreover, high levels of HMGB-1 have been detected in the blood of patients with sepsis and in the synovial fluid of rheumatoid arthritis (RA) patients [12]. Proinflammatory mediators, such as tumor necrosis factor α (TNF α) and interleukin-1 β (IL-1 β), dose-dependently induce the release of HMGB-1 from monocytes and macrophages [10]. Furthermore, once released, HMGB-1 activates an additional downstream cascade by stimulating monocytes to produce proinflammatory cytokines and chemokines [13].

The receptor for advanced glycation end products (RAGE) is a transmembrane protein that belongs to the immunoglobulin superfamily and is expressed in vascular smooth muscle cells, neurons, and certain phagocytes such as monocytes and macrophages [14]. Recently, the expression of RAGE has been reported in articular chondrocytes, synoviocytes, and RA synovial macrophages [15–17]. Membrane-associated HMGB-1 mediates cellular proliferation and growth by signaling through the receptor for RAGE [18].

Previous reports have suggested the HMGB-1 upregulation in OA cartilage and induction of inflammatory cytokines such as IL-6 or IL-8 [19]. In the current study, we investigated the expression and localization of nuclear and cytoplasmic HMGB-1 in normal human cartilage and OA cartilage. We further investigated the effect of HMGB-1 stimulation on TNF α and IL-1 β production, as well as the effect of TNF α and IL-1 β on HMGB-1 production by human OA chondrocytes. Our data strongly suggest the involvement of HMGB-1 in proinflammatory cytokines in cartilage destruction.

Materials and Methods

Production of HMGB-1-specific monoclonal antibody. Rats were immunized with HMGB-1/HMGB-2 (Sigma, St. Louis, MO, USA) emulsified with Freund's complete adjuvant. A booster injection with incomplete adjuvant was administered to the rats 3 weeks later. After confirming the elevation of anti-

HMGB-1 antibody, we produced hybridomas as previously described [20]. The epitope recognized by each monoclonal antibody was determined by dot blotting, using synthetic overlapping peptides derived from a human HMGB-1 sequence 15 amino acids long. The clone (Nos. 10–22, subclass IgG2a) used for the experiments recognized the C-terminal sequence of the HMGB-1 molecule, DEDEEEE, as specific for HMGB-1 but not for HMGB-2.

Recombinant human HMGB-1 (rHMGB-1). rHMGB-1 was produced in Sf9 cells to obtain LPS-free HMGB-1. In brief, full-length human HMGB-1 DNA was amplified by PCR using Cap Site cDNA dT from human microvascular endothelial cells (Nippon Gene, Tokyo, Japan) and primers (forward 5'-GCA GAA TTC ATG GGC AAA GGA GAT CCT A-3', reverse 5'-CAT CTC GAG TCA TTA TTC ATC ATC ATC ATC-3'). The fragment was digested with EcoRI and XhoI and cloned into a pFastBacHTA (Invitrogen, Carlsbad, CA, USA) expression vector. The transfection of the Sf9 cells with the pFastBacHTA-HMGB-1 bacmid was performed according to the manufacturer's instructions (Bac-to-Bac Baculovirus Expression System, Invitrogen). The infected SF9 cell extract containing His-tagged HMGB-1 protein was applied to Ni-NTA agarose (Qiagen, Hilden, Germany) and incubated for 3 hours at room temperature. After extensive washing, rHMGB-1 was eluted with imidazole buffer. The rHMGB-1 was collected and dialyzed overnight at 4°C against PBS. Purified rHMGB-1 protein was identified by SDS-PAGE and Western blotting with anti-HMGB-1 mAb. The final HMGB-1 preparation contained LPS of less than 2.0 pg/ μ g protein.

Human cartilage samples. Thirty-one human OA cartilage samples were obtained at the time of total knee arthroplasty (TKA) surgery from 27 OA patients (aged 48 to 82 years; average, 70.7 years), who lacked a medical history of other inflammatory diseases. Normal cartilage was obtained from 4 patients with osteosarcoma (aged 9 to 29 years; average 15.5 years) who lacked any inflammatory diseases at the time of amputation surgery. Written informed consent was obtained from the patients or their parents.

RNA isolation from OA cartilage. OA cartilage samples from OA patients (n = 4) and normal cartilage from osteosarcoma patients (n = 4) were

divided in half; one half was immediately frozen in liquid nitrogen and stored at -180°C until required for RNA isolation. At that point the frozen articular cartilage (10–40 mg wet weight) was milled with Micro Smash (Tomy Seiko Co, Tokyo, Japan) at 2 cycles of 1 min at 4,500 rpm and was suspended in 1 mL Isogen (Nippon Gene Co, Tokyo, Japan). Total RNA was isolated according to the manufacturer's protocol.

cDNA synthesis and Quantitative Real-Time PCR. First-strand cDNA was synthesized from 1 μg total RNA using the ReverTra Ace- α according to the manufacturer's (Toyobo Co., Ltd., Osaka, Japan) instructions. cDNA (1.5 μL) was amplified by real-time PCR using Brilliant[®] II SYBR Green QPCR Master Mix (Stratagene, La Jolla, CA, USA). Quantitative PCR was performed on a 25- μL samples. Mixtures were preincubated at 95°C for 10 min, followed by 40 cycles of PCR at 95°C for 30 sec and 60°C for 30 sec, and normalized to GAPDH. In every case, the cycle threshold (C_T) taken for quantitation was in the linear portion of the amplification range. PCR primers were HMGB-1 sense, 5'-TAT GAA AAG AAG GCT GCG AAG-3'; and HMGB-1 antisense, 5'-CTG CGC TAG AAC CAA CTT ATT C-3', GAPDH sense, 5'-CAT CAA GAA GGT GGT GAA GCA G-3'; and GAPDH; antisense, 5'-CTG CAA AGG TGG AGG AGT GG-3'. All PCRs were performed in triplicate using the Mx3000P quantitative PCR system (Stratagene). RNA levels are reported as fold changes compared with the control using the comparative quantitation analysis software available with the Mx3000P. Changes in expression (fold) for HMGB-1 were calculated as $2^{-\Delta(\Delta C_T)}$, where $\Delta C_T = C_T(\text{target}) - C_T(\text{housekeeping})$, and $\Delta(\Delta C_T) = \Delta C_T(\text{treated}) - \Delta C_T(\text{control})$. Reaction product purity was confirmed by examination of the melting curves for a single peak.

Immunohistochemistry for HMGB-1 and RAGE. Twenty-seven OA and 4 normal cartilage specimens were used in an immunohistochemical study for HMGB-1 and RAGE. The samples were immediately fixed in 4% paraformaldehyde in 0.1 M phosphate-buffered saline (PBS), decalcified in 0.3 M EDTA (pH 7.5) for 2–3 weeks, and embedded in paraffin. All sections were used for the histological and immunohistochemical studies.

The paraffin sections were soaked in xylene to be deparaffinized, then dehydrated in a graded alcohol

series (50–100%). Antigen retrieval was performed by autoclaving for 5 min at 97°C . After it was cooled, endogenous peroxidase activity was blocked by treatment with 0.3% H_2O_2 in PBS at room temperature for 30 min. It was then incubated with either rat monoclonal anti-HMGB-1 antibody (10 $\mu\text{g}/\text{mL}$) or goat anti-RAGE polyclonal antibody (10 $\mu\text{g}/\text{mL}$) (Chemicon, Temecula, CA, USA) with PBS/bovine serum albumin (BSA) overnight at 4°C . After washing, the slides were incubated with biotinylated anti rat IgG antibody diluted 1:200 with PBS. After rinsing in PBS, the specimens were incubated with avidin-biotinylated enzyme complex (ABC) reagent (Vectastain Elite ABC Kit, Vector Laboratories, Burlingame, CA, USA), then washed with distilled water. For RAGE, a chemical reagent marketed as Histofine Simple Stain MAX PO (G) (Nichirei Biosciences, Tokyo, Japan) was applied as a secondary antibody at room temperature for 30 min. The reaction was visualized by diaminobenzidine, which turned brown, after which the samples were counterstained with hematoxylin.

Quantitative analysis for HMGB-1 and RAGE-positive cells. Immunoreactivity was evaluated by light microscopy. The chondrocytes with definite, diffusely stained cytoplasm or nuclei were regarded as positively stained. The populations of HMGB-1-positive, or RAGE-positive cells in the superficial, middle, and deep layers of OA cartilage, were quantified by counting the number of the cells within the 3 layers. Cell counts were performed in at least 3 fields, at $\times 100$ magnification, and then averaged. Judgment was based on the consensus of at least 2 of the authors (C.T. and A.Y.) without reference to the patients' clinical information. The number of positive chondrocytes was divided by the total number of chondrocytes within all 3 layers to calculate the positive chondrocyte ratio. The marginal areas between each layer were avoided for cell counting to reduce inter-observer errors. All sections were also stained with Safranin O and observed by light microscopy. Histological classification of the severity of OA lesions was graded 1–6, using the OARSI cartilage OA histopathology grading system reported by Pritzker *et al.* [21].

Chondrocyte isolation from OA cartilage. For the *in vitro* study, OA cartilage samples were obtained from another 6 sets of patients at TKA. The

ages of patients ranged from 62 to 88 years (mean \pm SEM 72.1 ± 8.2 years). Chondrocytes were isolated by digestion of cartilage specimens in 0.1% chymotrypsin (Wako Pure Chemical Industries, Osaka, Japan) and 0.2% collagenase (Sigma, St. Louis, MO, USA) following the method of Bruckner *et al.* [22]. Chondrocytes were seeded in 6 - well plates (5×10^4 /mL), cultured in 3mL Dulbecco's modified Eagle medium (Wako Pure Chemical Industries, Osaka, Japan) containing 10% fetal bovine serum, 100U/mL penicillin (Wako Pure Chemical Industries, Osaka, Japan), and 100 μ g/mL streptomycin (Wako Pure Chemical Industries, Osaka, Japan), and incubated in a 5% CO₂ humidified incubator at 37°C for 14 days before starting the experiments.

Quantification of IL-1 β and TNF α production by HMGB-1-stimulated chondrocytes. After exposure of the cells by recombinant human HMGB-1 (10, 100, and 1,000ng/mL) (Biological Industries, Kibbutz, Israel) for 6, 12, and 24h, the concentrations of IL-1 β and TNF α in the supernatant were measured using a commercially available enzyme-linked immunosorbent assay (ELISA) kit (R&D Systems, Minneapolis, MN, USA), following the manufacturer's protocol.

Quantitative Real-Time PCR for HMGB-1 mRNA in cytokine-stimulated chondrocytes. Recombinant human IL-1 β and TNF α were purchased from R&D Systems. The cytokines were stored at -180°C and diluted in a culture medium immediately before being used. Total RNA was isolated from primary OA chondrocytes after treatment with IL-1 β (10ng/mL) and TNF α (1ng/mL) for 0, 12, and 24h. HMGB-1 mRNA expression was analyzed by real-time PCR as previously described.

Immunocytochemistry for HMGB-1 in cytokine-stimulated chondrocytes. Primary cultured OA chondrocytes were treated with IL-1 β (10ng/mL) and TNF α (1ng/mL) for 48h, and the distribution of the HMGB-1 protein was investigated by immunocytochemistry. Cells were fixed in 4% paraformaldehyde for 10min, blocked with 1% BSA for 10min at room temperature, then incubated with an anti-HMGB-1 antibody (1 μ g/mL) for 30min at room temperature. A BSA solution without the primary antibody was used as a negative control. Alexa Fluor 488-conjugated antibody (Molecular Probes, Eugene, OR, USA) and Alexa-Fluor 594-conjugated phalloidin

(Molecular Probes), both incubated for 30min at room temperature, and Hoechst 33342 (ICN Biomedicals, Aurora, OH, USA), incubated for 5min at room temperature, were used for detection. Fluorescence images were captured using an inverted Leica DMRIII microscope (Leica, Bannockburn, IL, USA) equipped with epifluorescence filters and a charge-coupled device camera using Leica CW4000 software.

Statistical analysis. Statistical comparisons were made by Mann-Whitney *U* test using Stat-view software. All values are expressed as means \pm SEM. *P* values less than 0.05 were considered significant.

Results

Expression of HMGB-1 in OA cartilage. HMGB-1 was detected in normal and OA cartilage at both the mRNA and protein level. HMGB-1 mRNA expression was significantly higher in OA cartilage than in normal cartilage (Fig. 1).

Expression and localization of HMGB-1 in OA cartilage. The protein expression of HMGB-1 was seen in all 27 OA cartilage samples (Fig. 2). HMGB-1-positive cell ratios relevant to the OA histopathology were 12.6% in Grade 1, 20.1% in Grade 2, 21.8% in Grade 3, and 34.2% in Grade 4 (Fig. 3A). In Grades 2, 3, and 4, HMGB-1-positive cell ratios were significantly higher than that in Grade 1. There was no significant difference between ratios in Grade 2 and 3. In Grade-4 OA, the HMGB-1-positive cell

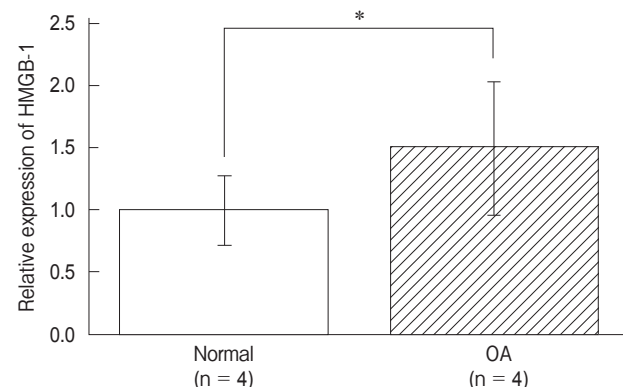


Fig. 1 Expression of HMGB-1 in OA cartilage. HMGB-1 expression in normal cartilage (n = 4) and OA cartilage (n = 4). HMGB-1 mRNA was significantly expressed. **p* < 0.05 vs. normal (*p* = 0.043).

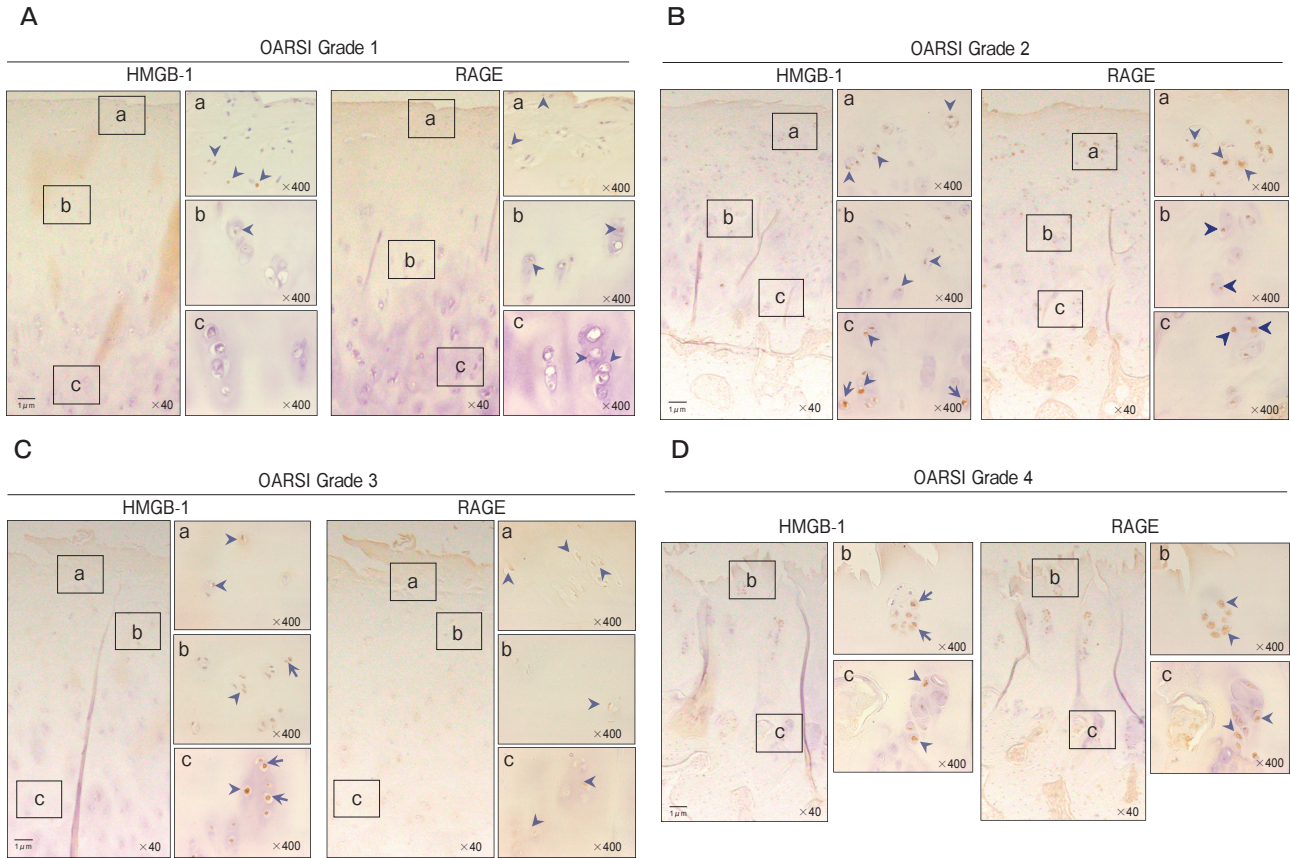


Fig. 2 Immunohistochemistry for HMGB-1 and RAGE. Expression and localization of HMGB-1 and RAGE in OA cartilage in relation to the level of cartilage destruction. Twenty-seven OA and 4 normal cartilage specimens were used for the immunohistochemical study of HMGB-1 and RAGE. HMGB-1 was expressed in the nucleus. The cytoplasmic HMGB-1-positive cell rate tended to increase as the OARSI grade increased. RAGE was expressed in the cytoplasm, and the RAGE-positive cell ratio tended to be higher according to the OARSI grade, as with HMGB-1. Arrow heads indicate nuclear HMGB-1- and RAGE-positive cells, and arrows indicate cytoplasmic HMGB-1-positive cells. Inset a, b and c show the superficial, middle and deep layer, respectively.

ratio was significantly higher than those in Grade 2 or 3 (Fig. 3A). The cytoplasmic HMGB-1-positive cell rates were 1.4% in Grade 1, 2.3% in Grade 2, 11.1% in Grade 3, and 13.4% in Grade 4. In Grades 3 and 4, the cytoplasmic HMGB-1-positive cell ratios were significantly higher than those in Grade 1 and 2, respectively (Fig. 3A). There was no significant difference in ratios between Grade 1 and 2, or between Grade 3 and 4.

Expression of RAGE in OA cartilage. We also investigated RAGE expression in OA cartilage by immunohistochemical analysis. The RAGE-positive cell ratios relevant to OA histopathology were 20.4% in Grade 1, 36.6% in Grade 2, 37.3% in Grade 3, and 35.3% in Grade 4 (Fig. 3B). The ratios of RAGE

expression by chondrocytes in Grade 2, 3, and 4 were significantly higher than those in Grade 1 (Fig. 3B). There was no significant difference among ratio in Grades 2, 3, and 4. (Fig. 3B).

Effect of proinflammatory cytokines on HMGB-1 expression and localization in OA chondrocytes. IL-1 β (10 ng/mL) significantly up-regulated the expression of HMGB-1 mRNA up to 24h by OA chondrocytes (Fig. 4A); however, TNF α (1 ng/mL) up-regulation of HMGB-1 mRNA did not reach significance. The change in localization of HMGB-1 after treatment with IL-1 β and TNF α was examined by immunocytochemistry. In the unstimulated group, HMGB-1 was localized in the nucleus (Fig. 4B). However, HMGB-1 was translocated from

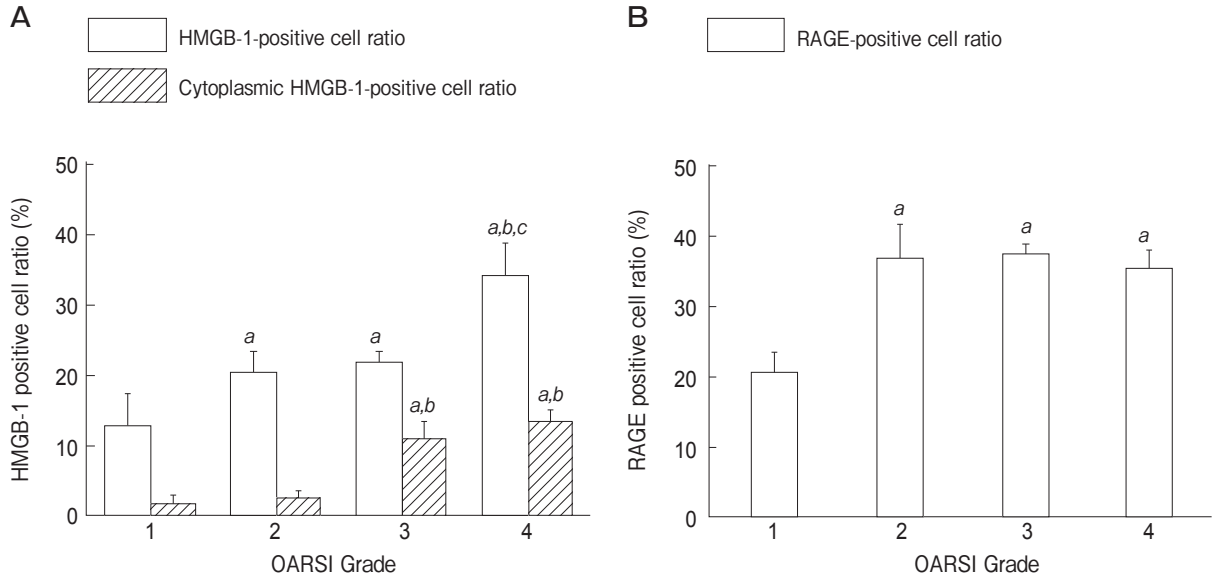


Fig. 3 Population of HMGB-1-and RAGE positive chondrocytes. (A) Cytoplasmic HMGB-1-positive cell ratios tended to be higher in the specimens having the higher OARSI grade. (a, $p < 0.05$ vs. Grade 1; b, $p < 0.05$ vs. Grade 2; c, $p < 0.05$ vs. Grade 3.), (B) The ratios and localization of RAGE expression by chondrocytes in Grades 2, 3 and 4 were significantly higher than those in Grade 1. There was no significant difference between Grades 2/3 and 4, nor was there any significant difference between Grades 3 and 4.

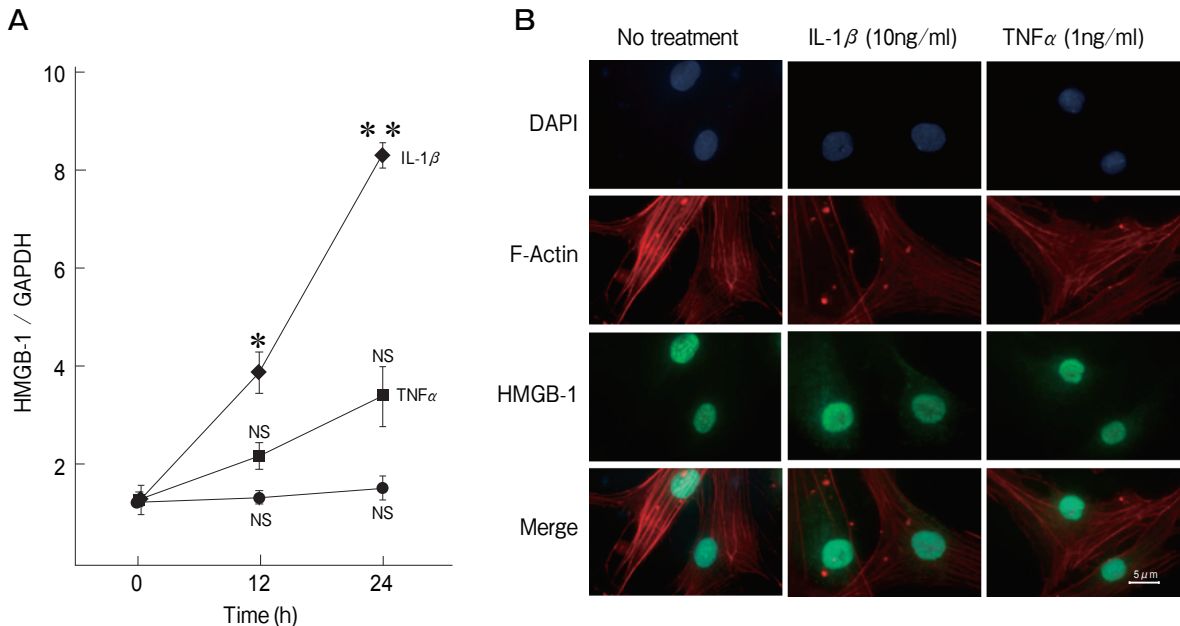


Fig. 4 Effect of IL-1β and TNFα on HMGB-1 and localization in OA chondrocyte. Expression of HMGB-1 mRNA by real-time PCR. Cultured chondrocytes were stimulated with IL-1β (10 ng/μL), and total RNA was isolated. Up-regulation of HMGB-1 was still increasing after 12h, but not TNFα (A). (* $p < 0.05$ vs. 0h ($p = 0.033$), ** $p < 0.05$ vs. 12h ($p = 0.002$), NS = not significant.) Translocation of HMGB-1 in chondrocytes after IL-1β and TNFα stimulation. The immunofluorescence assay indicated that HMGB-1 was translocated from the nucleus to the cytosol following stimulation with both IL-1β and TNFα (B). Chondrocytes were stimulated with IL-1β (10 ng/mL) and TNFα (1 ng/mL) for 48h and then compared with unstimulated chondrocytes. Chondrocytes stimulated with IL-1β or TNFα exhibited HMGB-1 staining in the cytosol. Note that unstimulated chondrocytes exhibited HMGB-1 staining only in the nucleus.

the nucleus to the cytosol after stimulation by IL-1 β (10 ng/mL) and TNF α (1 ng/mL) at 48 h.

Effect of HMGB-1 on IL-1 β and TNF α release by chondrocytes. The effects of HMGB-1 on IL-1 β and TNF α production by chondrocytes were analyzed by ELISA. HMGB-1 stimulated the production of IL-1 β up to 24 h (Fig. 5A), and 1,000 ng/mL of HMGB-1 significantly up-regulated the production of IL-1 β by OA chondrocytes at 12 h (Fig. 5B). Peak TNF α levels after stimulation with HMGB-1 (100 ng/mL) occurred at 12 h and tended to decrease for 24 h thereafter. (Fig. 5C). At 12 h, the amount of TNF α released into the cultured medium increased in a dose-dependent manner after stimulation by HMGB-1 (Fig. 5D).

Discussion

A previous report demonstrated the significant increase of HMGB-1 mRNA expression in human OA cartilage as compared with normal cartilage [19]. In the present study, we examined the expression and localization of HMGB-1 in OA cartilage. Our results confirmed that HMGB-1 expression in OA cartilage was significantly higher than that in the normal cartilage for both mRNA and protein levels. In the immunohistochemical analysis, it was revealed that the HMGB-1-positive cell ratio and cytoplasmic HMGB-1 positive cell ratio tended to increase along with the OA histological grade, but the nuclear HMGB-1 positive cell ratio was unchanged. These results coincide with a report of Heinola *et al.* in which they studied bovine

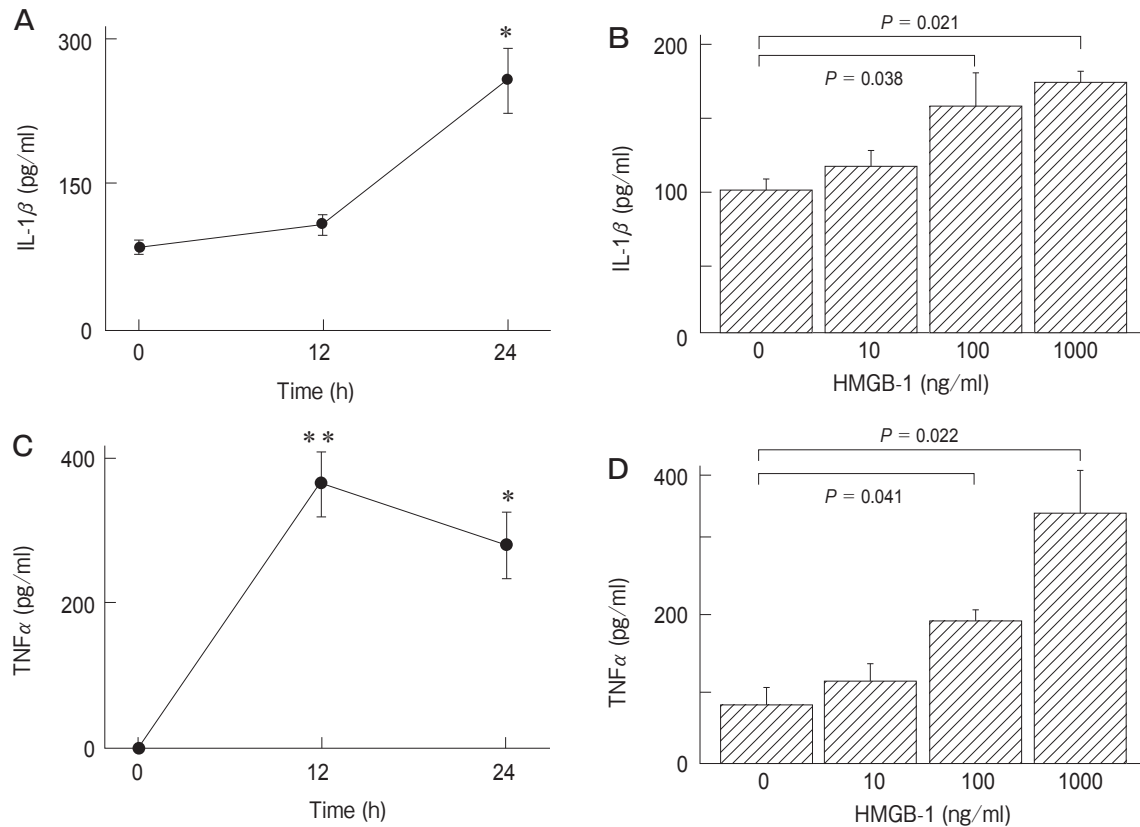


Fig. 5 Effect of HMGB-1 on IL-1 β and TNF α expression by human chondrocytes. Chondrocytes were stimulated with HMGB-1, and ELISA was used to measure levels of IL-1 β and TNF α . After stimulation with HMGB-1 (100 ng/mL), IL-1 β levels increased in a time-dependent manner (A). IL-1 β was released in a dose-dependent manner for 24 h after stimulation with HMGB-1, and the amount of IL-1 β and TNF α production increased significantly at doses of > 100 ng/mL (B). The peak TNF α levels after stimulation with HMGB-1 (100 ng/mL) occurred at 12 h and tended to decrease at 24 h (C). TNF α was released in a dose-dependent manner after stimulation for 12 h (D). (A) * p < 0.05 vs. 0 h (p = 0.025). (C) * p < 0.05 vs. 0 h (p = 0.039), ** p < 0.05 vs. 0 h (p = 0.018).

cartilage [23].

It is not fully understood what stimulates HMGB-1 production or cytoplasmic translocation from the nucleus in chondrocytes. In normal joint cartilage, HMGB-1 has been reported to be located in the nuclei of chondrocytes and synovial cells [12]. However, in inflammatory synovial cells, HMGB-1 shows both nuclear and cytoplasmic distribution [12]. It was also shown that TNF α stimulation caused translocation of HMGB-1 from the nucleus to the cytosol in CD68-positive cells [14]. In the present study, we demonstrated that IL-1 β but not TNF α up-regulated the expression of HMGB-1 at mRNA levels. Treatment with both IL-1 β and TNF α caused translocation of HMGB-1 from the nucleus to the cytosol 48h after stimulation.

On the other hand, previous studies have demonstrated that extracellular HMGB-1 induced the production of IL-1 β , TNF α , and IL-6 in macrophages [10, 14]. Intra-articular injection of HMGB-1 into murine knee joints resulted in synovitis, but mice rendered deficient in the IL-1 receptor did not develop synovitis after injection. These results suggest that HMGB-1 is not a mere expression of the inflammatory response, but a trigger for joint inflammation by activating macrophages and inducing production of IL-1 via NF- κ B activation [13]. In the current study, IL-1 β and TNF α production by OA chondrocytes was stimulated by HMGB-1 in a dose-dependent manner. It is speculated that proinflammatory cytokines stimulate the expression and release of HMGB-1 by chondrocytes and that the HMGB-1 released by chondrocytes further promotes the expression of proinflammatory cytokines [14]. This speculation is supported by our current result that the HMGB-1-positive cell ratio increases along with the OA cartilage grade. It is reported that the combination of HMGB-1 and heparin induced marked angiogenesis in a heparin-dependent manner [24]. The HMGB-1 increase in the deep layers of OA cartilage might be related to angiogenesis at the osteochondral junction.

RAGE and toll-like receptors 2 and 4 are known ligands of HMGB-1 [25]. RAGE has been found to be expressed in cartilage [16] and RA synovium [14]; however, RAGE and HMGB-1 do not have a one-to-one correspondence. A study reported by Loeser *et al.* [16] showed that RAGE expression was higher in OA cartilage than in normal cartilage. Our immunohis-

tochemical results also demonstrated that expression of RAGE was significantly higher in cartilage with Grades 2, 3, and 4 OA than that in Grade 1 cartilage. In a separate experiment we used 5 other human OA cartilage specimens to perform double immunohistochemistry for HMGB-1 and RAGE to clarify the interrelationship of the 2 proteins. The result showed that some populations of chondrocytes showed a positive reaction only for HMGB-1 or for RAGE, whereas some were positive for both HMGB-1 and RAGE (data not shown). These findings suggest that HMGB-1 might act on chondrocytes in both an autocrine and paracrine manner. Stimulation of RAGE signaling from HMGB-1 might lead to upregulation of catabolic enzymes such as matrix metalloproteinase 13 production and contribute to cartilage matrix degradation in arthritis [16].

In conclusion, we demonstrated the increased expression of HMGB-1 in OA cartilage relevant to the histological OA grade. *In vitro* experiments using human OA chondrocytes suggested the close interrelationship between HMGB-1 and IL-1 β . These results indicate the possible involvement of the proinflammatory cytokine-HMGB-1-RAGE cascade in the development of human osteoarthritis. Whether the blockade of this cascade could contribute to the moderation of cartilage degeneration requires further investigation.

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