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Retinoic acid blocks pro-inflammatory cytokine-induced matrix metalloproteinase production by down-regulating JNK-AP-1 signaling in human chondrocytes

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Abstract

The development of osteoarthritis (OA) has recently been implicated as a result of immune-mediated damage of chondrocytes and their supporting matrixes. Pro-inflammatory cytokines like interleukin (IL)-1 and tumor necrosis factor alpha (TNF-α) play pivotal roles in immunopathogenesis of OA. Because vitamins preserving anti-oxidative effects are suggested to provide protection in OA patients from joint damage, in the present study, we examined the effects and mechanisms of all-*trans* retinoic acid (*t*-RA) in suppressing pro-inflammatory cytokine-induced matrix metalloproteinases (MMPs) production in human chondrocytes. Chondrocytes were prepared from cartilage specimens of OA patients receiving total hip or total knee replacement. The protein concentration was measured by ELISA, the mRNA expression by reverse transcriptase-polymerase chain reaction, the protein expression by Western blotting, the transcription factor DNA-binding activity by electrophoretic mobility shift assay and the protein kinase activity by kinase assay. We showed that both MMP-1 and MMP-13 mRNA expression, protein production and enzyme activity induced by either IL-1 or TNF-α were suppressed by *t*-RA or different retinoid derivatives. The molecular investigation revealed that the *t*-RA-mediated suppression was likely through blocking p38 kinase and c-Jun N-terminal kinase-activator protein-1 signaling pathways. In contrast, *t*-RA had no effect on extracellular signal-regulated kinase activity, nuclear factor kappaB (NF-κB) DNA-binding activity and IκBα degradation. Furthermore, we showed that *t*-RA could reduce IL-1-induced TNF-α production in chondrocytes. Our results suggest that vitamin A may protect OA patients from pro-inflammatory cytokine-mediated damage of chondrocytes and their supporting matrixes.

Keywords: Osteoarthritis; Cytokines; Chondrocytes; Matrix metalloproteinases; C-Jun N-terminal kinase; Activator protein-1

1. Introduction

Osteoarthritis (OA) is a joint disorder that happens mainly in aged people. Although both biomechanical stress and ageing process have long been considered as two major factors contributing to OA, recent studies highlight the critical role of inflammation in pathogenesis of this disorder [1,2]. Evidence that suggests inflammatory process in OA progression can also be found in clinical observations such as the presence of joint swelling, joint effusion as well as resting stiffness of the involved joints. When synovial tissues of OA joints are examined, the hyperplasia of synovial lining cells, the deposition of immunoglobulins

Abbreviations: AP-1, activator protein-1; EMSA, electrophoresis mobility shift assay; ERK, extracellular signal-regulated protein kinase; 4-HPR, *N*-(4-hydroxyphenyl)retinamide; IκBα, I kappa B alpha; IL, interleukin; JNK, c-jun N-terminal kinase; mAb, monoclonal antibody; MMP, matrix metalloproteinase; NF-κB, nuclear factor kappa B; OA, osteoarthritis; RT-PCR, reverse transcriptase-polymerase chain reaction; *t*-RA, all-*trans* retinoic acid; TIMP-1, tissue inhibitor of matrix metalloproteinase 1; TNF-α, tumor necrosis factor alpha

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and the invasion of mononuclear cells can be readily observed [3]. Furthermore, the biomechanical stress by itself can induce inflammatory response in cartilage and lead to OA development [4,5].

Cytokines secreted from infiltrating mononuclear cells, synovial cells and chondrocytes play pivotal roles in cartilage destruction of OA. Both IL-1 and TNF-α are well-known pro-inflammatory cytokines that induce production of matrix enzymes that also damage cartilage [6,7]. In addition, these and other pro-inflammatory cytokines can induce chondrocyte apoptosis [8,9]. Products like nitric oxide and growth-related oncogene alpha chemokine, both are upregulated by pro-inflammatory cytokines in OA, are also shown to cause apoptosis of chondrocytes [10,11]. By genetic approaches, Meulenbelt et al. [12] recently showed that IL-1 gene cluster polymorphisms may be associated with the pathogenesis of OA involving hip joints.

It is generally accepted that vitamins preserving antioxidative properties such as vitamins A, C, D and E may provide benefits for several ageing-related degenerative disorders, including OA [13,14]. Animal studies also reveal that a diet supplemented with vitamins E, C, A, B6, B2 and selenium can help prevent or treat mechanically induced OA [15]. Because there is currently no effective yet specific therapy for OA, a great proportion of aged people are still choosing to take vitamins or sometimes are encouraged to take vitamins for preventing OA progression [16]. In this context, retinoic acid (RA), the most biologically active natural metabolite of vitamin A, has been shown to mediate a variety of cellular effects such as vertebrate development, cellular differentiation and homeostasis [17]. These properties make vitamin A an attractive candidate as a potential OA-protector. In the present study, we examined the possible effects and mechanisms of *t*-RA in pro-inflammatory cytokine-induced MMP production in human chondrocytes.

2. Materials and methods

2.1. Reagents

The recombinant human IL-1β and TNF-α were purchased from R&D (St. Paul, MN). Four retinoid derivatives, including *t*-RA, *N*-(4-hydroxyphenyl)retinamide (4-HPR), 9-*cis* RA and 13-*cis* RA were purchased from Sigma–Aldrich Chemical Company (St. Louis, MO). Polyclonal antisera against total extra-cellular signal-regulated kinase (ERK)1, ERK2, p38 and JNK (for Western blotting) were purchased from Santa Cruz Biotechnology (Santa Cruz, CA). The antibodies recognizing phosphorylated ERK, phosphorylated p38 and JNK (for kinase assays) were purchased from Cell Signaling (Beverly, MA). The JNK substrate, GST-c-Jun fusion protein, was a kind gift from Dr. S.-F. Yang (Academia Sinica, Taiwan). ELISA kits for determination of protein amounts or enzyme activities of MMP-1 and MMP-13 were purchased from Amersham-Pharmacia (Piscataway, NJ). ELISA kits for measuring concentrations of TNF-α or tissue inhibitor of matrix metalloproteinase 1 (TIMP-1) were purchased from R&D. Unless specified, the rest of the reagents were purchased from Sigma–Aldrich Chemical Company.

2.2. Preparation of chondrocyte specimens

The cartilage from, in average, 60–80 OA patients who received total knee or total hip joint replacement were obtained aseptically with prior approval of the Institutional Review Board, Tri-Service General Hospital. The preparation of first passage chondrocytes from cartilage was performed according to the published report [18]. In brief, the full thickness articular cartilage was removed from the underlying bone and cut into pieces of around 0.5 cm^2. After enzymatic digestion with 2 mg/ml Pronase (Calbiochem, La Jolla, CA) in serum-free DMEM/antibiotics (Gibco-BRL, Gaithersberg, MD) for 1 h at 37 °C in 5% CO_2 atmosphere, the specimens were then digested with collagenase I at 0.25 mg/ml in DMEM medium containing 5% fetal bovine serum for overnight. Finally, the cells in monolayer culture were suspended and cultured in DMEM medium containing 10% fetal bovine serum and antibiotics for 5–7 days before use.

2.3. ELISA

Standard ELISA methods were used to measure TNF-α, TIMP-1 and MMP protein concentrations as described by the manufacturers. The Biotrak™ activity assay system was used to determine the enzyme activities of MMPs (Amersham-Pharmacia) in harvested supernatants according to the instructions of the manufacturers. All determinants were performed in duplicates or triplicates and expressed as means ± S.D.

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

The RT/PCR was performed as follows. In brief, total RNA was isolated after lysing the cells by Trizol (Gibco). After reverse transcription of RNA to cDNA, samples were subjected to PCR reactions. The primer designs and PCR reactions were performed as described by other reports [19]. The consensus primers for MMP-1 were 5′-CAC AGC TTT CCT CCA CTG CTG CTG C-3′ and 5′-GGC ATG GTC CAC ATC TGC TCT TGG C-3′; for MMP-3 were 5′-CCT CTG ATG GCC CAG AAT TGA-3′ and 5′-GAA ATT GGC CAC TCC CTG GGT-3′; for MMP-13 were 5′-GAC TTC ACG ATG GCA TTG CTG-3′ and 5′-GCA TCA ACC TGC TGA GGA TGC-3′; for TIMP1 were 5′-CAC CCA CAG ACG GCC TTC TGC AAT-3′ and 5′-AGT GTA GGT CTT GGT GAA GCC-3′; for GPADH

were 5′-CCA CCC ATG GCA AAT TCC ATG GCA-3′ and 5′-TCT AGA CGG CAG GTC AGG TCC ACC-3′.

2.5. Nuclear extract preparation

Nuclear extracts were prepared according to our published work [20]. Briefly, the cells (in average, 2×10^6) were left at 4 °C in 50 μl of buffer A [10 mM HEPES, pH 7.9, 10 mM KCl, 1.5 mM $MgCl_2$, 1 mM dithiothreitol (DTT), 1 mM PMSF and 3.3 μg/ml aprotinin] for 15 min with occasional gentle vortexing. The swollen cells were centrifuged at 15,000 rpm, 3 min. After removal of the supernatants (cytoplasmic extracts), the pelleted nuclei was washed with 50 μl buffer A and subsequently, the cell pellets were resuspended in 20 μl buffer C (20 mM HEPES, pH 7.9, 420 mM NaCl, 1.5 mM $MgCl_2$, 0.2 mM EDTA, 25% glycerol, 1 mM DTT, 0.5 mM PMSF and 3.3 μg/ml aprotinin) and incubated at 4 °C for 30 min with occasional vigorous vortexing. Then the mixtures were centrifuged at 15,000 rpm, 30 min and the supernatants were used as nuclear extracts.

2.6. Electrophoretic mobility shift assay (EMSA)

The EMSA was performed as detailed in our previous report [20]. The oligonucleotides containing NF-κB binding site (5′-AGT TGA GGG GAC TTT CCC AGG C-3′) and activator protein 1 (AP-1) binding site (5′-CGC TTG ATG AGT CAG CCG GAA-3′) were purchased and used as DNA probes (Promega, Mandison, WI). The DNA probes were radio-labeled with [γ-^{32}p]ATP using the T4 kinase according to the manufacturer's instructions (Promega). For the binding reaction, the radio-labeled NF-κB or AP-1 probe was incubated with 5 μg of nuclear extracts. The binding buffer contained 10 mM Tris–HCl (pH 7.5), 50 mM NaCl, 0.5 mM EDTA, 1 mM DTT, 1 mM $MgCl_2$, 4% glycerol and 2 μg poly(dI-dC). The reaction mixture was left at room temperature to proceed with binding reaction for 20 min. The final reaction mixture was analyzed in a 6% non-denaturing polyacrylamide gel with 0.5× Tris–borate/EDTA (TBE) as an electrophoresis buffer.

2.7. Western blotting

ECL Western blotting (Amersham-Pharmacia) was performed as described [21]. Briefly, after extensive wash, the cells were pelleted and resuspended in lysis buffer. After periodic vortexing, the mixture was centrifuged and the supernatant was collected and the protein concentration measured. Equal amounts of whole cellular extracts were analyzed on 10% SDS-polyacrylamide gel electrophoresis (SDS-PAGE) and transferred to the nitrocellulose filter. For immunoblotting, the nitrocellulose filter was incubated with TBS-T containing 5% non-fat milk (milk buffer) for 2 h, and then blotted with antisera against specific proteins for another 2 h at room temperature. After washing with milk buffer twice, the filter was incubated with goat anti-mouse IgG conjugated to horseradish peroxidase at a concentration of 1:5000 for 30 min. The filter was then incubated with the substrate and exposed to X-ray film.

2.8. Immunoprecipitation kinase assay

This assay has been described in our previous work [20]. In brief, the whole cellular extract 50–100 μg was incubated with 5 μl of specific antibody in incubation buffer containing 25 mM HEPES (pH 7.7), 300 mM NaCl, 1.5 mM MgCl2, 0.2 mM EDTA, 0.1% Triton X-100, 20 mM β-glycerophosphate, 0.1 mM Na_3VO_4, 2 μM leupeptin and 400 μM PMSF overnight. The mixture was then immunoprecipitated by addition of protein A beads and rotated at 4 °C for 2 h. After extensive wash, twice with HEPES washing buffer containing 20 mM HEPES (pH 7.7), 50 mM NaCl, 2.5 mM $MgCl_2$, 0.1 mM EDTA and 0.05% Triton X-100, twice with LiCl washing buffer containing 500 mM LiCl, 100 mM Tris (pH 7.6), 0.1% Triton X-100 and 1 mM DTT and twice with kinase buffer containing 20 mM MOPS (pH 7.2), 2 mM EDTA, 10 mM $MgCl_2$, 0.1% Triton X-100 and 1 mM DTT, the beads were resuspended in 40 μl kinase buffer with addition of cold ATP (30 μM), 8 ng of substrate (GST-c-jun) and 10 μCi of [γ-^{32}P]ATP. The mixture was incubated at 30 °C with occasional gentle mixing for 30 min. The reaction was then terminated by resuspending in 1% SDS solubilizing buffer and boiled for 5 min and analyzed in SDS-PAGE.

2.9. Statistical analysis

When necessary, the results were expressed as mean ± S.D. Unpaired Student's *t*-test was used to determine the difference which was thought to be significant when $P < 0.05$.

3. Results

3.1. t-RA inhibited MMP-1 and MMP-13 gene expression and protein production induced by IL-1 and TNF-α

Human chondrocytes were pre-incubated in the presence or absence of various concentrations of *t*-RA and then stimulated with IL-1 or TNF-α. After treatment, the total cellular RNA of each sample was obtained and RT/PCR was performed to evaluate the expression of MMP-1, MMP-13, MMP-3, TIMP-1 and GAPDH genes. As shown in Fig. 1A, *t*-RA effectively down-regulated the IL-1-induced expression of both MMP-1 and MMP-13 genes but had ignored effect on MMP-3 and TIMP-1 gene expression. Similarly, *t*-RA inhibited both MMP-1 and

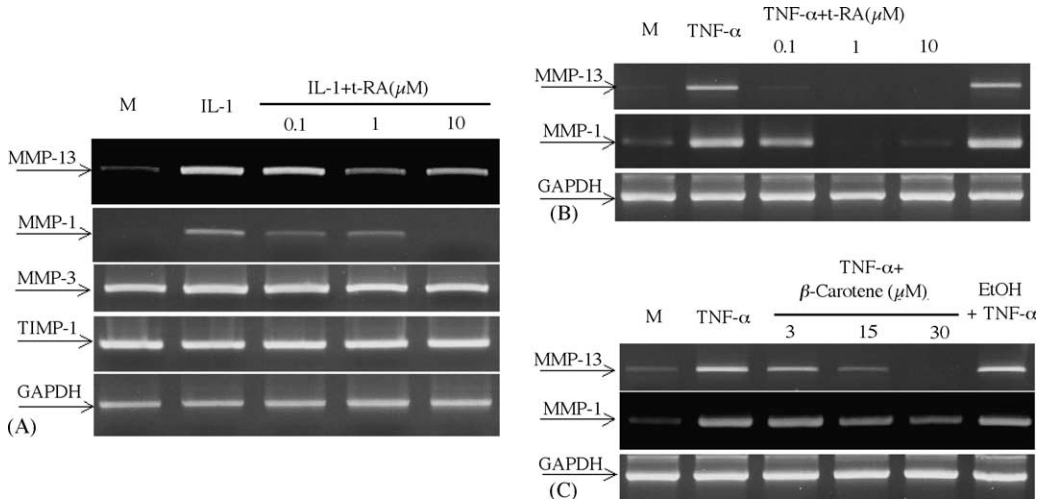

Fig. 1. Regulation of pro-inflammatory cytokine-induced MMPs and TIMP-1 gene expression by *t*-RA. Chondrocytes, 3×10^5 for each condition, were treated with various doses of *t*-RA (A and B) or beta-carotene (C) or ethanol (B and C) for 72 h and then stimulated with IL-1 (5 ng/ml) or TNF-α (10 ng/ml) for 72 h. The total RNA was prepared and RT/PCR was performed to determine mRNA expression of individual proteins. The representative data out of at least three independent experiments are shown.

MMP-13 gene expression induced by TNF-α stimulation (Fig. 1B). In consistent, beta-carotene, a pro-vitamin A, also effectively reduced TNF-α-induced MMP-1 and MMP-13 gene expression (Fig. 1C). In addition, *t*-RA significantly suppressed the IL-1-induced MMP-1 (Fig. 2A) and MMP-13 (Fig. 2B) protein production but did not affect TIMP-1 protein levels (Fig. 2C). At 1 and 10 μM concentrations, *t*-RA could suppress around 50–60% of control levels of both MMP-1 and MMP-13 protein production. Furthermore, the 80–100% of enzymatic activities of both MMP-1 and MMP-13 induced by IL-1 were also suppressed by *t*-RA (data not shown). Similarly, in addition to the suppression of TNF-α-induced gene expression, *t*-RA also inhibited the TNF-α-induced protein production and enzyme activities of both MMP-1 and MMP-13 (data not shown).

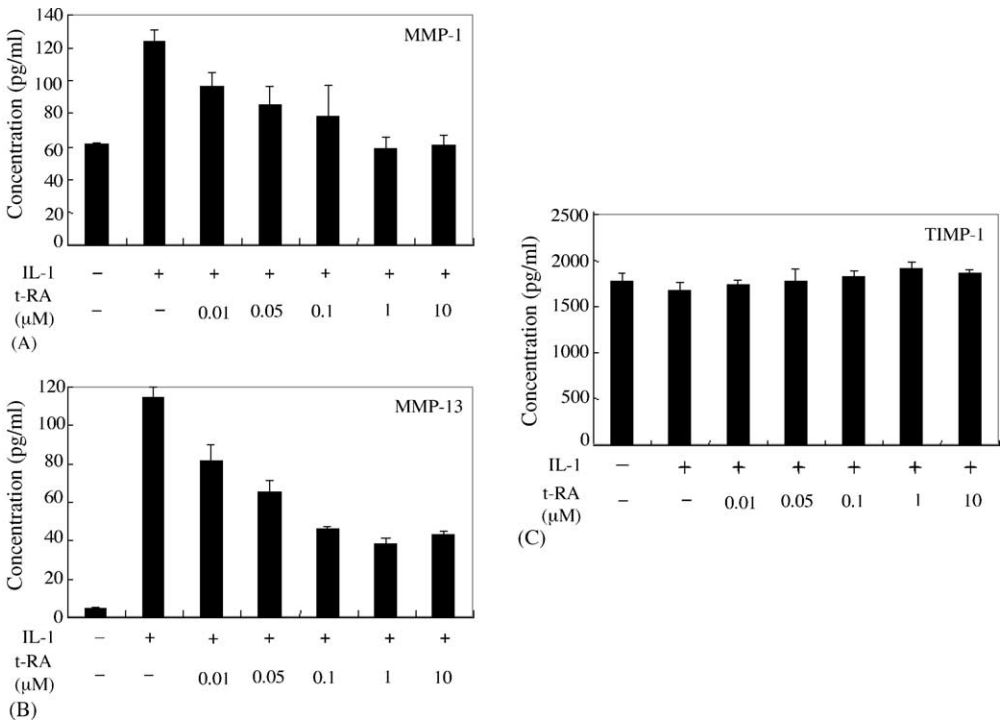

Fig. 2. Inhibition of IL-1-induced MMP protein production by *t*-RA. After pretreatment with *t*-RA at various doses for 72 h, chondrocytes were stimulated with IL-1 or not for 72 h and then the supernatants were collected for the measurement of MMP-1 (A), MMP-13 (B) and TIMP-1 (C) concentrations. The representative data out of at least three independent experiments are shown.

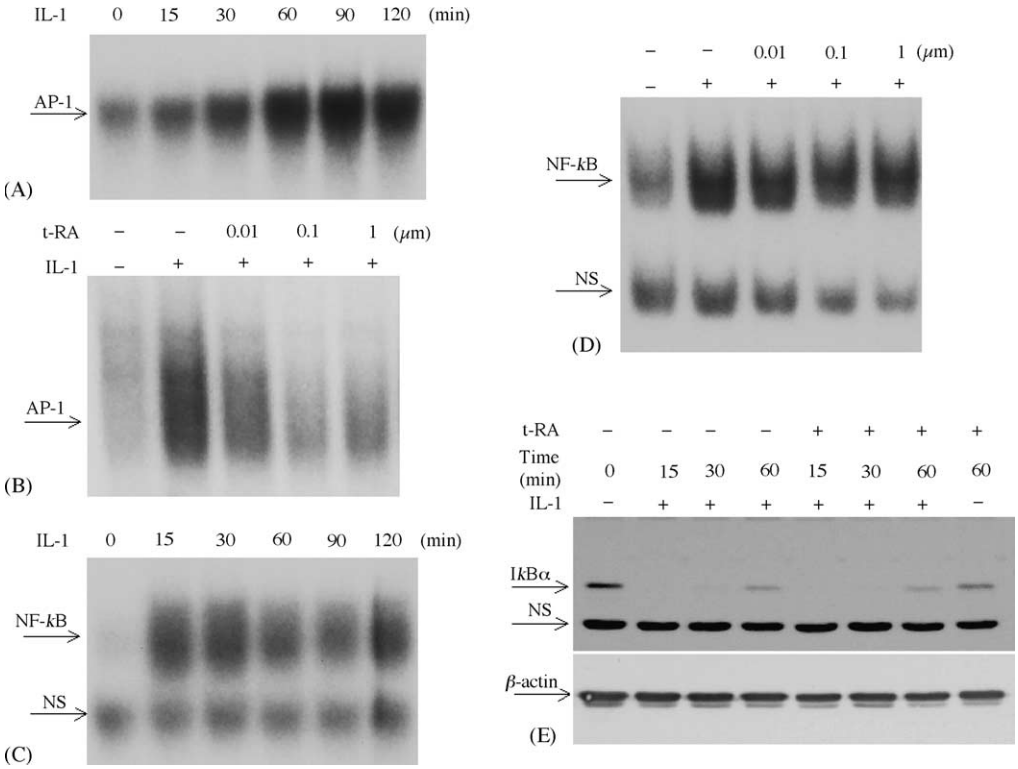

Fig. 3. Suppression of IL-1-induced AP-1 but not NF-κB activation by *t*-RA. Chondrocytes, 1–2 × 10^6 for each condition, were pretreated or not with various doses of *t*-RA and then stimulated with IL-1 for various time points (A and C) or for 2 h (B and D). The DNA-binding activities of AP-1 (A and B) and NF-κB (C and D) were determined with EMSA. In (E), chondrocytes were stimulated, in the presence or absence of *t*-RA (0.1 μM) pretreatment, with IL-1 for different time points and both IκBα and β-actin levels were determined by Western blotting; NS, non-specific. The representative data out of at least three independent experiments are shown.

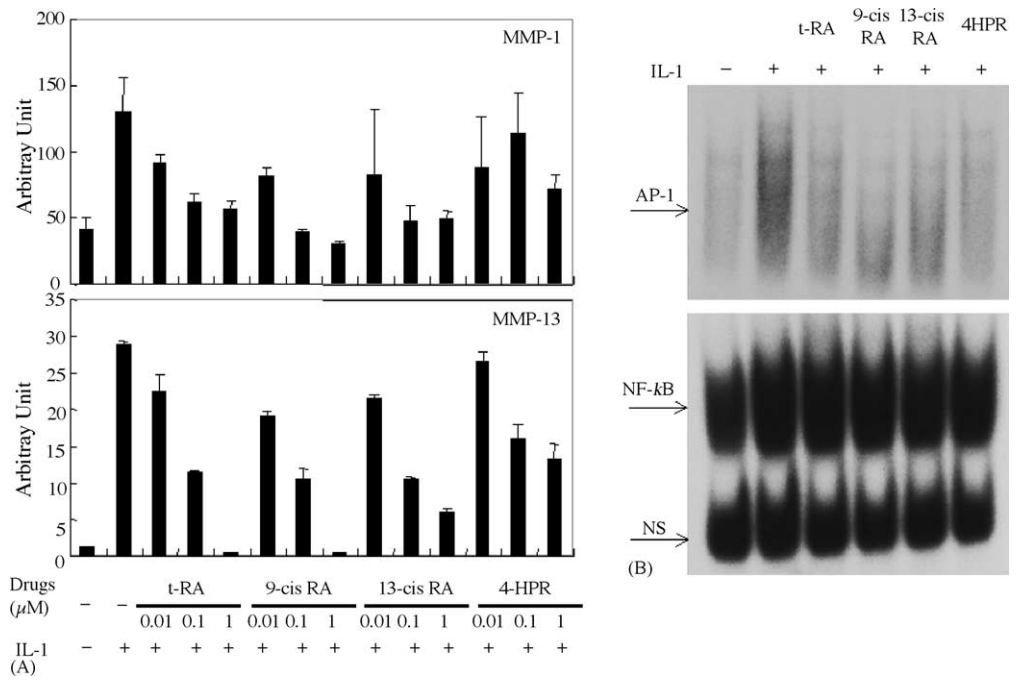

Fig. 4. Suppression of IL-1-induced MMP enzyme activities and AP-1 DNA-binding activities by retinoid derivatives. Chondrocytes were pretreated with various doses of four retinoid derivatives and then stimulated with IL-1 for 72 h (A) or for 2 h (B). In (B), the concentration of retinoid derivatives was 1 μM. The supernatants were collected and the enzyme activities of MMP-1 and MMP-13 were determined (A). The cellular nuclear extracts in individual conditions were analyzed for DNA-binding activities of AP-1 and NF-κB with EMSA (B). The representative data out of at least three independent experiments are shown.

3.2. t-RA inhibited IL-1-induced AP-1 but not NF-κB DNA-binding activities

The molecular mechanisms of *t*-RA-mediated suppression of IL-1 effects were investigated. We focused on examining the possible effects of *t*-RA on two families of transcription factors, namely NF-κB and AP-1, which are commonly involved in pro-inflammatory cytokine-mediated signaling pathways in a variety of tissue cells. As shown in Fig. 3A, IL-1 induced strong AP-1 DNA-binding activities in chondrocytes with a peak effect detectable 90 min after stimulation. In the presence of *t*-RA, such an activity was significantly suppressed (Fig. 3B). In contrast, although NF-κB DNA-binding activity was also induced by IL-1 (Fig. 3C), it was not inhibited by *t*-RA (Fig. 3D, compare both specific and non-specific band intensities). In consistent, *t*-RA did not reverse IκBα degradation induced by IL-1 (Fig. 3E). Similar conclusions were obtained when TNF-α was used as stimuli instead (data not shown).

3.3. Suppression of IL-1-induced MMP-1 and MMP-13 enzymatic activities by different retinoid derivatives

To further examine the suppressive effects of vitamin A on IL-1-induced MMPs, we included examining another three retinoid derivatives, namely 9-*cis* RA, 13-*cis* RA and 4-HPR. Consistently, the IL-1-induced MMP-1 and MMP-13 protein productions (data not shown) and enzyme activities were effectively suppressed by four retinoid derivatives (Fig. 4A). The subsequent experiments further demonstrated that all four retinoid derivatives could block IL-1-induced AP-1 but not IL-1-induced NF-κB DNA-binding activities (Fig. 4B).

3.4. Inhibitory effects of t-RA on IL-1- and TNF-α-induced MAP kinase activation

Because the activation of AP-1 relies on its upstream kinases, we determined the effects of *t*-RA on MAP kinases, including ERK, p38 and JNK activities induced by IL-1. As shown in Fig. 5A, although IL-1 greatly induced ERK activity, it was not suppressed by *t*-RA. In contrast, *t*-RA suppressed IL-1-induced phosphorylated p38 protein levels (Fig. 5B) and JNK activities (Fig. 5C) determined by Western blotting and immunoprecipitation kinase assays, respectively. Consistently, the TNF-α-induced activation of both p38 and JNK was also suppressed by *t*-RA (Fig. 6A and B). In comparison, *t*-RA seemed to have relatively stronger suppressive effect on TNF-α-induced than on IL-1-induced enzyme activities of these MAP kinases. Altogether, these results demonstrated that *t*-RA could inhibit both IL-1- and TNF-α-induced activation of chondrocytes through at least down-regulating p38 and JNK-AP-1 signaling pathway.

3.5. t-RA reduced TNF-α production induced by IL-1

In order to enhance the conclusions of this study, we also examined whether *t*-RA could inhibit the production of TNF-α-induced by IL-1 stimulation in chondrocytes. As shown in Fig. 7, while the solvent ethanol pretreatment did not show any suppressive effect, *t*-RA pretreatment effec-

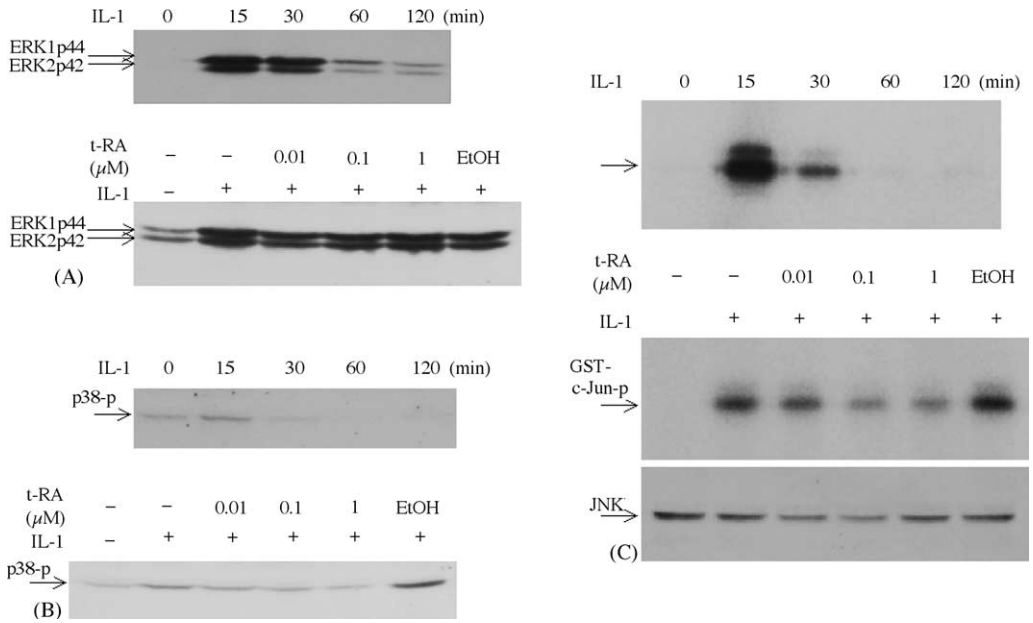

Fig. 5. Inhibition of IL-1-induced p38 and JNK activities by *t*-RA. Chondrocytes were pretreated or not with various concentrations of *t*-RA and then stimulated with IL-1 for various time points or for 15 min (un-specified) as indicated. The total cellular lysates were prepared and the kinase activities were determined by either Western blotting (A and B) or kinase assays (C). The total amount of JNK was measured by Western blotting (C, lower panel). p38—p stands for phosphorylated p38. The representative data out of at least three independent experiments are shown.

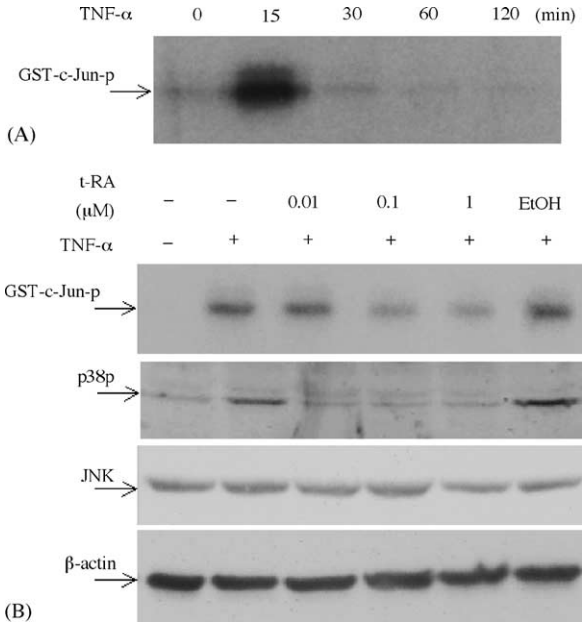

Fig. 6. Inhibition of TNF-α-induced p38 and JNK activities by *t*-RA. Chondrocytes were pretreated or not with various concentrations of *t*-RA and then stimulated with TNF-α for various time points as indicated (A) or for 15 min (B). The cellular extracts were prepared and the kinase activities for p38 and JNK were measured. The representative data out of at least three independent experiments are shown.

tively reduced IL-1-induced TNF-α production in a dose-dependent manner in chondrocytes.

4. Discussion

There are evidences suggesting that some vitamins may not only help relieve the symptoms of OA but also bring a favorable influence on the course of OA [14]. The major drawback to conclude the therapeutic benefits of taking vitamins in OA patients has been the requirement of long-term clinical observations. In this context, the evidence from basic research provides an alternative yet reasonable clue for considering the potential therapeutic benefits of vitamins in OA. In this study, we examined the possible effects and mechanisms of vitamin A in pro-inflammatory cytokine-induced production and activation of MMPs in human chondrocytes. The results indicated that physiological concentrations of vitamin A appeared to be enough to provide certain protection from the immunopathogenesis of OA. Our observations further demonstrated that the suppression of MMPs by *t*-RA might be associated with the inhibition of p38 and JNK-AP-1 signaling pathways. In contrast, the NF-κB signaling seemed not to be involved in *t*-RA-mediated suppression of IL-1-induced MMP protein production and enzyme activities in chondrocytes.

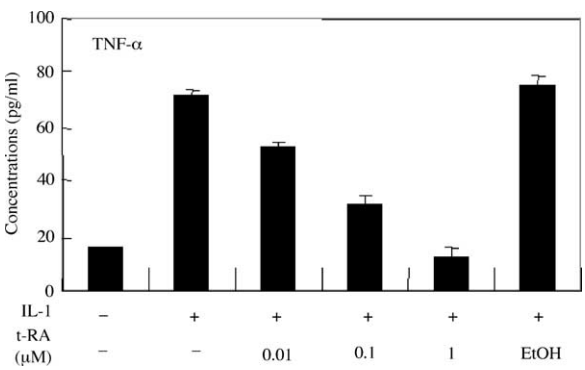

Fig. 7. Inhibition of IL-1-induced TNF-α production by *t*-RA. After pretreatment with the solvent ethanol or *t*-RA at various doses for 72 h, chondrocytes were stimulated with IL-1 or not for 72 h and then the supernatants were collected for the measurement of TNF-α concentrations. The representative data out of at least three independent experiments are shown.

IL-1 has been considered to be the most critical pro-inflammatory cytokine in mediating joint damage [22]. At lower concentrations in the tens of picomolar range, IL-1 effectively inhibits matrix synthesis by cartilage; higher concentrations of IL-1 cause direct matrix breakdown [23]. Studies in animal models reveal that transection of the anterior cruciate ligament, one of the mechanisms responsible for OA development, induces IL-1 production in both synovium and cartilage of dogs [24]. The cartilage damage induced in this model can be successfully prevented by injection of a recombinant IL-1 receptor antagonist [25]. In ex vivo studies using human cartilage, researchers demonstrated that the production of nitric oxide, MMPs and prostaglandin E_2, commonly involved in OA pathogenesis, is effectively blocked by the injection of recombinant type II IL-1 decoy receptor [26–28]. Furthermore, immunohistochemistry analysis performed in human OA knees reveals tense expression of IL-1β in OA cartilage but not in OA synovium [29]. Moreover, the IL-1 staining is particularly strong in chondrocytes where the proteoglycan in cartilage areas has been depleted [29,30]. Therefore, in light of the significance of IL-1 in OA pathogenesis, the inhibition of IL-1-induced MMP protein production and enzyme activities by *t*-RA and other different retinoid derivatives in chondrocytes should conclude a therapeutic potential of vitamin A in prevention of OA joint damages.

Through binding to nuclear receptors such as retinoic acid receptor (RAR) and retinoid X receptor (RXR), RA regulates many genes that contain RA-responsive elements [31,32]. Because of ligand specificity of different retinoid derivatives, the suppressive effects of *t*-RA used in most of this study have been shown mainly through interaction with RAR but not RXR [32]. After receiving signals from RA, RAR binds c-Jun or interferes c-Jun DNA-binding and blocks the transactivation of respective genes by c-Jun/c-Fos [33–36]. However, different mechanisms may be involved in different tissue cells in response to RA treatment. For example, RAR suppressing AP-1 activities through disrupting c-Jun/c-Fos dimerization is only observed in Hela cells but not in Cos cells nor in yeasts [37]. In human monocytic cells, RA inhibits LPS-induced tissue factor gene transcription through a mechanism that

does not involve suppression of AP-1 activities [38]. In addition, RA inhibits expression of the cartilage phenotype in epiphyseal chondrocytes via positive regulation of AP-1-responsive MMP genes and induction of c-fos and c-Jun gene expression [39]. In the present report examining human chondrocytes, we provide evidence that RA might suppress pro-inflammatory cytokine-mediated cartilage and matrix damages through a mechanism of suppression of AP-1 signaling pathway.

The inhibition of IL-1-induced p38 and JNK activities by *t*-RA further suggests that the underlying suppressive mechanisms might also involve more upstream molecules rather than simply interfere AP-1 activities through RAR. Indeed, both RAR-dependent and RAR-independent mechanisms have been observed in RA-mediated pharmacological effects [40–42]. In this context, using mesangial cells, Xu et al. [43] demonstrated the induction of MAPK phosphatase 1 by *t*-RA that results in inhibition of JNK activities and prevention of cellular apoptosis. The inhibition of JNK activities by *t*-RA might also provide evidence of additional benefits for vitamin A in OA because aside from induction by pro-inflammatory cytokines, JNK can also be induced under mechanical compression of cartilage [44].

Depending on the use of different tissue cell models, the role of NF-κB in inflammatory cytokine-induced MMP production is somewhat disputable. We and other researchers demonstrated that both TNF-α and IL-1-induced NF-κB activation could not be suppressed by retinoids and chitinase 3-like protein human cartilage glycoprotein 39 in human chondrocytes ([45] and this report). However, through genetic approaches, there are also evidence suggesting that NF-κB is required for the induction of MMPs in human rheumatoid arthritis synovial fibroblasts, human umbilical vein endothelial cells and chondrosarcoma cells [46,47]. It suggests that the use of different tissue cells and different model systems may come up with different results. Given the existence of these arguments, our results demonstrating that through blocking JNK-AP-1 signaling events, *t*-RA inhibited both IL-1 and TNF-α-induced MMP-1 and MMP-13 protein production and enzyme activities in human chondrocytes should provide useful scientific evidence that is close to the disease and therapeutic status in humanbeings.

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