Inhibitory effect of JAK inhibitor on mechanical stress-induced protease expression by human articular chondrocytes

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Abstract

Objective. To investigate whether janus kinase (JAK) inhibitor exhibites a chondroprotective effect against mechanical stress-induced expression of a disintegrin and metalloproteinase with thrombospondin motifs (ADAMTS) and matrix metalloproteinase (MMPs) in human chondrocytes.

Materials and methods. Normal human articular chondrocytes were seeded onto stretch chambers and incubated with or without tofacitinib (1000 nM) for 12 h before mechanical stimulation or cytokine stimulation. Uni-axial cyclic tensile strain (CTS) (0.5 Hz, 10% elongation, 30min) was applied and the gene expression levels of type II collagen α1 chain (*COL2A1*), aggrecan (*ACAN*), *ADAMTS4*, *ADAMTS5*, *MMP13*, and runt-related transcription factor 2 (*RUNX-2*) were examined by real-time polymerase chain reaction. Nuclear translocation of RUNX-2 and nuclear factor-κB (NF-κB) was examined by immunocytochemistry, and phosphorylation of mitogen-activated protein kinase (MAPK) and signaling transducer and activator of transcription (STAT) 3 was examined by western blotting. The concentration of interleukin (IL)-1β, IL-6 and tumor necrosis factor -α in the supernatant were examined by enzyme-linked immunosorbent assay.

Results. *COL2A1* and *ACAN* gene expression levels were decreased by CTS, but these catabolic effects were canceled by tofacitinib. Tofacitinib significantly down-regulated CTS-induced expression of *ADAMTS4*, *ADAMTS5*, *MMP13* and *RUNX2* ~~in chondrocytes~~, and the release of IL-6 in supernatant by chondrocytes. Tofacitinib also reduced CTS-induced nuclear translocation of RUNX-2 and NF-κB, and

phosphorylation of MAPK and STAT3.

Conclusion. *Tofacitinib suppressed mechanical stress-induced expression of ADAMTS4, ADAMTS5, and MMP13* by human chondrocytes through inhibition of the JAK/STAT and MAPK cascades.

Keywords

Janus kinase, Chondrocyte, Mechanical stress, Tofacitinib, Rheumatoid arthritis

Introduction

Oral low-molecular-weight compounds that inhibit janus kinase (JAK) (1) and spleen tyrosine kinase (2) have been introduced as targeted synthetic disease-modifying anti-rheumatic drugs (DMARDs) and have demonstrated good therapeutic efficacy in terms of rheumatoid arthritis (RA) disease progression. JAKs constitute a family of non-receptor tyrosine kinases of approximately 130-kDa comprising JAK1, JAK2, JAK3, and Tyk2. Cytokine binding causes two separate receptor polypeptide chains to dimerize, bringing together the associated JAKs, which then phosphorylate each other on tyrosines to become fully activated. The activated JAKs then phosphorylate various intracellular transmitters such as signaling transducer and activator of transcription (STAT) proteins and mitogen-activated protein kinases (MAPKs), including extracellular signal-regulated protein kinase (ERK), p38 MAPK, and c-Jun N-terminal kinase (JNK), thus controlling the expression of various genes in the nucleus (3, 4). Recently, it has been shown that Tofacitinib, a pan-JAK inhibitor, exhibits consistent efficacy in patients with an inadequate response and intolerance to conventional synthetic DMARDs (5, 6), while another study demonstrated that tofacitinib monotherapy had comparable effects to biologic DMARDs (7-9). However, there has been no report that examined the effects of tofacitinib on the protease expressions by chondrocytes.

Damaged articular cartilage is exposed to mechanical loading in inflammatory joint diseases such as RA and osteoarthritis, and the molecular mechanisms of cartilage breakdown in these diseases show considerable overlap, particularly with respect to

matrix-degrading enzymes, such as a number of matrix metalloproteinase (MMP) (10), however almost all clinical trials involving MMP inhibitors have failed, including a trial of a novel inhibitor targeting disease-relevant MMPs (11). We previously reported (12-14) that catabolic mechanical stress induced the expression of MMP, a disintegrin and metalloproteinase with thrombospondin motifs (ADAMTS), and other proteases in cultured human chondrocytes. Runt-related transcription factor 2 (RUNX-2) and inflammatory cytokines such as IL-1β play important roles in regulating expression of these proteases via nuclear factor-κB (NF-κB) and MAPKs. In the current study, we investigated the effects of tofacitinib on mechanical stress-induced expression of transcription factors and proteases in human chondrocytes. The results of the study suggest that tofacitinib may have a role for cartilage protection from further deterioration promoted by mechanical loading by inhibiting chondrocyte-derived matrix proteases.

Materials and Methods

Cells and cell culture

Normal human articular chondrocytes from the knee joint (NHAC-kn) were purchased from Lonza (Walkersville, MD, USA). The cells were cultured in chondrocyte basal medium (Lonza) containing chondrocyte growth medium, fetal bovine serum, transforming growth factor-β, R^3 insulin-like growth factor, transferrin, insulin, gentamicin, and amphotericin-B (CDM™BulletKit®, Lonza) at 37°C. The medium was changed every 2 days, and NHAC-kn cells were used at passage three.

Cyclic tensile strain with or without JAK inhibitor tofacitinib

Tofacitinib (CP-690550) citrate was purchased from Selleck Chemicals (Houston, TX, USA), dissolved in dimethyl sulfoxide, and then diluted with phosphate-buffered saline (PBS) to 100 or 1000 nM. The maximum concentration (Cmax) when tofacitinib is administrated with 5mg twice daily (BID) (167nM) is higher than 100 nM, and 1000 nM of tofacitinib is equivalent to the serum Cmax (1006 nM) when tofacitinib is administrated with 30mg BID dose in human (18). We applied tofacitinib treatment for 1, 6, and 12 h before cyclic tensile strain (CTS), and found that the samples with 12h treatment had the most reproducible data. Thus, we used 12h treatment for all experiments in this study. NHAC-kn cells were seeded onto stretch chambers with a type I collagen-coated culture surface of 2 × 2 cm for isolation of RNA, and 3 × 3 cm for immunocytochemistry and protein isolation. CTS was applied using an ST-140 mechanical stretch system (STREX, Osaka, Japan). In this system, the chamber was

attached to the stretching apparatus, which has a fixed side opposite a movable side driven by a computer-controlled motor. This apparatus allows the entire silicon membrane to be stretched uniformly (15, 16). In the current study, CTS (0.5 Hz, 10% elongation) was applied for 30 min to cause catabolic stress, as described previously, (12-14, 17). The current particular force (10% elongation) and frequency (0.5Hz; one second on, one second off) has been used for analysis for chondrocyte metabolism, and reported that CTS with this condition for 30 min result in the catabolic reaction by chondrocytes with decreased type II collagen and aggrecan expression, and increased expression of MMP-3, -13, ADAMTS-4, and -5 (12-14). Cells incubated without mechanical stress were used as a control.

Reverse transcription PCR and real-time PCR analysis

The cells were washed three times with PBS at 1, 6, 12, and 24 h after CTS, and total RNA was isolated using an miRNeasy® Mini Kit (Qiagen, Hilden, Germany). RNA samples (1000 ng) were reverse-transcribed with ReverTra Ace (Toyobo, Osaka, Japan). The resulting cDNAs were used for PCR amplification in the presence of 10 pmol of specific primers using ExTaq DNA polymerase (TaKaRa, Ohtsu, Japan). The specific primers used are described in Table 1 (*RUNX2*, *ADAMTS4*, *ADAMTS5*, *MMP13*, type II collagen α1 chain [*COL2A1*], aggrecan [*ACAN*] and glyceraldehyde-3-phosphate dehydrogenase [*G3PDH*]). Each reverse transcription (RT) PCR reaction was allowed to proceed for 30–37 cycles.

Real-time PCR was performed using an Mx3000P QPCR System (Agilent Technologies, Santa Clara, CA, USA) with TaqMan Gene Expression Assays for human *ADAMTS4* (Hs00192708_m1), *ADAMTS5* (Hs00199841_m1), *MMP13* (Hs00233992_m1), *RUNX2* (Hs01047977_m1), and *G3PDH* (Hs03929097_g1) (Applied Biosystems, Foster City, CA, USA). Amplification of the housekeeping gene *G3PDH* was used to normalize the efficiency of cDNA synthesis and the amount of RNA. We calculated the final expression levels by dividing the expression levels of *ADAMTS4*, *ADAMTS5*, *MMP13*, and *RUNX2* by the expression level of *G3PDH*. Each value obtained for the control cells (unstretched cells without tofacitinib) was set to 1.

Immunocytochemistry

The mechanical stress-induced nuclear translocation of RUNX-2 and NF-κB p65 was examined by immunocytochemistry. Cells were stretched for 30 min with or without tofacitinib, according to the protocols described above, and then fixed with 1% paraformaldehyde solution. The membranes of the culture chambers were then removed and incubated with anti-RUNX-2 antibody (1:100, ab76956, Abcam, Cambridge, UK), and anti-NF-κB p65 antibody (1:100, C22B4, Cell Signaling, Danvers, MA, USA), for 2 h at room temperature. Bovine serum albumin-containing solutions without primary antibodies were used as negative controls. We used Alexa Fluor 488-conjugated antibody (10 μg/ml, anti-mouse/rabbit) as secondary antibodies, Alexa Fluor 568-conjugated phalloidin (2 μg/ml, Molecular Probes, Eugene, OR, USA) for actin staining, and Hoechst 33342 (1 μg/ml, ICN Biomedicals, Aurora, OH, USA) for nuclear

staining. Cells were observed under a fluorescence microscope (Leica, Wetzlar, Germany), and nuclear translocation was evaluated by the positive-cell ratios for RUNX-2 and NF-κB p65 (number of positive cells/all cells), calculated as the mean value from four fields at 100× magnification.

Western blot analysis

Cells were resuspended in Mammalian Protein Extraction Buffer (GE Healthcare, Piscataway, NJ, USA) 30 min after CTS. Cell lysates (15 μg total protein/lane) were loaded onto sodium dodecyl sulfate–polyacrylamide gels using BioRad Any kD™ Mini-PROTEAN® TGX™ Gels (Bio-Rad, Munchen, Germany) and run for 40 min at 150 V and then transferred to polyvinylidene difluoride membranes using a Trans-Blot® Turbo™ Blotting System (Bio-Rad). The membranes were incubated with blocking reagent (Toyobo) and incubated overnight at 4°C with antibodies to ERK, JNK, p38 MAPK (Abnova, Taipei, Taiwan), STAT3 (Santa Cruz Biotechnology, Santa Cruz, CA, USA), and phosphorylated ERK, JNK, p38, and STAT3 (Cell Signaling Technology, Beverly, MA, USA) at dilutions of 1:1000. After washing with washing buffer, the membranes were incubated with IRDye Goat Anti-Rabbit IgG (LI-COR Biosciences, Lincoln, NE, USA) or IRDye Goat Anti-Mouse IgG (LI-COR Biosciences) as secondary antibodies at room temperature for 1 h. Immunoreactive proteins were detected using the OdysseyFc Imaging System (LI-COR Biosciences). We also analyzed the densities of the obtained western blotting fragments using the

OdysseyFc Imaging System. The levels of phosphorylated p38, ERK, JNK, and STAT3 were indicated as ratios, and normalized to the densities of the p38, ERK, JNK, and STAT3 fragments.

ELISA for cytokines in the culture medium

Cell culture supernatants were collected at 12 and 24 h after CTS. The concentration of IL-1β, IL-6 and tumor necrosis factor -α (TNF-α) in the supernatant was measured using a high sensitivity IL-1β and IL-6 enzyme-linked immunosorbent assay (ELISA) kit (Quantikine® HS ELISA Human IL-1β/IL-1F2 Immunoassay, Quantikine® HS ELISA HumanIL-6 Immunoassay and Quantikine® HS ELISA Human TNF-α Immunoassay, R&D Systems, Inc., Minneapolis, MN, USA), according to the manufacturer's protocol. Cell culture supernatants without mechanical stress were used as a control.

Statistical analysis

The results are expressed as mean ± standard deviation. Statistical comparisons were performed using Student's *t*-tests. All experiments were repeated at least four times and similar results were obtained. All differences were considered statistically significant at a P value < 0.05.

Ethics approval

We didn't acquire any data from patients or animals in this study. Thus, the ethics approval was not required in accordance with the policy of our institution.

Results

Effects of tofacitinib on CTS-induced gene expression

We previously reported that *RUNX2* and *ADAMTS5* mRNA expression levels increased 1 h after CTS (early phase), *ADAMTS4* and *MMP13* increased 24 h after CTS (late phase), and *COL2A1* and *ACAN* levels decreased 15–30 h after CTS (12, 14). We therefore examined the effects of different concentrations of tofacitinib on the expression levels of *RUNX2* and *ADAMTS5* 1 h after CTS, and *COL2A1*, *ACAN*, *ADAMTS4*, and *MMP13* 24 h after CTS.

COL2A1 and *ACAN* expression levels were reduced by CTS, but these down-regulation were canceled by treatment with tofacitinib, as demonstrated by RT–PCR. In the absence of tofacitinib, CTS up-regulated *RUNX2*, *ADAMTS4*, *ADAMTS5*, and *MMP13* expression levels, but these were down-regulated in tofacitinib-treated samples. RT-PCR demonstrated a significant difference in *RUNX2* mRNA levels after treatment with 100 and 1000 nM tofacitinib, but no differences in *ADAMTS4*, *ADAMTS5*, and *MMP13* levels (Fig. 1A, B). As presented in Fig. 1B, 1000 nM of tofacitinib showed significantly stronger effect than 100 nM of tofacitinib on the

expression of *RUNX-2* mRNA, which has been demonstrated in our previous study to be an important transcription factor in regulation of *MMP-13* and *ADAMTS-5* in the similar experiment (12). We therefore used tofacitinib 1000 nM for the following experiments.

RUNX2 mRNA expression was increased 1 h after CTS (Fig. 2A), and *ADAMTS4* and *MMP13* were increased 24 h after CTS (Fig. 2B, D) in the absence of tofacitinib. *ADAMTS5* induction in the absence of tofacitinib was biphasic, with early and late phases at 1 and 12 h, respectively, after CTS (Fig. 2C). Both early- and late-phase increases were significantly down-regulated by treatment with tofacitinib (Fig. 2A–D). Significant differences were noted at 1 h for *RUNX2*, at 24 h for *ADAMTS4*, at 12 h for *ADAMTS5*, and at 24 h for *MMP13*.

Effects of tofacitinib on CTS-induced nuclear translocation of RUNX-2 and NF-κB p65 in chondrocytes

CTS induced RUNX-2 and NF-κB p65 translocation to the nucleus, as demonstrated by immunocytochemistry, but this effect was inhibited in cells treated with tofacitinib (Fig. 3A). The percentages of chondrocytes positive for nuclear RUNX-2 and NF-κB p65 were significantly decreased by treatment with tofacitinib (Fig. 3B).

Effects of tofacitinib on CTS-induced activation of MAPK and JAK/STAT pathways

Mechanical stress can activate stress-response signaling pathways, such as MAPK and

JAK/STAT. We therefore investigated MAPK (ERK, JNK, and p38) and STAT3 phosphorylation in the presence and absence of tofacitinib at 30 min after CTS. CTS significantly increased MAPK and STAT3 phosphorylation compared with non-stretched controls, as shown by western blot analysis, while tofacitinib inhibited the CTS-induced phosphorylation of ERK, JNK, p38, and STAT3 (Fig. 4A–D).

Effects of tofacitinib on CTS-induced expression of cytokines from chondrocytes

The concentration of IL-1β and IL-6 in the supernatant after CTS increased in a time-dependent manner in samples without tofacitinib, and the production of IL-6 24 h after CTS was significantly increased compared with that without CTS. The concentration of IL-6 in samples with tofacitinib treatment 12 and 24 h after CTS were significantly decreased compared with that in samples without tofacitinib treatment. In contrast, no significant effects of tofacitinib were seen on the concentration of IL-1β (Table. 2). The concentrations of TNF-α were under detection limit of ELISA in the current experimental protocol.

Discussion

Articular cartilage is exposed to proinflammatory cytokines and matrix proteinases derived from the synovial membrane in joints with active RA. Once cartilage degeneration has started, mechanical loading further promotes cartilage damage even if the synovitis is effectively treated by DMARDs. Larsen grade II joint destruction (19)

with joint-space narrowing represents a critical point, especially in weight-bearing large joints, and joints with pre-existing damage of Larsen grade III or higher are disposed to progressive joint destruction, despite tumor necrosis factor-blocking therapies (20). Early diagnosis and intervention with DMARDs aimed at suppressing synovitis before the initiation of cartilage damage are thus the optimal strategies (21, 22). In this study, we used normal chondrocytes to confirm the effects of JAK inhibitor on the mechanical-stress in the normal or less damaged cartilage. Clinically, we should prevent the cartilage degeneration by early treatment and control of disease activity, because damaged cartilage would not stand against mechanical stress especially in the weight-bearing joint.

JAKs play important roles in intracellular signal transduction for various cytokines (1, 3, 4). Tofacitinib is the first oral JAK inhibitor approved for the treatment of RA (23), and has been demonstrated to act on JAK1, JAK2 and JAK3. The dominant effects of tofacitinib are mediated through dimers of JAK1 and/or JAK3 (24, 25). Although the anti-inflammatory effects of tofacitinib on synovial inflammation have been demonstrated *in vitro* (7, 24) and among RA patients (9, 26), its direct effects on chondrocyte metabolism remain unclear. Tofacitinib has been reported to increase levels of adenosine, which possesses anti-inflammatory activity (27), in human chondrocytes, and partially to prevent over-glycosylation of collagen by inhibiting the IL-1β-stimulated production of procollagen-lysine, 2-oxoglutarate 5-dioxygenase 2, and regulate the IL-1β stimulated changes of protein profile in chondrocytes (28). These reports showed tofacitinib have potential chondro-protective effects, and gave support

to our result.

Mechanical stress is known to regulate cell metabolism via ion channels or cell surface integrins (29). Involvement of JAK/STAT activation in mechano-transduction has been identified in some cell types such as cardiomyocytes and fibroblasts (30, 31), but rarely in chondrocytes (32). In the current study, we showed that mechanical stress activated the JAK/STAT3 pathway. Furthermore, as we expected, tofacitinib reasonably inhibited ADAMTS-4, ADAMTS-5, and MMP-13 gene expression and suppressed the down-regulation of type II collagen and aggrecan by blocking at least the part of the downstream of mechanical stress-induced activation of JAK-mediated signaling. Obviously, our results suggest the beneficial effects derived by JAK-STAT3 pathway inhibition on chondrocyte metabolism under mechanical stress, and not by the specific effects of tofacitinib used in the study.

Aging is known to promote cartilage-matrix stiffening via the accumulation of advanced glycation end-products (AGEs) (33, 34). Huang et al. (35) previously reported that blocking the JAK/STAT3 signaling pathway with selective JAK-2 and JAK-3 inhibitors inhibited AGE-induced activation of MMP-13 and ADAMTS and prevented AGE-mediated decreases in collagen II and aggrecan. We speculate JAK inhibition by tofacitinib may also attenuate aging-related AGE-mediated protease expression by chondrocytes and might be a useful information for the development of the disease modifying osteoarthritis drugs.

Regarding changes in transcriptional activity caused by mechanical stress, Marcu et al. (36) reported that NF-κB was triggered by mechanical stress and that activated

NF-κB regulated the expression of several cytokines and matrix-degrading enzymes. In addition, the MAPK pathway, which involves ERK, JNK, and p38, is known to be modulated by various external stimuli (37, 38). We previously reported that the application of CTS to human chondrocytes induced nuclear translocation of NF-κB and RUNX-2 via activation of MAPKs such as ERK, p38, and JNK, and evoked downstream expression of proteases such as ADAMTS-4, ADAMTS-5, and MMP-13,(14-16). The present *in vitro* study demonstrated that tofacitinib inhibited protease expression, at least partly by inhibiting the mechanical stress-induced nuclear translocation and activation of NF-κB and RUNX-2.

The JAK-STAT pathway and STAT signaling have been shown to be activated in chondrocytes and chondrocyte-like cells, and to induce MMP expression via several cytokines, including IL-1β, oncostatin M (39), IL-7 (40), and IL-6/sIL-6R in combination with IL-1β (41). We previously reported that CTS (0.5 Hz, 10% elongation for 30 min) induced IL-1β production by human chondrocytes 12 h after stimulation (late phase) (14). In this study, the concentration of IL-1β in the supernatant after CTS increased at 12 after CTS, and this might influence on the biphasic expression of ADAMTS-5. However, tofacitinib did not show significant inhibition on CTS-induced IL-1β production by chondrocytes. Wang et al. reported that IL-6 was up-regulated by fluid-induced shear stress (42-44) via up-regulation of cyclooxygenase-2 (COX-2) and prostaglandin E_2 (PGE_2) production in chondrocytes (42). In this study the concentration of IL-6 in the supernatant significantly increased at 24h after CTS, and it was significantly decreased by treatment with tofacitinib. Thus, tofacitinib could inhibit

the IL-6-induced up-regulation of COX-2 and PGE_2 production. It might therefore be reasonable to speculate that tofacitinib may not inhibit the IL-1β-mediated catabolic effect directly, but may contribute to the reduction of NF-κB activation evoked by the cytokine loop after mechanical stress.

There were several limitations to this study. First, the stretching system used was a simple model because cells were cultured in monolayers. Thus, our findings of the current study are limited to the vitro effects of JAK-STAT signaling inhibition on CTS-induced ADAMTS / MMP production in monolayer cultured chondrocytes. The results might be different if investigated in chondrocytes cultured under three-dimensional condition and in tissue, or by other mechanical-stress such as shear and compression. Second, the study focused on the effects of tofacitinib mainly on the expression of ADAMTS-4, 5 and MMP-13 at mRNA levels in vitro, and did not investigate other selective JAK inhibitors, and whether such drugs might exert similar chondro-protective effects thus remains unknown. Third, the inflammatory condition within the RA joint is complicated, and factors other than mechanical stress were excluded in the current *in vitro* model. Fourth, it would be difficult to obtain the reproducible results in OA or RA tissue;.OA tissue may show various stages of cell differentiation and chondrocytes from RA tissue may be affected by cytokines or drug treatment.

In conclusion, JAK is involved in the mechanical stress-induced signal transduction pathway in chondrocytes. Our in vitro study showed that inhibition of JAK-mediated STAT and MAPK activation could ameliorate the expression of proteases in mechanical

stress-loaded human chondrocytes in vitro.

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Figure legends

Table 1.

Primer sequences used for RT–PCR

Table 2.

The effect of tofacitinib on the CTS induced expression of cytokines from chondrocyte. The data are presented as the mean ± standard deviation (n=6 per experimental group). Tofa, tofacitinib. *P < 0.01 relative to CTS (−), §P < 0.05 between Tofa (−) and Tofa (+)

Figure 1.

(A) Results of RT–PCR showing CTS-induced gene expression in chondrocytes treated with various concentrations of tofacitinib. CTS reduced the expression of *COL2A1* and *ACAN* and induced the expression of *RUNX2*, *ADAMTS4*, *ADAMTS5*, and *MMP13*. Tofacitinib up-regulated the expression of *COL2A1* and *ACAN* and down-regulated the CTS-induced *RUNX2*, *ADAMTS4*, *ADAMTS5*, and *MMP13* expression. PCR amplifications were repeated at least three times with similar results. (B) Results of real-time PCR (n=8 per experimental group) showing CTS-induced gene expression in chondrocytes treated with various concentrations of tofacitinib. Tofacitinib showed concentration-dependent effects on *RUNX2*, *ADAMTS4*, and *ADAMTS5* expression. Tofacitinib 1000 nM inhibited *RUNX2* expression significantly more than 100 nM. Tofa, tofacitinib. *P < 0.01

Figure 2.

Results of real-time PCR (n=8 per experimental group) showing effects of tofacitinib (1000 nM) on time-dependent changes in expression of *RUNX2*, *ADAMTS4*, *ADAMTS5*, and *MMP13* (A-D). CTS induced *RUNX2* expression after 1 h (A), *ADAMTS4* and *MMP13* after 24 h (B, D), and *ADAMTS5* after 1 h and 12 h (C). Tofacitinib significantly down-regulated these expressions at both early and late phases. Tofa, tofacitinib. *$P < 0.05$ relative to 0 h, **$P < 0.01$ relative to 0 h, $^{§}P < 0.01$ between Tofa (−) and Tofa (+), $^{§§}P < 0.01$ between Tofa (−) and Tofa (+)

Figure 3.

(A) Results of immunocytochemistry showing that tofacitinib (1000 nM) inhibited CTS-induced nuclear translocation of RUNX-2 and NF-κB p65 subunit in chondrocytes (30 min after CTS). In the absence of tofacitinib, RUNX-2 and NF-κB both translocated to the nucleus following CTS (green signals), but translocation was suppressed by tofacitinib. Right panels show merged images stained with Hoechst 33342. (B) Percentages of chondrocytes positive for nuclear RUNX-2 and NF-κB p65 subunit were significantly reduced by tofacitinib. Tofa, tofacitinib. *$P < 0.01$

Figure 4.

Fold increases in phosphorylation based on analysis of western blots by densitometry.

(A) Total and phosphorylated ERK (P-ERK), (B) total and phosphorylated JNK (P-JNK), (C) total and phosphorylated p38 (P-p38), and (D) total and phosphorylated STAT3 (P-STAT3). CTS up-regulated phosphorylation of ERK, JNK, p38, and STAT3, and these effects were down-regulated by tofacitinib. Tofa, tofacitinib. (n=4 per experimental group) *P < 0.05, **P < 0.01

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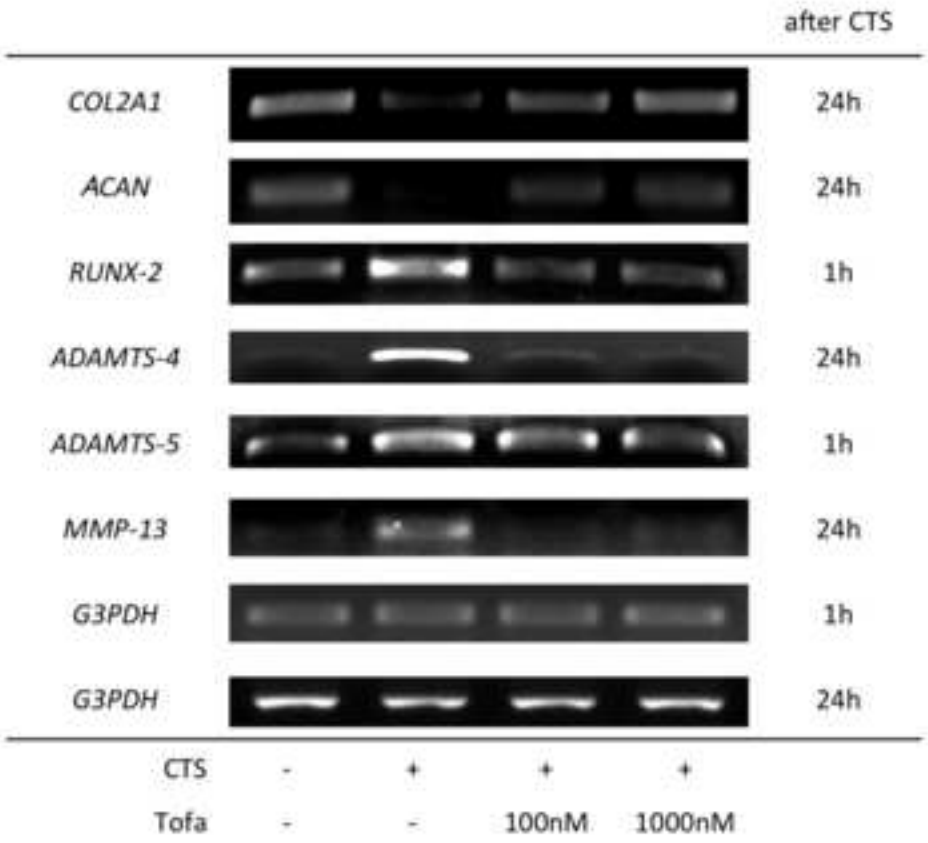
Figure 1.

A



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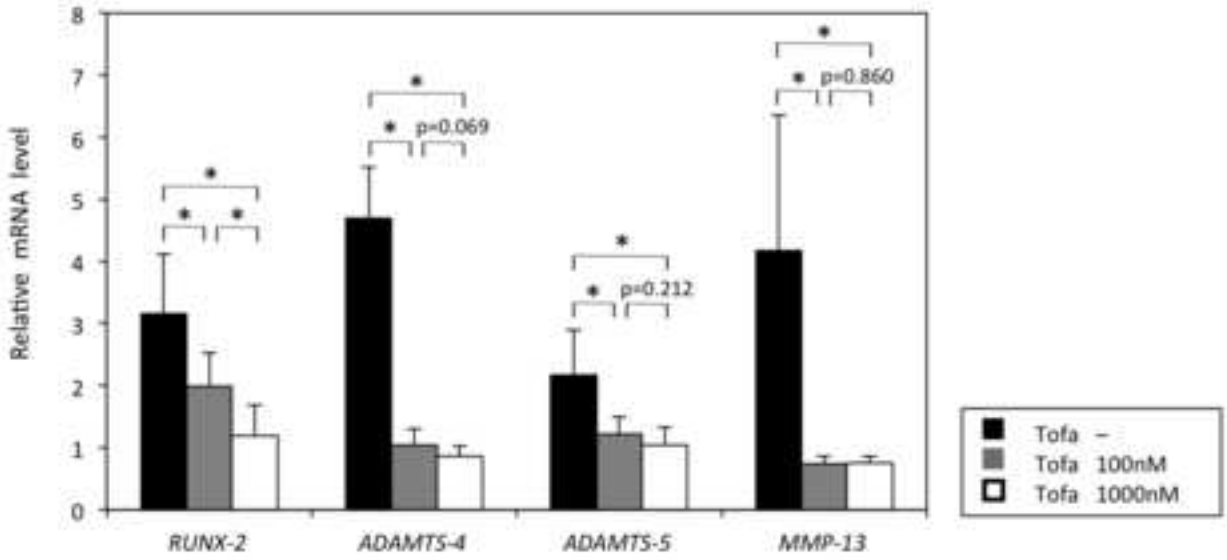

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Figure 2.

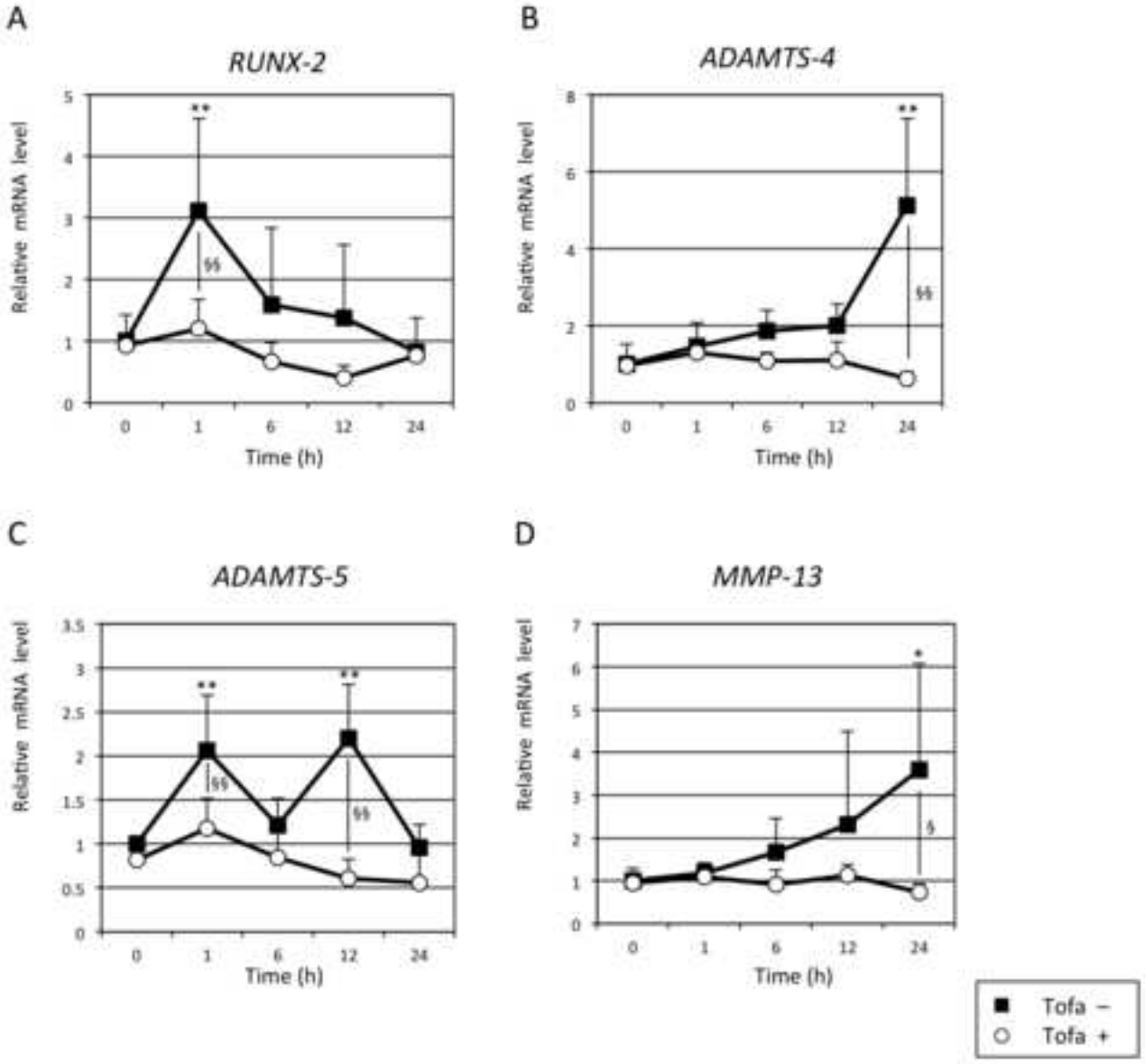

figure3 Click here to download Figure figure3.tiff

Figure 3.

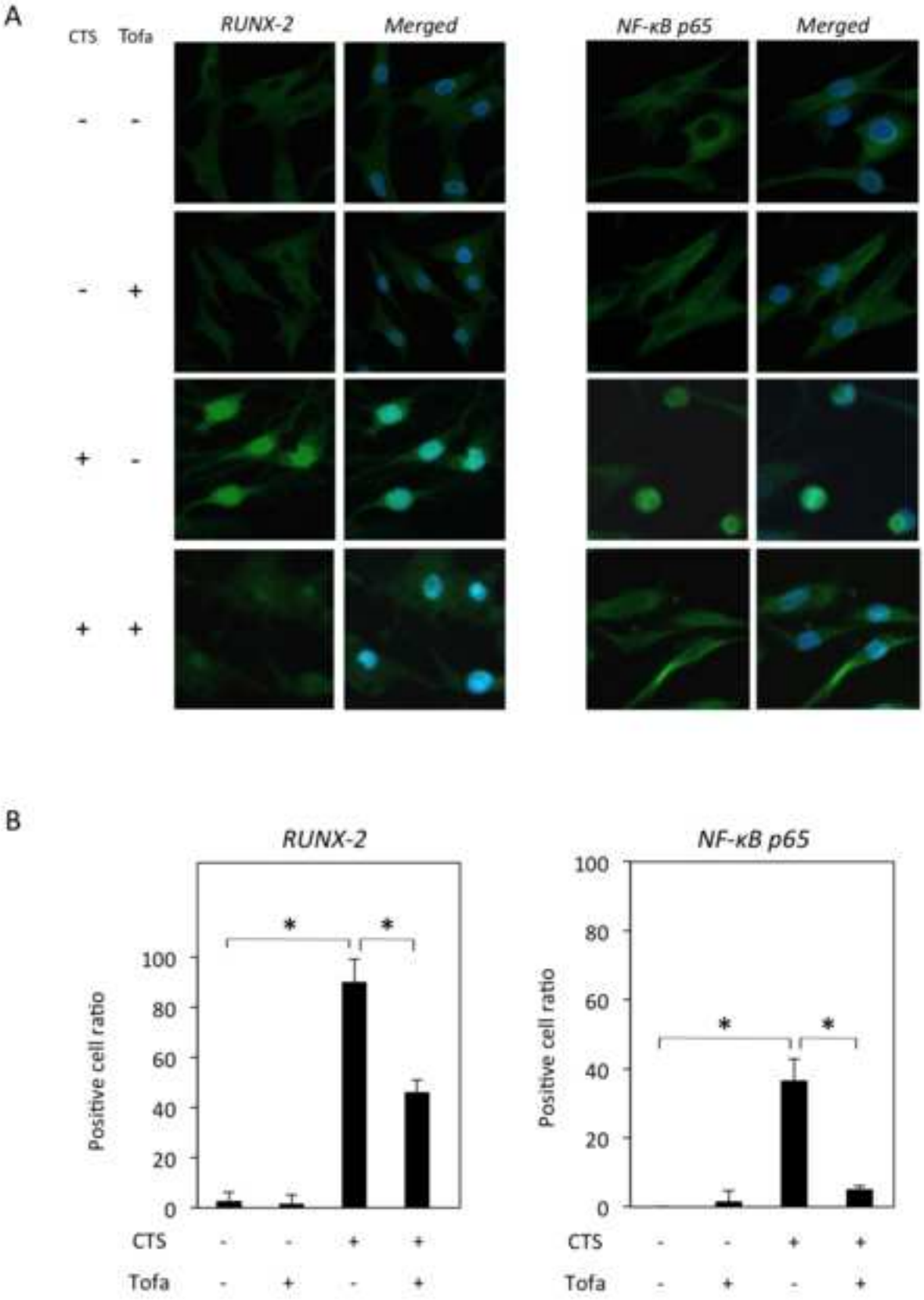

Figure 4.

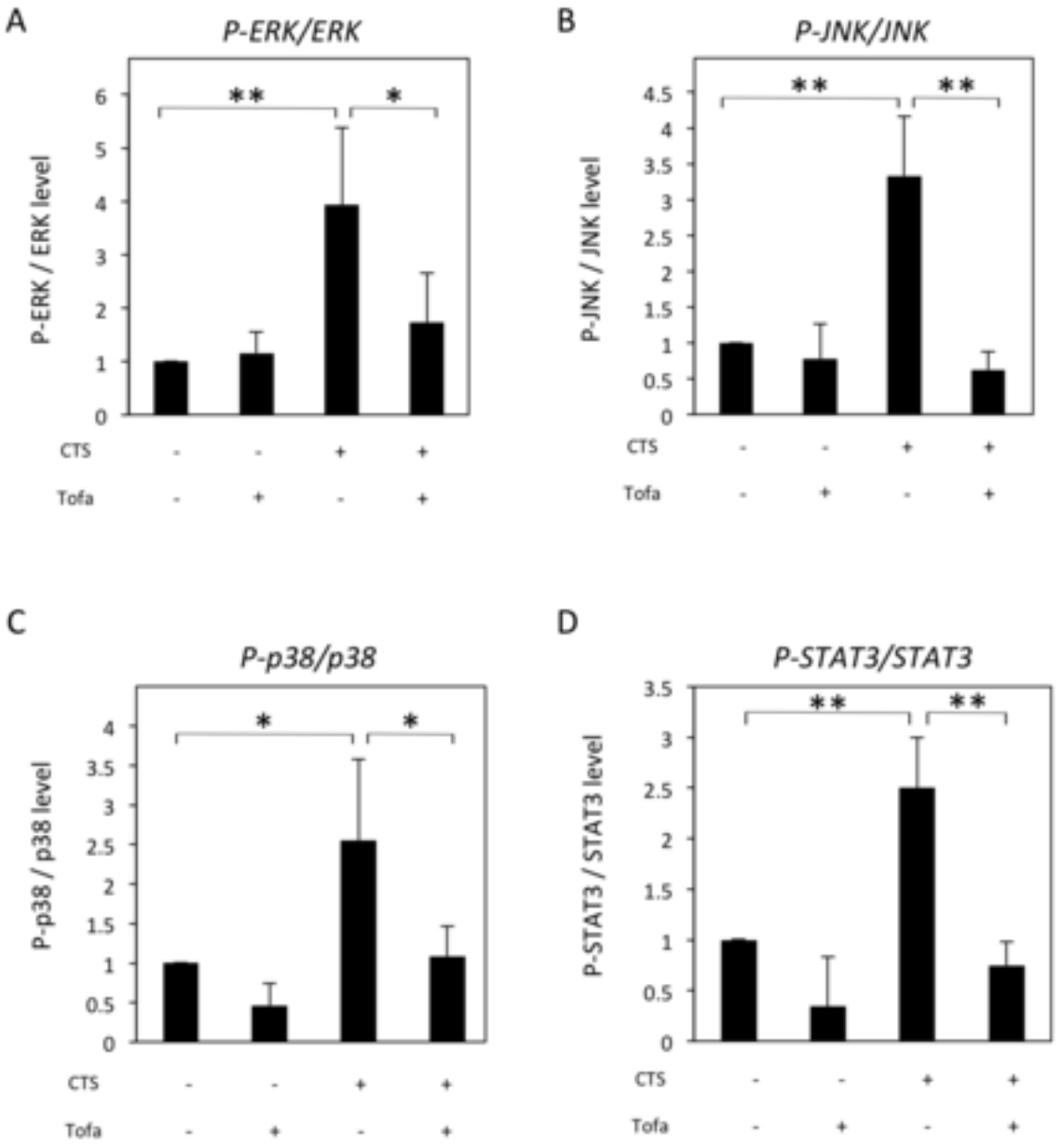

Table 1.

Gene		Nucleotide sequence	Annealing Tm
RUNX-2	F	CTC TAC CAC CCC GCT GTC TT	58°C
	R	CAC CTG CCT GGC TCT TCT TAC	
ADAMTS-4	F	AGG CAC TGG GCT ACT ACT AT	60°C
	R	GGG ATA GTG ACC ACA TTG TT	
ADAMTS-5	F	TAT GAC AAG TGC GGA GTA TG	60°C
	R	TTC AGG GCT AAA TAG GCA GT	
MMP-13	F	ACC CTG GAG CAC TCA TGT TTC CTA	60°C
	R	TGG CAT CAA GGG ATA AGG AAG GGT	
ACAN	F	ACT TCC GCT GGT CAG ATG GA	58°C
	R	TCT CGT GCC AGA TCA TCA CC	
COL21	F	AAT TCC TGG AGC CAA AGG AT	55°C
	R	AGG ACC AGT TGC ACC TTG AG	
G3PDH	F	CAT CAA GAA GGT GGT GAA GCA G	60°C
	R	CGT CAA AGG TGG AGG AGT GG	

Table 2.

Concentration of IL-1β and IL-6 in the supernatant after CTS on cultured human normal chondrocyte

		CTS −				12h after CTS				24h after CTS			
		IL-1β		IL-6		IL-1β		IL-6		IL-1β		IL-6	
Tofa	−	0.032	± 0.090	3.95	± 4.50	0.072	± 0.172	4.45	± 5.77	0.080	± 0.145	17.60 *	± 16.71
	1000nM	0.042	± 0.088	3.54	± 5.17	0.009	± 0.015	3.66 §	± 5.01	0.140	± 0.153	8.27 §	± 5.18

(pg/ml)