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Hyaluronan regulates PPARγ and inflammatory responses in IL-1β-stimulated human chondrosarcoma cells, a model for osteoarthritis

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ABSTRACT

The carbohydrate polymer, hyaluronan, is a major component of the extracellular matrix in animal tissues. Exogenous hyaluronan has been used to treat osteoarthritis (OA), a degenerative joint disease involving inflammatory changes. The underlying mechanisms of hyaluronan in OA are not fully understood. Pro-inflammatory interleukin (IL)-1β downregulates peroxisome proliferator-activated receptor gamma (PPARγ), and increases expression of matrix metalloproteinases (MMPs) which are responsible for the degeneration of articular cartilage. The effects of low- and high-molecular-weight hyaluronan (oligo-HA and HMW-HA) on the inflammatory genes were determined in human SW-1353 chondrosarcoma cells. HMW-HA antagonized the effects of IL-1β by increasing PPARγ and decreasing cyclooxygenase (COX)-2, MMP-1, and MMP-13 levels. It promoted Akt, but suppressed mitogen-activated protein kinases (MAPKs) and nuclear factor kappa B (NFκB) signaling, indicating anti-inflammatory effects. In contrast, the cells had overall opposite responses to oligo-HA. In conclusion, HMW-HA and oligo-HA exerted differential inflammatory responses *via* PPARγ in IL-1β-treated chondrosarcoma cells.

1. Introduction

Hyaluronan (also called hyaluronic acid or HA) is a ubiquitous carbohydrate polymer and a major component of the extracellular matrix in multiple tissues (Toole, 2000). It is composed of repeating GlcNAcβ(1→4)-GlcUAβ(1→3) disaccharide units which molecular weight often reaches million Dalton. Studies showed that patients with an arthropathy have a lower concentration and molecular weight of hyaluronan in their joints than healthy humans (Dahl, Dahl, Engstrom-Laurent, & Granath, 1985; Wu, Shih, Hsu, & Chen, 1997). Intra-articular injection of hyaluronan has been used in diverse clinical applications, including ECM regeneration, wound healing, and treatment of osteoarthritis (OA) (Altman, 2010; Mei-Dan et al., 2010; Muzzarelli, Greco, Busilacchi, Sollazzo, & Gigante, 2012). Injection of hyaluronan into OA-afflicted joints helps relieve pain in some, but not all, patients (Mei-Dan et al., 2010). The underlying mechanisms of hyaluronan in cells are not fully understood.

OA accounts for the major pain and disability of older adults. It is a gradually progressing disorder affecting mammalian joints and is characterized by the destruction of articular cartilage, resulting in discomfort and dysfunction of the affected joint (Cahue et al., 2007; Yelin, 1992). The pathologic changes that occur during OA development include proteoglycan degradation at the early stages, type II collagen degradation, and eventual localized or complete loss of the cartilage matrix (Homandberg, 2001). Cytokines and their downstream targets play major roles in the pathogenesis of OA (Daheshia & Yao, 2008; Kardel, Ulfgren, Reinholt, & Holmlund, 2003). For example, pro-inflammatory cytokine such as interleukin (IL)-1β is produced by activated synoviocytes and articular chondrocytes. IL-1β activates cyclo-oxygenase-2 (COX-2), and increases expression

Abbreviations: MMP-1, matrix metalloproteinase 1; MMP-13, matrix metalloproteinase 13; ECM, extracellular matrix; HMW-HA, high-molecular-weight hyaluronan; oligo-HA, oligomer hyaluronan; OA, osteoarthritis; COX-2, cyclooxygenase-2; PPARγ, peroxisome proliferator-activated receptor; NF-κB, nuclear factor kappa B; IκBα, nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor, alpha; MAPK, mitogen-activated protein kinase; JNK, c-Jun N-terminal kinase; ERK, extracellular signal-regulated kinase.

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of several matrix metalloproteinases (MMPs), including MMP-1, MMP-3, and MMP-13 from normal articular human chondrocytes (Fan, Yang, Bau, Soder, & Aigner, 2006; Hiramitsu et al., 2006). Its signal transduction utilizes three classical mitogen-activated protein kinase (MAPK) pathways: p38, extracellular signal regulated kinase (ERK), and Jun terminal kinase (JNK) (Saklatvala, 2007). Upon activation, MAPKs then regulate the expression of MMPs (Ancha et al., 2007; Im et al., 2007). In OA cartilage, MMP-1, MMP-3, and MMP-13 levels are increased, and participate in the degradation of the extracellular matrix (ECM) under various physiological and pathological conditions (Sternlicht & Werb, 2001).

Chondrocytes may differentially respond to hyaluronan derivatives. Researchers observed that production of MMP-1 was significantly suppressed by a 1900-kDa hyaluronan (high-molecular-weight hyaluronan; HMW-HA) (Tanaka, Masuko-Hongo, Kato, Nishioka, & Nakamura, 2006). In contrast, others found that hyaluronan oligosaccharides (oligo-HA) increased MMP-13 expression which induced the loss of extracellular matrix proteoglycan and collagen of human articular cartilage (Ohno, Im, Knudson, & Knudson, 2006). A recent study also showed better protection of articular cartilage by hyaluronan with higher MW *via* inhibiting MMP-2, MMP-9 and plasminogen activator pathways (Hsieh, Yang, Lue, Chu, & Lu, 2008). Studies suggested that hyaluronan regulates MMPs expression *via* MAPK and nuclear factor kappa B (NF-κB) pathways (Campo et al., 2009; Noble, McKee, Cowman, & Shin, 1996; Ohno et al., 2006). HMW-HA may be degraded into hyaluronan oligosaccharides (oligo-HA) *in vivo*, and activate an NF-κB/IκBα auto-regulatory loop and induce transcription of MMP-9 and MMP-13 (Fieber et al., 2004; Noble et al., 1996). Although many effects of hyaluronan have been reported, the mechanism of the differential effects by its size remains elusive.

Peroxisome proliferator-activated receptors (PPARs) are members of the nuclear hormone receptor superfamily and ligand-activated transcription factors. Studies suggest that PPARγ plays an important role in the pathogenesis of OA (Fahmi, Martel-Pelletier, Pelletier, & Kapoor, 2011). Among the two PPARγ isoforms, human cartilage mainly expresses PPARγ1, and its expression is suppressed by IL-1β and significantly lower in OA tissue than in normal tissue (Afif et al., 2007). Since oligo-HA and HMW-HA modulate inflammation in OA and MMPs differentially, we hypothesized that they may exhibit divergent effects on the regulation of PPARγ and inflammatory responses in chondrocytes.

The human chondrosarcoma SW-1353 cells respond to IL-1β stimulation by activation of MAPKs and induction of MMPs (Afif et al., 2007; Chao, Hsieh, Cheng, Lin, & Chen, 2011; Wang, Lin, Qin, Chen, & Chen, 2010). These cells resembled properties of primary chondrocytes from OA subjects. Using this cell model, we examined the effects of HMW-HA and oligo-HA pretreatment in IL-1β-stimulated SW-1353 cells on (1) expression levels of MMP-1, MMP-13, COX-2, and PPARγ; and (2) intracellular signaling pathways (MAPK, NF-κB, and PI3K/Akt). Our data further elucidated the mechanisms of hyaluronan in human chondrocytes.

2. Methods

2.1. Reagents

Medical grade high-molecular-weight sodium hyaluronate (HMW-HA, MW = 600–1200 kDa) was purchased from Seikagaku Co (Japan). This hyaluronan product is endotoxin-free. Because the molecular weight of HMW-HA encompassed a wide range, molar solutions were not formulated, and doses were in mg/mL. The endotoxin-free hexamer sodium hyaluronate oligosaccharide (oligo-HA, MW = 1.22 kDa) was purchased from Cosmobio Co., Ltd. Most chemicals were purchased from Sigma (St. Louis, MO), unless indicated otherwise. Leibovitz's L-15 medium (L-15), fetal bovine serum (FBS), L-glutamine, penicillin/streptomycin, and amphotericin were purchased from Life Technologies (Invitrogen Corp; Carlsbad, CA). Antibodies specific for MMP-1, MMP-13, pAkt, pJNK, IκBα and GAPDH were purchased from Cell Signaling Technology (Danvers, MA). Horseradish peroxidase-conjugated secondary antibodies were purchased from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA). Recombinant Human IL-1β was purchased from R&D Systems, Inc. (Minneapolis, MN).

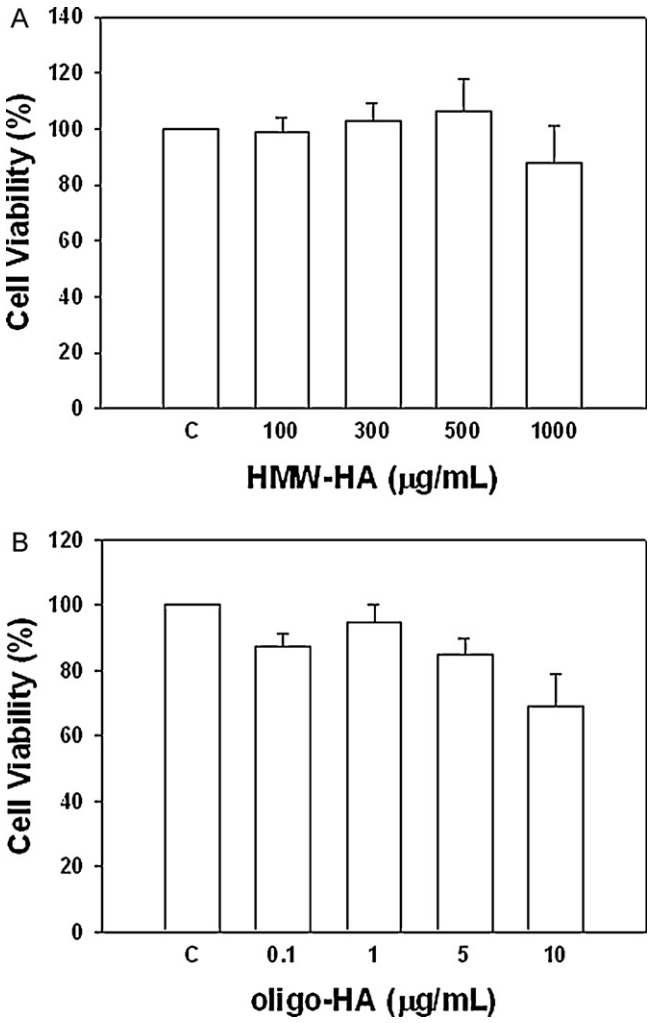

Fig. 1. Viability of human chondrosarcoma cells with hyaluronan treatments. SW-1353 cells were treated with various concentrations of hyaluronan for 24 h. The extent of cell viability was determined by WST-1 assays. The result from cells without hyaluronan was used as 100% control. (A) Cells were treated with HMW-HA at 100, 300, 500 and 1000 μg/mL, respectively, and the relative viabilities were 98 ± 4.5%, 102 ± 6.5%, 106 ± 11% and 87 ± 13%. (B) Cells were treated with oligo-HA at 0.1, 1, 5 and 10 μg/mL, respectively, and the relative viabilities were 88 ± 3.7%, 95 ± 5.6%, 85 ± 5.2% and 70 ± 9.3%.

2.2. Culture of chondrosarcoma cells and preparation of cell lysates

SW-1353 chondrosarcoma cells (ATCC HTB-94; Wang et al., 2010) were cultured in L-15 medium supplemented with 2 mM L-glutamine, 10% heat-inactivated FBS, and penicillin (100 U/mL)/streptomycin (100 mg/mL) (Invitrogen Corp; Carlsbad, CA) in a humidified incubator with 5% CO_2 at 37 °C. Cells were plated at a concentration of 10^5 cells/mL and cultures were used for experiments at 80% confluence and moved to an incubator without CO_2. After reaching confluence, cells were pretreated with various

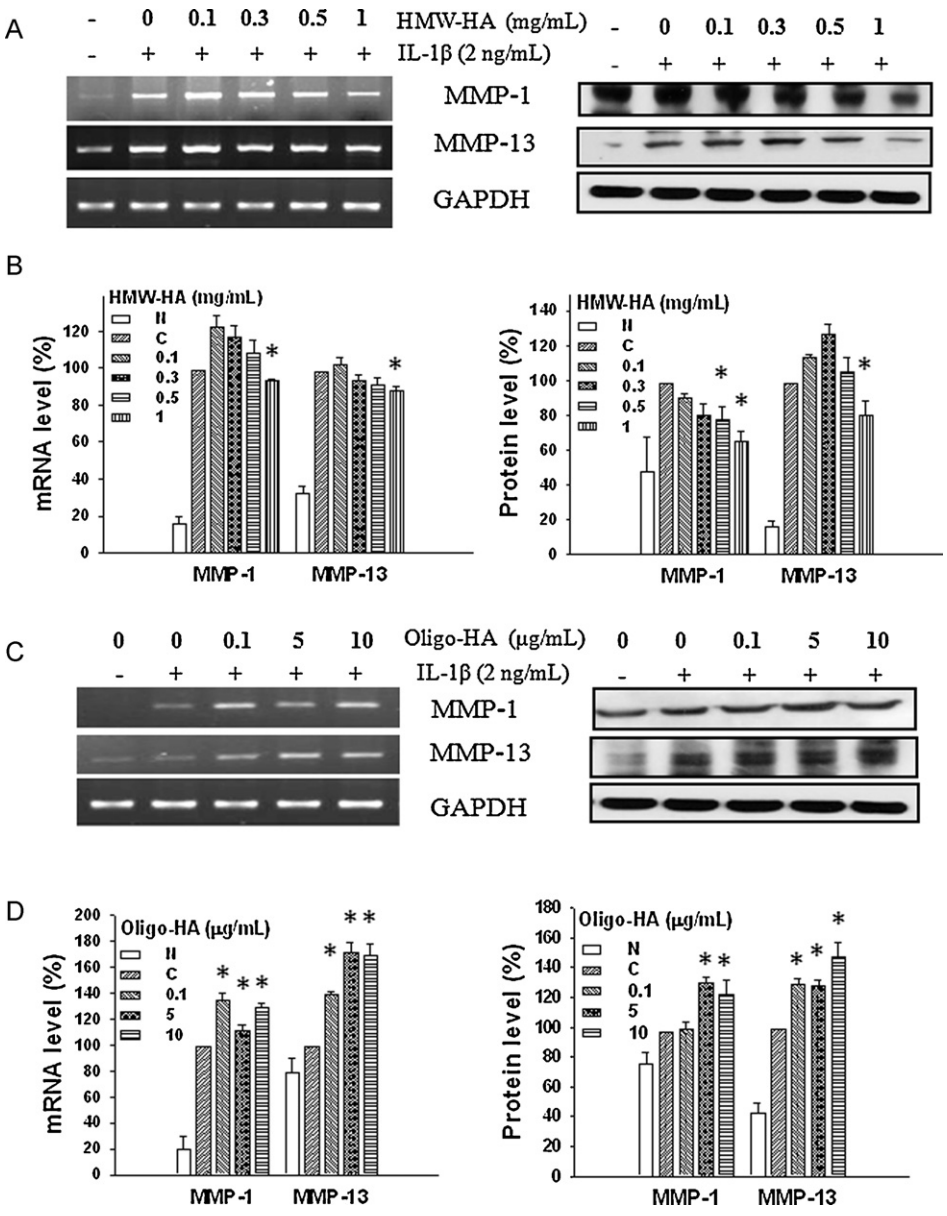

Fig. 2. Effects of hyaluronan on IL-1β-induced MMPs expression in chondrosarcoma cells. Cells were pre-incubated with various concentrations of HMW-HA or oligo-HA for 30 min and then stimulated with 2 ng/mL IL-1β for 24 h. Cell lysates were prepared and analyzed for MMP-1 and MMP-13 expression levels. The mRNA levels were analyzed by RT-PCR (left panels) and protein levels by Western blotting (right panels). GAPDH was used as a loading control. For quantitative analysis, the results from samples with only IL-1β treatment were used as 100% control. (A) Electrophoretic analysis of MMP-1 and MMP-13 from HMW-HA-treated cells. (B) Quantitative analysis of MMP-1 and MMP-13 expression levels in HMW-HA-treated cells. (C) Electrophoretic analysis of MMP-1 and MMP-13 from oligo-HA-treated cells. (D) Quantitative analysis of MMP-1 and MMP-13 expression levels in oligo-HA-treated cells. N, no treatment; C, control with IL-1β only. $^{*}p < 0.05$.

concentrations of hyaluronan for 30 min, stimulated with 2 ng/mL recombinant human IL-1β (R&D Systems Inc.) and incubated for the indicated time intervals. After incubation, cells were assayed for viability and proliferation, or were rinsed with PBS and harvested for RNA or protein analysis.

2.3. Cell viability

Cell viability and proliferation of the treated and untreated cells were measured by the WST-1 assay, as described (QIAGEN). Briefly, cells were incubated with the medium containing various concentrations of hyaluronan for 24 h. After incubation, the medium was replaced with the WST-1 reagent and fresh medium in a ratio of 1:9. Cells were then incubated at 37 °C for 2 h before being analyzed at an absorbance of 450 nm.

2.4. Reverse transcriptase-polymerase chain reaction (RT-PCR)

Total RNA was extracted (TRIzol) from chondrosarcoma cells treated with either vehicle, HMW-HA, or oligo-HA for 24 h. cDNA was synthesized from 0.5 μg of total RNA using TaqMan reverse transcription reagents (Applied Biosystems). Reverse transcription, which was performed in the Creacon technology PCR System, began with a 10-min incubation period at 25 °C, continued for 30 min at 48 °C, and was inactivated at 95 °C for 5 min. RT-PCR was carried out with the indicated primers (Table 1) in the Creacon technology PCR System thermal cycler/detector in the following sequence: 5 min at 95 °C, 30 cycles consisting of 30 s at 94 °C, 40 s at 50 °C, and 30 s at 72 °C, and finally 7 min at 72 °C, followed by cooling to 4 °C. GAPDH was amplified as an internal control. The PCR products were analyzed on 1% agarose gel in triplicate, and the results of each sample were normalized to GAPDH.

Table 1
Primers for RT-PCR.

Gene	Forward	Reverse
MMP1	5′-GTCAGGGGAGATCATCGG-3′	5′-GCCCAGTACTTATTCCCT-3′
MMP13	5′-GCTTAGAGGTGACTGGCAA-3′	5′-CCGGTGTAGGTGTAGATAGGAA-3′
COX-2	5′-TTCAAATGAGATTGTGGGAAAT-3′	5′-AGATCATCTCTGCCTGAGTATCTT-3′
PPARγ	5′-GTATGACTCATACATAAAGT-3′	5′-TCTCGTGGACTCCATATTTG-3′
GAPDH	5′-CTGCCGTCTAGAAAAACC-3′	5′-CCAAATTCGTTGTCATACC-3′

2.5. Protein isolation, SDS-PAGE, and Western blotting

Cells were lysed by adding buffer containing 10 mM Tris HCl (pH 7.5), 1 mM EGTA, 1 mM $MgCl_2$, 1 mM sodium orthovanadate, 1 mM DTT, 0.1% mercaptoethanol, 0.5% Triton X-100, and protease inhibitor cocktail (final concentrations of 0.2 mM PMSF, 0.1% aprotinin, and 50 μg/mL leupeptin). Cells adhering to the plates were scraped off using a rubber policeman and stored at −70 °C for further analysis. Proteins were separated by using different percentages of SDS-polyacrylamide and electrotransferred onto a polyvinyldifluoride (PVDF) membrane. After washed once with phosphate-buffered saline (PBS) and twice with PBS plus 0.1% Tween 20 (PBST), the PVDF membrane was blocked with either PBST plus 3% bovine serum albumin (BSA) or Tris-buffered saline Tween 20 (TBST) plus 5% milk at room temperature for 1 h. The membrane was then incubated with the primary antibodies in the same buffer for another 1 h. Primary antibodies specific for MMP-1, MMP-13, pAkt, pJNK, CD44, and IκBα, as well as apoptosis specific antibodies and GAPDH were purchased from Cell Signaling Technology (Beverly, MA). The PVDF membrane was incubated with peroxidase-linked anti-mouse IgG antibodies (Santa Cruz Biotechnology Inc., Santa Cruz, CA) for 1 h and then developed using a LumiGLO chemiluminescence kit (Amersham, UK).

2.6. Statistical analysis

The band densities on gels and blots were determined using UN-SCAN-IT gel 6.1. Plot illustrations were performed using SigmaPlot 9. Data were expressed as mean ± SD. Statistical analyses of triplicates were performed using the analysis of variance (ANOVA).

3. Results

3.1. Dosage testing for hyaluronan on cell viability

Treatment of SW-1353 cells with HMW-HA up to 1000 μg/mL slightly but not significantly reduced viability or proliferation (Fig. 1A). In the contrary, oligo-HA less than 10 μg/mL reduced SW1353 cell viability although without significance (Fig. 1B). Therefore doses up to 1000 μg/mL HMW-HA and 10 μg/mL oligo-HA were used in the following experiments.

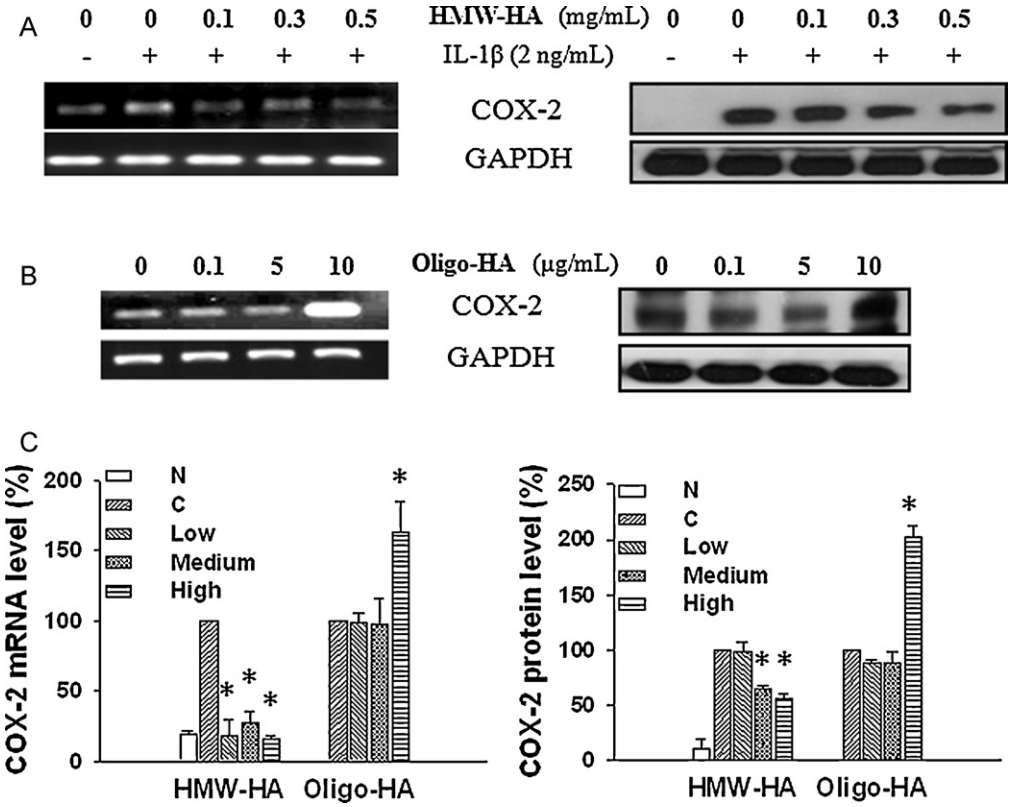

Fig. 3. Effects of hyaluronan on IL-1β-induced COX-2 expression in chondrosarcoma cells. Cells were pre-incubated with various concentrations of HMW-HA or oligo-HA for 30 min, and then stimulated with 2 ng/mL IL-1β for 24 h. Cell lysates were collected and analyzed for COX-2 mRNA levels by RT-PCR (left panels) and protein levels by Western blotting (right panels). GAPDH was used as a loading control. (A) Electrophoretic analysis of HMW-HA-mediated COX-2 mRNA and protein levels. (B) Electrophoretic analysis of oligo-HA-mediated COX-2 mRNA and protein levels. (C) Quantitative analysis of COX-2 levels affected by hyaluronan. The results from samples with only IL-1β treatment were used as 100% control. N, no treatment; C, control with IL-1β only. $^{*}p < 0.05$.

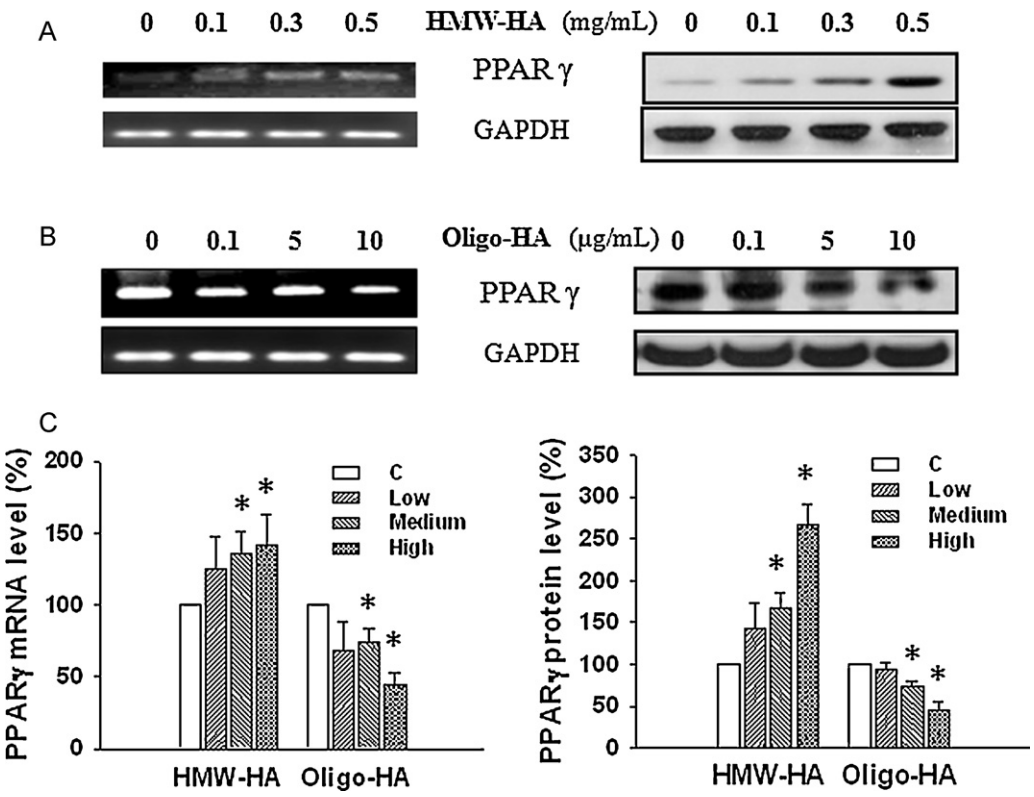

Fig. 4. Effects of hyaluronan on IL-1β-induced PPARγ expression in chondrosarcoma cells. Cells were pre-incubated with various concentrations of HMW-HA or oligo-HA for 30 min, and then stimulated with 2 ng/mL IL-1β for 24 h. Cell lysates were collected and analyzed for PPARγ mRNA levels by RT-PCR (left panel) and protein levels by Western blotting (right panel). GAPDH was used as a loading control. (A) Electrophoretic analysis of HMW-HA-affected PPARγ mRNA and protein levels. (B) Electrophoretic analysis of oligo-HA-affected PPARγ mRNA and protein levels. (C) Quantitative analysis of PPARγ levels affected by hyaluronan. The results from samples with only IL-1β treatment were used as 100% control. $^{*}p < 0.05$.

3.2. Differential modulation of IL-1β-induced MMPs and COX-2 by HMW- and oligo-HA

OA joints exhibited high MMP1 and MMP13 levels which are induced by IL-1β (Fan et al., 2006; Hiramitsu et al., 2006). To evaluate the effects of HMW-HA and oligo-HA on MMP expression, SW-1353 cells were incubated with various concentrations of HMW-HA or oligo-HA for 30 min prior to IL-1β stimulation. MMP-1 and MMP-13 expressions at mRNA and protein levels were assessed. As expected, IL-1β induced mRNA and protein levels of MMP-1 and MMP-13 in the cells (Fig. 2A and C). Pretreatment with high doses of HMW-HA reduced their protein levels significantly in IL-1β-stimulated cells (Fig. 2B). Unlike HMW-HA treatment, oligo-HA further increased the IL-1β-induced MMP-1 and MMP-13 expression at both mRNA and protein levels (Fig. 2C and D). Our data were consistent with that IL-1β-mediated up-regulation of MMP-1 and MMP-13 was inhibited by HMW-HA and enhanced by oligo-HA.

COX-2 plays a major role in joint inflammation (Afif et al., 2007). In our experiment, COX-2 expression levels were induced by IL-1β treatment (Fig. 3). Pre-treatment with HMW-HA abolished the IL-1β-mediated up-regulation of COX-2 expression levels (Fig. 3A and C). In contrast, SW-1353 cells treated with oligo-HA (10 μg/mL) prior to IL-1β exhibited a further increase in COX-2 expression at both mRNA and protein levels (Fig. 3B and C). These results are consistent with an anti-inflammatory effect of HMW-HA and pro-inflammatory effect of oligo-HA in chondrocytes.

3.3. Differential effects of HMW- and oligo-HA on the expression of PPARγ

Because of anti-inflammatory and chondroprotective properties of PPARγ (Afif et al., 2007), we investigated the effect of hyaluronan on PPARγ expression levels in chondrosarcoma SW-1353 cells. HMW-HA significantly augmented PPARγ mRNA and protein expressions in a dose dependent manner in the IL-1β-treated cells (Fig. 4A and C). In contrast, oligo-HA significantly decreased PPARγ expression at both mRNA and protein levels (Fig. 4B and C). It is consistent with an anti-inflammatory role for HMW-HA and pro-inflammatory activity for oligo-HA.

3.4. Effects of HMW- and oligo-HA on the phosphorylation of IκBα, Akt and MAPKs

In order to elucidate the mechanism underlying the modulation of IL-1β activity by HMW-HA and oligo-HA, we examined the activation of the key downstream signaling proteins, IκBα, Akt and MAPKs in SW-1353 cells (Fig. 5). By treatment with IL-1β alone, the levels of the examined signaling proteins in cells were not significantly changed up to 60 min. When co-treated with a high concentration (1 mg/mL) of HMW-HA, the cells expressed low levels of phosphorylated IκBα (p-IκBα) at 30 and 60 min, while oligo-HA at a low concentration (10 μg/mL) enhanced p-IκBα expression (Fig. 5A). This observation correlated well with the changes of total IκBα protein levels (Fig. 5B), and suggested

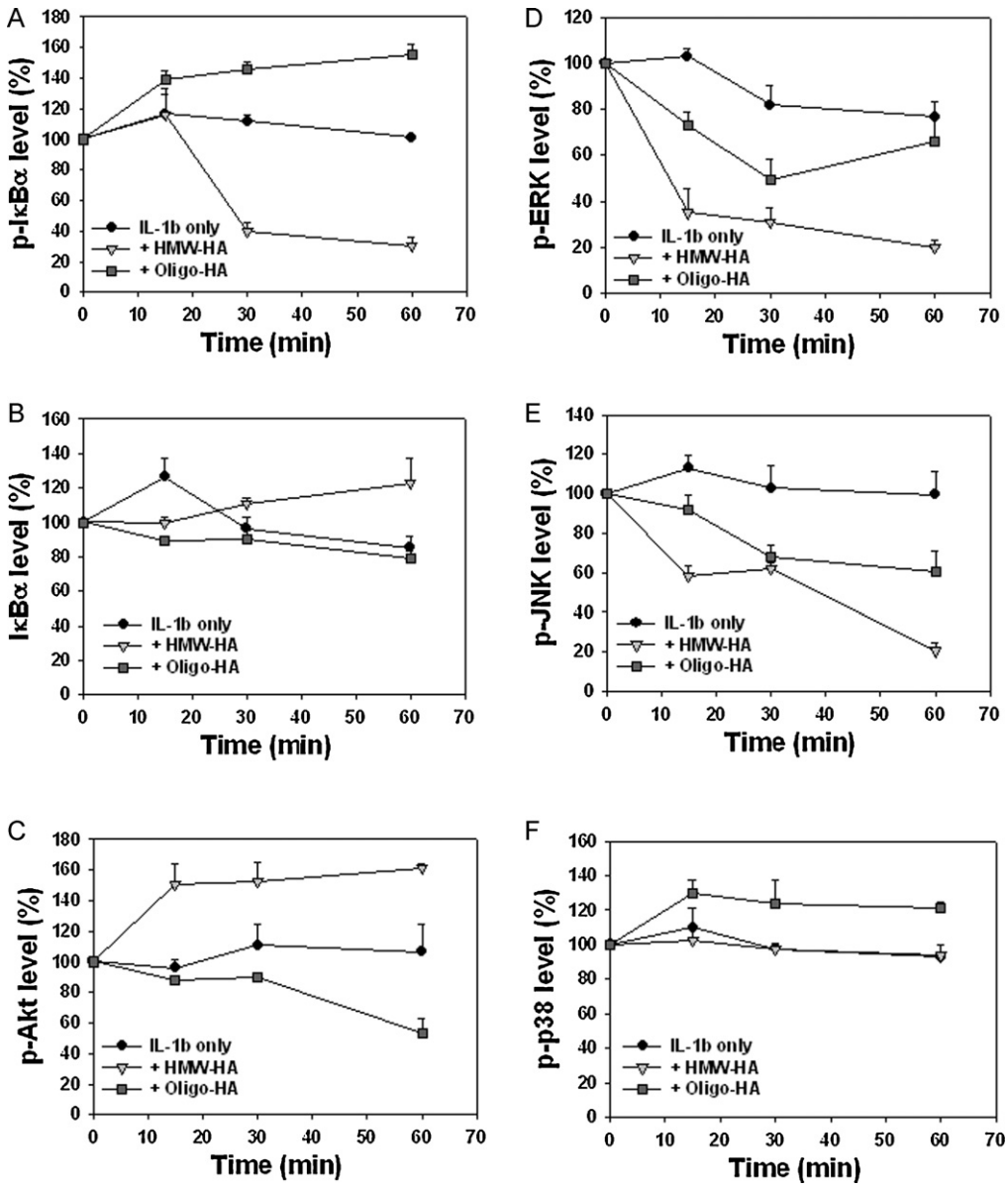

Fig. 5. Effects of hyaluronan on IL-1β-induced phosphorylation of IκBα, Akt and MAPKs in chondrosarcoma cells. Cells were pre-incubated with 1 mg/mL HMW-HA or 10 μg/mL oligo-HA for 30 min, and then stimulated with 2 ng/mL IL-1β for 15, 30 and 60 min. The protein levels were analyzed by Western blotting, and then quantified as described in Section 2. The level of α-tubulin was used as a loading control in Western blotting. The quantified results were normalized to those at time zero as 100%. (A) Phosphorylated IκBα (p-IκBα). (B) Total IκBα. (C) Phosphorylated Akt (p-Akt). (D) Phosphorylated ERK (p-ERK). (E) Phosphorylated JNK (p-JNK). (F) Phosphorylated p38 (p-p38).

that NF-κB activation may be inhibited by HMW-HA, and induced by oligo-HA.

Phosphorylated Akt was increased by the co-treatment with HMW-HA, while decreased by oligo-HA (Fig. 5C). It suggests that HMW-HA is able to induce Akt signaling, but the degraded form of hyaluronan (oligo-HA) at a moderate concentration may reverse this effect.

IL-1β signal transduction utilizes one of the three MAPKs: p38, ERK and JNK. To determine the IL-1β signaling pathway modulated by hyaluronan, the levels of phosphorylated p38, c-JNK and ERK in hyaluronan-pretreated IL-1β-stimulated SW-1353 cells were also determined by Western blotting analysis. Treatment with either HMW- or oligo-HA reduced phosphorylation of ERK and JNK (Fig. 5D and E), indicating similar effect of both types of hyaluronan on ERK and JNK activation. Interestingly HMW-HA had no effect on the phosphorylation of p38; however, oligo-HA increased phosphorylation of p38 (Fig. 5F).

4. Discussion

In this study, we investigated the role of HMW-HA and oligo-HA on modulation of IL-1β-induced expression of MMPs, COX-2, PPARγ and other inflammation-related genes in chondrosarcoma SW-1353 cells. In agreement with previous reports, HMW-HA suppressed the IL-1β-induced expression of MMP-1, MMP-13, and COX-2 (Mitsui et al., 2008; Tanaka et al., 2006). HMW-HA exerted an anti-inflammatory role through significant activation of Akt and suppression of NF-κB. Additionally we found that HMW-HA upregulated the expression of PPARγ.

In contrast to HMW-HA, oligo-HA induced production of inflammatory mediators in agreement with previous reports (Fieber et al., 2004). The pro-inflammatory activity of oligo-HA was further supported by evidence on activation of the NF-κB pathway in the cells. Oligo-HA arises due to destruction of tissue during injury. Its interaction with CD44 receptor and the toll like receptor 4 (TLR4)

on human chondrocytes both lead to NF-κB activation and the pro-inflammatory response (Campo et al., 2010b). Our data indicated that HMW-HA and oligo-HA have opposing influences on the inflammatory response of chondrosarcoma cells, in agreement with previous studies on human and mouse chondrocytes (Campo et al., 2010a; Ohno et al., 2006; Tanaka et al., 2006). Furthermore, to our knowledge, this is the first study showing the differential effects of HMW- and oligo-HA on PPARγ expression levels.

Recently, PPARs were strongly linked to the inflammatory response (Afif et al., 2007; Ramanan, Kooshki, Zhao, Hsu, & Robbins, 2008). Inducers of inflammation such as lipopolysaccharide (LPS) and TNFα activate NF-κB, and promote the secretion of several pro-inflammatory cytokines. PPARγ and its ligands block the NF-κB pathway, and modulate this inflammatory response (Ramanan et al., 2008). In this study, we showed that PPARγ expression was augmented by HMW-HA treatment. HMW-HA-treated cells with a suppressed NF-κB pathway, exhibited a reduced ability of the IL-1β stimulation to activate inflammation-related genes such as MMPs, and COX-2. It remains unknown whether PPARγ expression acts as a main mediator of the effects of hyaluronan. On the other hand, we demonstrated that PPARγ expression was suppressed by oligo-HA, resulting in activation of NF-κB along with a concomitant increase in inflammatory response-related gene expression.

The PI3K/Akt signaling pathway was recently shown to negatively regulate NF-κB-mediated inflammatory responses *in vitro*. Researchers showed that α-lipoic acid attenuates LPS-induced inflammatory responses by activating the PI3K/Akt signaling pathway in cultured human monocytic cells (Zhang, Wei, Hagen, & Frei, 2007). PI3K/Akt negatively regulated NF-κB subunit p65 transactivation induced by TLR4 activation in cardiac myocyte (Li et al., 2004). Recent study indicated that oligo-HA induced inflammation by engaging TLR4 in human chondrocytes (Campo et al., 2010b). Similar to glucosamine activation of Akt (Lin et al., 2008), our findings showed that treatment with HMW-HA promoted Akt phosphorylation and inhibited NF-κB activation. These data suggest an important role for HMW-HA in negatively regulating NF-κB activity by activation of the PI3K/Akt pathway. We also demonstrated that treatment with oligo-HA (10 μg/mL) inhibited PI3K/Akt pathway.

Members of the MAPK pathway, including p38, JNK and ERK, are known to regulate the expression of MMP-1, MMP-13, as well as other MMPs (Ancha et al., 2007). Activation of kinases in the MAPK pathway, such as p38, is necessary for IL-1β-induced COX-2 gene expression *in vitro* and *in vivo* (Degousee et al., 2003). We showed that both HMW- and oligo-HA treatment resulted in a decrease in JNK and ERK phosphorylation in SW-1353 cells. This may be related to the suppression of some inflammation-related genes. We also showed that oligo-HA increased IL-1β-mediated p38 phosphorylation, suggesting that oligo-HA may augment the expression of inflammatory mediators through activation of p38-related pathways (Karna, Miltyk, Surazynski, & Palka, 2008; Ohno et al., 2006).

In conclusion, we demonstrated an anti-inflammatory role for HMW-HA in our model chondrosarcoma SW-1353 cells, while oligo-HA augmented the IL-1β inflammatory response, which was similar to the effects in osteoarthritis-associated inflammation. These distinct effects in SW-1353 cells may mimic their roles at the site of osteoarthritis and strongly support that HMW- and oligo-HA have opposite roles in the osteoarthritis-induced inflammatory response (Wobig et al., 1999). HMW-HA and oligo-HA also differentially modulated expression levels of MMPs which play important roles in the degradation of the ECM. Our data showed that these changes may be associated with modulation of PPARγ and activation of the PI3K/Akt signaling pathways.

The intra-articular injection of oligo- and HMW-HA has been used as supplementation for treatment of patients with OA (Wobig et al., 1999), but specific effects of HMW-HA treatment and endogenous oligo-HA on the inflammatory response have not been completely elucidated. Our results suggest that treatment with HMW-HA mediates its additional anti-inflammatory benefit to these patients *via* multiple mechanisms, and may support further clinical investigations on the use of these biopolymers.

Author's contributions

CCC designed research and wrote paper. MSH performed research and analyzed data. STL performed research. YHC performed research. CWC analyzed data. TKL analyzed data. PTH analyzed data. YFL analyzed data and wrote paper. CHC designed research and wrote paper.

Conflict of interest

The authors declare no conflict of interest.

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