Effects and relationship of ERK1 and ERK2 in interleukin-1β-induced alterations in MMP3, MMP13, type II collagen and aggrecan expression in human chondrocytes

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Received October 15, 2010; Accepted December 8, 2010

DOI: 10.3892/ijmm.2011.611

Abstract. Interleukin (IL)-1β plays an important role in the pathogenesis of osteoarthritis and catabolic processes in articular cartilage. Growing evidence suggests that ERK1/2 activation is involved in IL-1β-mediated matrix metalloproteinase (MMP) 3, MMP13, type II collagen and aggrecan expression in chondrocytes. To investigate the respective effects and the relationship of ERK1 and ERK2, knockdown of ERK1 and/or ERK2 was performed in human chondrocytes using specific small interfering RNAs (siRNAs), and the cells were treated with IL-1β (10 ng/ml) for 24 h. Uninfected chondrocytes treated with IL-1β (10 ng/ml) were used as a positive control. Other cells cultured without IL-1β or siRNA treatment were used as a negative control. The mRNA levels of MMP3, MMP13, type II collagen and aggrecan were evaluated by quantitative real-time PCR. The protein levels of MMP3 and MMP13 in the culture medium were examined by ELISA. The protein levels of type II collagen, aggrecan, ERK1/2 and phospho-ERK1/2 were evaluated by Western blotting. The results indicate that IL-1β enhances MMP3 and MMP13 expression and inhibits type II collagen and aggrecan expression. Activation of the MAPK/ERK pathway was observed. Knockdown of ERK1 or ERK2 significantly reversed these effects to similar degree. Combined knockdown of ERK1 and ERK2 displayed synergistic effects. ERK1 and phospho-ERK1 or ERK2 and phospho-ERK2 were inhibited by knockdown of ERK1 or ERK2, respectively. No compensatory effect by up-regulation of the opposite isoform was observed. The combined knockdown suppressed ERK1/2 and phospho-ERK1/2. The data suggest that although inhibition of both ERK1 and ERK2 is more effective, inhibition of either ERK isoform may be sufficient and could be used for novel therapies or as drug targets for pharmacological intervention in cartilage breakdown in osteoarthritis.

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Key words: interleukin-1β, ERK1/2, matrix metalloproteinase 3 and 13, type II collagen, aggrecan, chondrocytes

Introduction

Osteoarthritis (OA) is a progressive degenerative disease associated with articular cartilage erosion, synovial inflammation and subchondral bone remodeling, which may result in compromised joint function or even disability. Neither the etiology nor the pathology of OA is exactly understood. The pro-inflammatory cytokines interleukin (IL)-1β and tumor necrosis factor-α are highly increased in articular cartilage and synovial tissues in OA (1,2). In addition, there is growing evidence that IL-1β plays an important role in the pathogenesis of OA (3,4). IL-1β stimulates the release of catabolic factors by articular chondrocytes, such as matrix metalloproteinases (MMPs) and aggrecanases, which result in catabolic destruction of extracellular matrix proteins (5-8). The up-regulated MMP3 and MMP13 expression in articular chondrocytes induced by IL-1β comprise one of the mechanisms that lead to the degradation of articular cartilage (9-12). In addition, there is evidence that IL-1β induces the down-regulation of type II collagen and aggrecan expression in articular chondrocytes (13-16). Both of these molecules are major components of the extracellular matrix in articular cartilage.

Previous studies have indicated that the mitogen-activated protein kinase (MAPK) pathway is a fundamental pathway for IL-1β-induced catabolic factor gene transcription in articular chondrocytes (9,17-19). The MAPK pathway is comprised of ERK1/2, p38 and the JNK family. Other studies have demonstrated that IL-1β induces the release of MMP3 and MMP13 by articular chondrocytes through the activation of ERK1/2 (9,19,20). On the other hand, the MAPK/ERK pathway is also involved in IL-1β-induced decrease in type II collagen and aggrecan expression in chondrocyte-laden agarose constructs (21). Other studies have shown that inhibition of the MEK/ERK MAPK pathway can decrease the development of structural changes in experimental OA and rheumatoid arthritis *in vivo* (4,22). Taken together, these studies indicate that ERK may be an important therapeutic target for OA therapy. ERK1 and ERK2 are two isoforms of ERK. However, the roles of

ERK1 and ERK2 in the IL-1β-regulated expression of MMP3, MMP13, type II collagen and aggrecan in human chondrocytes are still poorly understood. Moreover, the relationship of ERK1 and ERK2 is unknown. Consequently, clarification of their respective roles and of the relationship between ERK1 and ERK2 is of major importance for providing new insights into the degeneration of articular cartilage induced by IL-1β.

In the present study, we examined the relationship of ERK1 and ERK2 and their possible effects in the IL-1β-mediated alterations in the expression of MMP3, MMP13, type II collagen and aggrecan in human chondrocytes.

Materials and methods

Chondrocyte isolation and culture. Human cartilage samples were obtained from the knee joints of OA patients undergoing arthroplasty, after receiving patient consent and in accordance with the hospital ethical guidelines. The study received ethics approval. The OA patients were diagnosed based on the criteria developed by the American College of Rheumatology Diagnostic Subcommittee for OA (23). Articular cartilage was dissected from the femoral condyle and tibial plateau. The cartilage was sliced into 1x1-mm pieces in Dulbecco's modified Eagle's medium (DMEM; Invitrogen, Carlsbad, CA, USA). The cells were released by incubation with 0.2 mg/ml collagenase (Sigma Chemical Co., Poole, UK) in serum-free DMEM overnight at 37˚C, collected by centrifugation (1,000 x g for 5 min) and washed twice with phosphate-buffered saline (PBS). Finally, the cells were resuspended and cultured in DMEM supplemented with 10% (vol/vol) fetal bovine serum (FBS; Invitrogen) plus 1% penicillin/streptomycin (Gibco-BRL, San Diego, CA, USA). The culture medium was changed every second day. The chondrocytic phenotype of the cultured cells was confirmed by positive immunostaining for type II collagen and toluidine blue staining of glycosaminoglycans. The first-passage chondrocytes were used in the experiments.

Design of ERK1 and ERK2 small interfering RNAs (siRNAs). siRNAs specific for human ERK1 and ERK2 were screened and selected based on the NCBI Reference Sequence database (GenBank: ERK1, NM002746.2; ERK2, NM002745.4). Three siRNA oligomers were respectively chosen to target the ERK1 and ERK2 CDS regions. A negative control siRNA was also selected. These siRNA oligomers were designated ERK1 siRNA1, 2, 3, ERK2 siRNA1, 2, 3 and negative control siRNA. Pairs of complementary oligonucleotides containing these sequences were synthesized (Invitrogen), annealed and cloned into the pSIH1-H1-copGFP shRNA vector. Cells of the 293 TN Producer Cell Line (System Biosciences, Mountain View, CA, USA) were seeded in 10-cm dishes in DMEM supplemented with 10% FBS. On the day before transfection, the medium was removed and replaced with DMEM containing 2.5% FBS. pPACK Packaging Plasmid Mix (System Biosciences) and lentivectors containing the three ERK1 siRNAs, three ERK2 siRNAs and negative control siRNA were transfected into 293 TN cells using the Lipofectamine™ 2000 transfection reagent (Invitrogen). After 24 h of culture, the transfection solution was removed and replaced with DMEM containing 1% FBS. The lentiviral supernatants were collected after 48 h of culture. Cell debris was eliminated by centrifugation at 5,000 x g for 5 min. Subsequently, the lentiviral supernatants were filtered through 0.45-μm PVDF filters (Millipore, Watford, UK). The titers of the lentiviruses (LVs) expressing the three ERK1 siRNAs, three ERK2 siRNAs and negative control siRNA were detected by infecting 293 TN cells with serial dilutions of the concentrated LVs. The lentiviral supernatants were adjusted to $1x10^4$ IFU/μl.

To select the most efficient siRNAs, the chondrocytes were cultured in 6-well plates ($3x10^5$ cells/well) overnight at 37˚C in a humidified atmosphere supplemented with 5% CO_2, and then infected with the LVs expressing the seven siRNAs. The optimum multiplicity of infection (MOI) was 30 based on the evaluation by fluorescence microscopy to examine the copGFP expression at 72 h after infection. Control cells were left untreated. At 96 h after infection, quantitative real-time PCR and Western blot analyses were performed to evaluate the ERK1 and ERK2 mRNA and protein levels, respectively. According to the results, ERK1 siRNA3 and ERK2 siRNA2 showed specificity and were the most efficient siRNAs for ERK1 and ERK2, respectively, without marked changes in GAPDH (data not shown) and therefore, were selected for subsequent experiments (Fig. 1). The sequences of the negative control siRNA, ERK1 siRNA3 and ERK2 siRNA2 were 5'-CGTTTAACTCTCCCAACCA-3', 5'-GTCCATCGACATCTGGTCT-3' and 5'-GATCTGTGACTTTGGCCTG-3', respectively. The corresponding oligonucleotide sequences were 5'-GATCCCGTTTAACTCTCCCAATTACTTCCTGTCAGATGGTTGGGAGAGTTAAACGTTTTTG-3', 5'-GATCCGTCCATCGACATCTGGTCTCTTCCTGTCAGAAGACCAGATGTCGATGGACTTTTTG-3' and 5-GATCCGCAATGACCATATCTGCTACTTCCTGTCAGATAGCAGATATGGTCATTGCTTTTTG-3', respectively. The quantitative real-time PCR and Western blot analyses indicated that the negative control siRNA caused negligible ERK1 and ERK2 suppression.

Chondrocyte infection and treatment. The chondrocytes were infected with LVs expressing negative control siRNA, ERK1 siRNA3 or ERK2 siRNA2 at a MOI of 30. Other chondrocytes were co-infected with LVs expressing ERK1 siRNA3 and ERK2 siRNA2 at a MOI of 30 each. The medium was removed after 24 h, and the infected chondrocytes were subsequently cultured in DMEM supplemented with 10% FBS for 72 h. After removal of the medium, the cells were washed once with PBS, trypsinized and seeded into 6-well plates ($1x10^5$ cells/well) for 6 h. The medium was removed and the cells were cultured in serum-starved DMEM. The next day, the infected chondrocytes were treated with 10 ng/ml IL-1β (Biomol, Hamburg, Germany). Uninfected chondrocytes treated with IL-1β (10 ng/ml) were used as a positive control. Other cells cultured without IL-1β or siRNA treatment were used as a negative control. Great care was taken to compare cells seeded at the same densities. After 24 h, the mRNA and protein levels of MMP3, MMP13, type II collagen and aggrecan were assessed. ERK1/2 and phospho-ERK1/2 were also assessed.

ELISA. The cumulative production of MMP3 and MMP13 in the cell culture supernatants were determined in undiluted

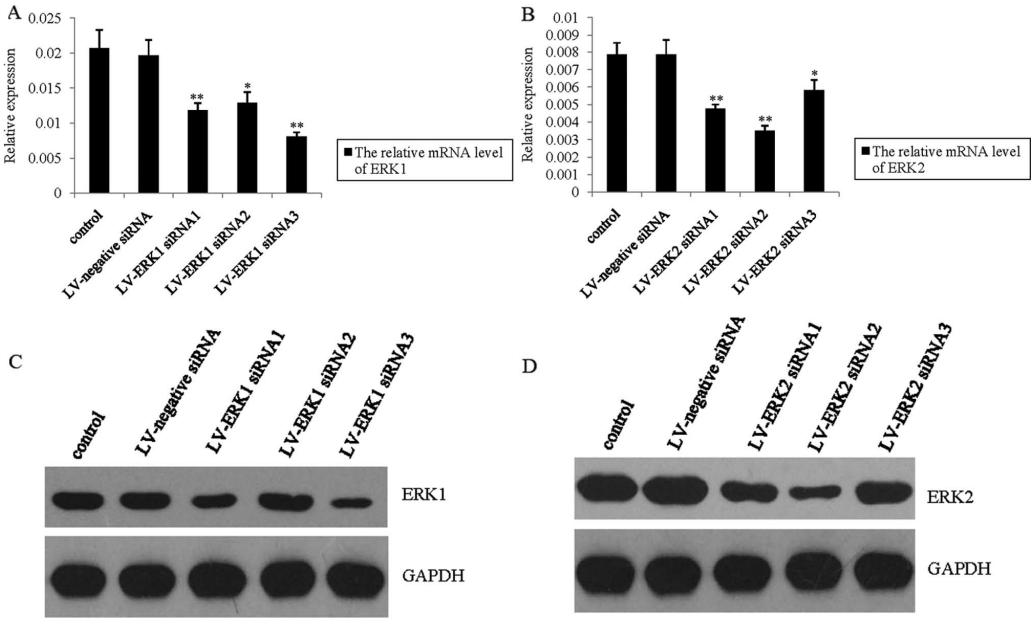

Figure 1. Lentiviruses (LVs) expressing ERK1 siRNA3 and ERK2 siRNA2 specifically and most efficiently target ERK1 and ERK2, respectively. Human chondrocytes were infected with LVs expressing ERK1 siRNA1, 2, 3, ERK2 siRNA1, 2, 3 or negative control siRNA at a MOI of 30. Control cells were left untreated. At 96 h after transfection, the mRNA and protein levels of ERK1 and ERK2 were examined by quantitative real-time PCR (A and B) and Western blotting (C and D), respectively. The data were obtained from three independent experiments. The mRNA levels of ERK1 and ERK2 are expressed as means ± SD. *P<0.05, **P<0.01, Significant difference vs. the control cells by the Mann-Whitney U test.

conditioned media using commercially available ELISA kits, according to the manufacturer's instructions (R&D Systems, Minneapolis, MN, USA for MMP3; RayBiotech, Atlanta, GA, USA for MMP13).

Protein extraction and Western blotting. Total cellular proteins were extracted in RIPA buffer (50 mM Tris-HCl pH 7.4, 150 mM NaCl, 1% Triton X-100, 0.1% SDS, 2 mM EDTA, 2 mM PMSF and protease inhibitors) at 4˚C. Aliquots (10 μl) of the cellular extracts were separated by 10% SDS-PAGE and electrotransferred onto polyvinylidene difluoride membranes. The membranes were blocked with 3% non-fat milk for 2 h at room temperature in Tris-buffered saline (pH 7.6) containing 0.5% Tween-20 (TBS-T). The membranes were then rinsed twice with TBS-T and incubated with the anti-human antibodies ERK1, ERK2, ERK1/2, phospho-ERK1/2, type II collagen or aggrecan (Santa Cruz, CA, USA) overnight at 4˚C. The membranes were washed and incubated with the corresponding anti-goat (Santa Cruz), anti-mouse or anti-rabbit HRP-conjugated IgG antibodies (Cell Signaling Technology Danvers, MA, USA) at room temperature for 2 h. Finally, the positive protein bands were detected with the ECL reagent (Pierce Biotech, Rockford, IL, USA). An anti-GAPDH antibody was used as a control to verify equal loading (Santa Cruz).

Quantitative real-time PCR. Total RNA was isolated using the TRIzol reagent (Invitrogen) according to the manufacturer's instructions. First-strand cDNA was synthesized using reverse transcriptase M-MLV cDNA synthesis kit (Takara, Tokyo, Japan). According to the manufacturer's recommended conditions, a 12-μl reaction mixture containing 2 μl of oligo d(T)18 primer (50 μM), 2 μg of total RNA and RNase-free dH_2O was incubated at 70˚C for 10 min. Subsequently, 1 μl of dNTP mixture (10 mM each), 4 μl of 5X M-MLV buffer, 0.5 μl ribonuclease inhibitor (40 U/μl), 1 μl of reverse transcriptase M-MLV (RNase H-free) (200 U/μl) and RNase-free dH_2O were added to a final volume of 20 μl and the reaction was incubated for 60 min at 42˚C. Next, the reaction was inactivated by heating at 70˚C for 15 min. The cDNA was collected and stored at -20˚C until analysis. Real-time PCR was performed with the GeneXpert® System (Cepheid, Sunnyvale, CA, USA) using the QuantiTect SYBR-Green RT-PCR kit (Takara). The reaction mixtures contained 10 μl of SYBR Premix Ex Taq, 0.4 μl of each target-specific primer pair, 2 μl of cDNA template and nuclease-free PCR grade water to a final volume of 20 μl. The following primers were used: ERK1, forward, 5'-CCTGCGACCTTAAGATTTG TGATT-3' and reverse, 5'-CAGGGAAGATGGGCCGGTTA GAGA-3'; ERK2, forward, 5'-GCGCGGGCCCGGAGAT GGTC-3' and reverse, 5'-TGAAGCGCAGTAAGATTTTT-3'; MMP3, forward, 5'-AGGCTGTATGAAGGAGAGGCTG AT-3' and reverse, 5'-AGTGTTGGCTGAGTGAAAGAGA CC-3'; MMP13, forward, 5'-AGCGCTACCTGAGATCATA CTACC-3' and reverse, 5'-AACCCCGCATCTTGGCTTTT TC-3'; type II collagen, forward, 5'-CTTCGGTGTCAGGG CCAGGATGTC-3' and reverse, 5'-GTGGCGAGGTCAGT TGGGCAGATG-3'; aggrecan, forward, 5'-GTCGGGAGCA GCAGTCACA-3' and reverse, 5'-GGGGCGCCAGTTCTC AAA-3'. The real-time PCR amplifications were performed using 40 cycles of 95˚C for 10 sec (denaturation), 60˚C for 20 sec (annealing) and 72˚C for 20 sec (elongation). GAPDH was amplified as a control using specific primers (forward, 5'-GCACCGTCAAGGCTGAGAAC-3' and reverse, 5'-ATGG TGGTGAAGACGCCAGT-3'). After the PCR amplification, dissociation curves were performed with one peak, thereby

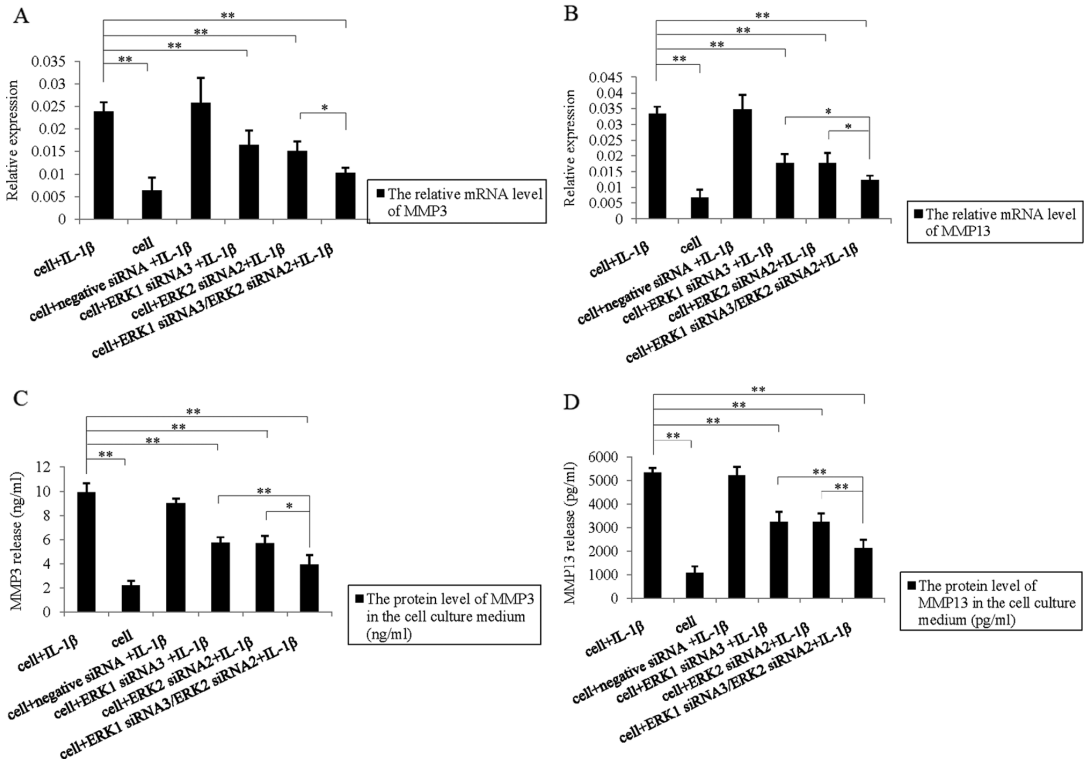

Figure 2. Knockdown of ERK1 and/or ERK2 suppresses the IL-1β-induced MMP3 and MMP13 expression. Uninfected human chondrocytes and human chondrocytes infected with lentiviruses (LVs) expressing negative control siRNA, ERK1 siRNA3, ERK2 siRNA2 or ERK1 siRNA3 plus ERK2 siRNA2 were incubated with IL-1β (10 ng/ml) for 24 h. Control cells were cultured for 24 h without IL-1β or siRNA treatment. The mRNA levels of MMP3 (A) and MMP13 (B) were assessed by quantitative real-time PCR. The protein levels of MMP3 (C) and MMP13 (D) in the culture medium were assessed by ELISA. The mRNA and protein levels of MMP3 and MMP13 are expressed as means ± SD. The data were obtained from three independent experiments. *P<0.05, **P<0.01, Significant difference by the Kruskal-Wallis ANOVA test.

demonstrating the specificity of each amplification. The $2^{-\Delta\Delta Ct}$ method was used to calculate the gene expression levels relative to the corresponding gene expression levels of GAPDH as previously described (24).

Statistical analysis. All the data were obtained from three independent experiments. The data were expressed as means ± SD. The statistical significance of differences among values was determined using the Mann-Whitney U test or the Kruskal-Wallis ANOVA test, as appropriate. All analyses were carried out using SPSS for Windows (Version 13.0; SPSS Inc., Chicago, IL, USA). Values of P<0.05 were considered to indicate statistical significance.

Results

Knockdown of ERK1 and/or ERK2 suppresses IL-1β-induced increases in MMP3 and MMP13 expression. As shown in Fig. 2A and B, in the presence of IL-1β, the mRNA levels of MMP3 and MMP13 were enhanced by 4.50-fold and 4.98-fold, respectively, in the treated cells compared with the untreated cells (P<0.01). The mRNA levels of MMP3 and MMP13 were significantly decreased by knockdown of ERK1 and/or ERK2 compared with IL-1β-treated cells. The basal protein levels of MMP3 and MMP13 were low in the absence of IL-1β, but increased by 3.70-fold and 4.90-fold, respectively, in the presence of IL-1β (P<0.01). The protein levels of MMP3 and MMP13 in the culture medium were also significantly decreased by knockdown of ERK1 and/or ERK2 compared with IL-1β-treated control cells (Fig. 2C and D). In addition, the combined knockdown led to a significant decrease in the MMP3 mRNA level compared to the ERK1 knockdown alone, while the MMP13 mRNA level was significantly decreased compared with ERK1 or ERK2 knockdown alone. The protein levels of MMP3 and MMP13 in the culture medium were significantly decreased by the combined knockdown compared with knockdown of ERK1 or ERK2 alone. There were no significant differences between the ERK1 and ERK2 knockdowns. In the cells infected with the LV expressing the negative control siRNA, the mRNA and protein levels of MMP3 and MMP13 were unchanged. These results suggest that ERK1 and ERK2 play similar roles in the IL-1β-induced MMP3 and MMP13 expression. In addition, synergistic effects of ERK1 and ERK2 are involved in the IL-1β-mediated MMP3 and MMP13 expression.

Knockdown of ERK1 and/or ERK2 reverses the IL-1β-induced suppression of type II collagen and aggrecan expression. The effects of ERK1 and/or ERK2 knockdown on type II collagen and aggrecan expression were determined in the presence of IL-1β. As shown in Fig. 3A and B, quantitative real-time PCR revealed that the mRNA levels of type II collagen and aggrecan were markedly decreased by IL-1β (0.26-fold and 0.31-fold relative to the cells, respectively, P<0.01). Knockdown of ERK1 and/or ERK2 significantly reversed the IL-1β-suppressed mRNA levels of type II

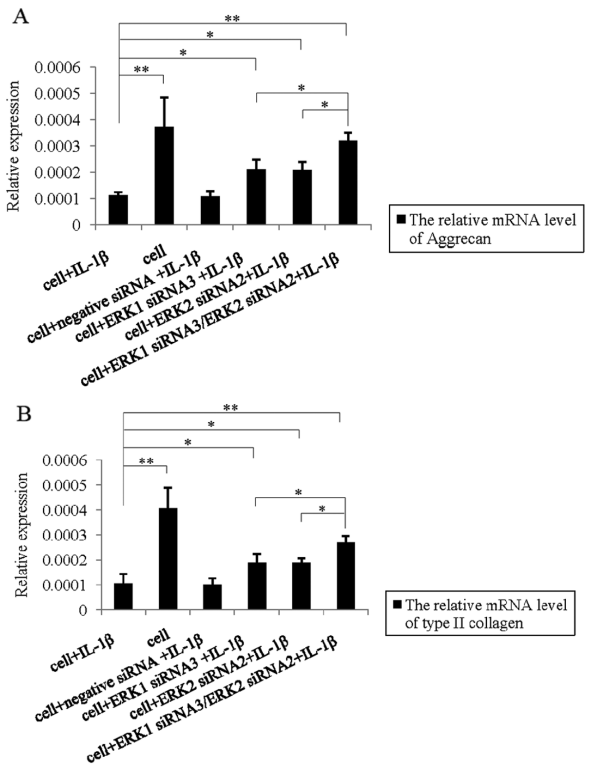

Figure 3. Knockdown of ERK1 and/or ERK2 reverses the IL-1β-suppressed type II collagen and aggrecan expression. Uninfected human chondrocytes and human chondrocytes infected with lentiviruses (LVs) expressing negative control siRNA, ERK1 siRNA3, ERK2 siRNA2 or ERK1 siRNA3 plus ERK2 siRNA2 were incubated with IL-1β (10 ng/ml) for 24 h. Control cells were cultured for 24 h without IL-1β or siRNA treatment. The mRNA levels of type II collagen (A) and aggrecan (B) were assessed by quantitative real-time PCR. The protein levels of type II collagen and aggrecan were assessed by Western blotting (C). The data were obtained from three independent experiments. The mRNA levels of type II collagen and aggrecan are expressed as the means ± SD. *P<0.05, **P<0.01, Significant difference by the Kruskal-Wallis ANOVA test.

collagen and aggrecan compared with IL-1β-treated cells. There were no significant differences in the mRNA levels of type II collagen and aggrecan between the ERK1 and ERK2 knockdowns. In addition, the mRNA levels of type II collagen and aggrecan were significantly increased by the combined knockdown compared with knockdown of ERK1 or ERK2 alone. Western blot analyses revealed similar results for the protein levels of type II collagen and aggrecan (Fig. 3C). In the cells infected with the LV expressing the negative control siRNA, the mRNA and protein levels of type II collagen and aggrecan were unchanged. These results imply that either ERK1 or ERK2 are involved in the IL-1β-suppressed type II collagen and aggrecan expression. ERK1 and ERK2 display synergistic effects.

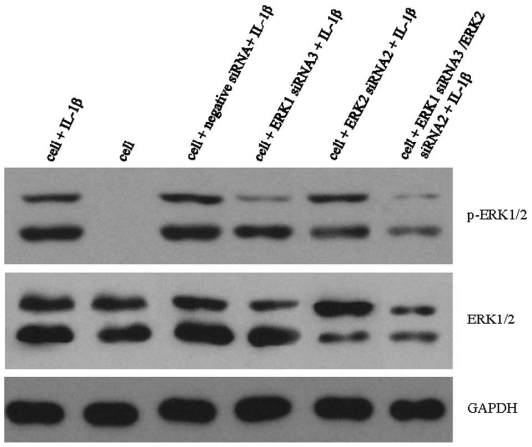

Figure 4. Knockdown of ERK1 and/or ERK2 suppresses the IL-1β-induced phospho-ERK1/2 and ERK1/2 expression. Uninfected human chondrocytes and human chondrocytes infected with LVs expressing negative control siRNA, ERK1 siRNA3, ERK2 siRNA2 or ERK1 siRNA3 plus ERK2 siRNA2 were incubated with IL-1β for 24 h. Control cells were cultured for 24 h without IL-1β or siRNA treatment. The levels of phospho-ERK1/2 and ERK1/2 were assessed by Western blotting. Representative data of three independent experiments are shown.

IL-1β-mediated alterations in the expression of MMP3, MMP13, type II collagen and aggrecan are dependent on the ERK activation level. We examined the ERK1/2 and phospho-ERK1/2 levels by Western blotting following knockdown of ERK1 and/or ERK2 in chondrocytes stimulated by IL-1β. As shown in Fig. 4, ERK1/2 and phospho-ERK1/2 were enhanced in chondrocytes stimulated by IL-1β. ERK1 and phospho-ERK1 or ERK2 and phospho-ERK2 were down-regulated, and these effects were related to knockdown of ERK1 or ERK2, respectively. No significant compensation occurred between the ERK isoforms after knockdown of one of the ERK isoforms. In addition, the combined knockdown suppressed ERK1/2 and phospho-ERK1/2. In cells infected with the LV expressing the negative siRNA, ERK1/2 and phospho-ERK1/2 were unchanged compared to IL-1β-treated cells. Taken together, these data strongly suggest that ERK1 and ERK2 are indistinguishable, yet have similar impacts on the IL-1β-mediated alterations in the expression of MMP3, MMP13, type II collagen and aggrecan. Therefore, each isoform appears to be necessary and contributes to the IL-1β-mediated alterations in the MMP3, MMP13, type II collagen and aggrecan expression. Moreover, these effects appear to be strongly related to the phospho-ERK levels, indicating that ERK1 and ERK2 display synergistic effects.

Discussion

It is well recognized that proinflammatory cytokines play important roles in the development of OA (25-27). IL-1β is considered to be one of the key cytokines involved in articular cartilage destruction. In the present study, we confirmed that IL-1β enhanced MMP3 and MMP13 expression and inhibited type II collagen and aggrecan expression in comparison to

untreated human chondrocytes. *In vitro*, IL-1 leads to the activation of ERK, p38 and JNK and is associated with the induction of MMPs in chondrocytes (9,28). MMP13 is capable of cleaving type II collagen while MMP3 is active against other components of the extracellular matrix, such as fibronectin and laminin. Synthesis of type II collagen and aggrecan is necessary for cartilage repair. Treatment of rabbit articular chondrocytes with IL-1β inhibited type II collagen expression (15), while proteoglycan synthesis was inhibited in cartilage disks from calves treated with IL-1α (13). This inhibition of collagen type II and aggrecan synthesis was also observed in bovine articular chondrocytes in the presence of IL-1β (14). Taken together, the up-regulated expression of MMP3 and MMP13 in articular chondrocytes induced by IL-1β comprises one of the mechanisms that lead to the degradation of articular cartilage. In addition, IL-1β exhibits inhibitory effects on collagen type II and aggrecan synthesis. Thus, IL-1β disrupts the balance of metabolism between catabolism and anabolism, which may result in articular cartilage destruction (29,30).

IL-1β was reported to induce the expression of MMP3 and MMP13 in chondrocytes through activation of the MAPK/ERK pathway (28). Avocado/soybean unsaponifiables (ASU), one of the most commonly used drugs for symptomatic OA, were also proven to decrease the IL-1β-induced expression of MMP3 and MMP13 in human chondrocytes through ERK1/2 inhibition (20). Moreover, Radons *et al* (31) reported that the expression of aggrecan was suppressed by IL-1 through activation of ERK1/2 and/or p38MAPK in human chondrocytes. IL-1β decreased the expression of type II collagen and aggrecan in chondrocyte-laden agarose constructs, and this effect was reversed by U0126, indicating the involvement of the MAPK/ERK pathway (21). Therefore, the MAPK/ERK pathway may be particularly important for the IL-1β-mediated regulation of catabolism and anabolism responsible for articular cartilage degradation. Our Western blot analyses also showed that the MAPK/ERK pathway was involved in the IL-1β-induced increase in the MMP3 and MMP13 expression and inhibition of the type II collagen and aggrecan expression. Therefore, ERK plays an important role in the pathology of OA. ERK1 and ERK2 are two isoforms of ERK. However, the possible specific roles and relationship of these two major ERK isoforms in the degeneration of articular cartilage induced by IL-1β are poorly understood. To clarify the involvement of ERK1 and ERK2 in OA, ERK1 and ERK2 siRNAs were designed and selected. The obtained data showed that ERK1 and ERK2 were specifically inhibited by LVs expressing ERK1 siRNA3 and ERK2 siRNA2 in human chondrocytes, respectively. Therefore, these LVs can be used to study the specific functions of these ERK isoforms. Knockdown of these two genes by the siRNAs in human chondrocytes provides an excellent means to study the specific and shared functions of these two isoforms.

A lack of ERK2 alone leads to early embryonic lethality. ERK2 deficiency cannot be compensated by ERK1 (32,33). These observations suggest that ERK2 has specific functions in developing mouse embryos. In addition, ERK2 has a positive role in controlling fibroblast proliferation, whereas ERK1 antagonizes this ERK2 activity (34). A recent study indicated ERK2 is involved in the regulation of hepatocyte survival, although ERK1 also displays a distinct role (35). Conversely, ERK1 plays a more prominent role in the pathogenesis of the Noonan syndrome-related valve disease compared to ERK2 (36). Regional differences in the total expression of ERK1 and ERK2 were detected across the primary somatosensory cortex, hippocampus and spinal cord (37). Although pain can initiate the activation of ERKs in all the three areas, pERK1 and pERK2 exhibited regional differences across the different areas. Taken together, these data suggest that the functions and relationship of ERK1 and ERK2 are extremely complex in different cell types. Therefore, we are tempted to speculate that either ERK1 or ERK2 alone can regulate the IL-1β-induced alterations in the MMP3, MMP13, type II collagen and aggrecan expression in human chondrocytes. Alternatively, one of the ERK isoforms may have a positive role in human chondrocytes in response to IL-1β, while the other isoform attenuates this role. In our study, we confirmed that knockdown of ERK1 or ERK2 alone could significantly reverse the altered expression of MMP3, MMP13, type II collagen and aggrecan induced by IL-1β in human chondrocytes. Inhibition of phospho-ERK1 and ERK1 or phospho-ERK2 and ERK2 was involved in these effects. In addition, knockdown of ERK1 or ERK2 alone exhibited similar effects. We did not observe that one ERK isoform was significantly up-regulated after knockdown of the other ERK isoform. Moreover, the combined knockdown displayed synergistic effects through the inhibition of phospho-ERK1/2 and ERK1/2. Similarly, IL-2 production in response to TCR stimulation in mouse 1B6 T cell hybridomas can be modulated by varying the level of cellular ERK protein via either isoform (38). Another study showed that when ERK2 was abolished, NIH 3T3 cell proliferation became dependent on ERK1, suggesting that ERK1 and ERK2 share functions in the regulation of this cell proliferation (39). Together, our data suggest that the expression of MMP3, MMP13, type II collagen and aggrecan in response to IL-1β stimulation may be equally modulated by varying the level of cellular ERK protein via either isoform. Furthermore, ERK1 and ERK2 display synergistic effects in regulating these effects.

In conclusion, the individual ERK isoforms exhibit similar effects by significantly reversing the IL-1β-induced alterations in the expression of MMP3, MMP13, type II collagen and aggrecan. ERK1 and ERK2 display synergistic effects. Therefore, ERK1 and ERK2 represent novel therapies or drug targets for pharmacological intervention in cartilage breakdown in OA and each may be sufficient although inhibition of both isoforms is more effective.

Acknowledgements

This study was supported by the Science and Technology Commission of the Shanghai Municipality (08JC1412600).

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