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# Analysis of isoform specific ERK signaling on the effects of interleukin-1β on COX-2 expression and PGE2 production in human chondrocytes

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ABSTRACT

The MAPK/ERK pathway is involved in IL-1β-induced cyclooxygenase (COX-2) expression and prostaglandin E2 (PGE2) production; two factors that play important roles in OA pathogenesis. In the present study, we find that IL-1β induced COX-2 expression and PGE2 production in human chondrocytes via a process that required the activation of the MAPK/ERK pathway. To evaluate the respective roles and relationship of ERK1 and ERK2 on IL-1β induced COX-2 expression and PGE2 production, small interfering RNA was used to knockdown ERK1, ERK2 or both in human chondrocytes. COX-2 expression and PGE2 production were significantly suppressed to a similar degree by the silencing of ERK1 or ERK2 alone. Moreover, the combined knockdown displayed a synergistic effect. Simultaneously, Western blotting indicated that the knockdown of ERK1 or ERK2 down regulated phospho-ERK1 and ERK1 or phospho-ERK2 and ERK2 levels, respectively. No significant compensatory mechanism through the upregulation of the other phospho-ERK and ERK isoform was observed. The combined silencing suppressed both phospho-ERK1/2 and ERK1/2. In conclusion, each ERK isoform similarly influenced IL-1β-mediated COX-2 expression and PGE2 production in human chondrocytes, and ERK1 and ERK2 displayed synergistic effects. Although, inhibition of both ERK1 and ERK2 would be a more effective, each ERK isoform may sufficiently regulate these effects in human chondrocytes. ERK1 or ERK2 may be potential therapeutic target for the inflammatory process of OA.



## 1. Introduction

Osteoarthritis (OA) is the most common type of disabling chronic condition in humans, but its precise pathology is unclear. Chondrocytes respond to a variety of stimuli, such as proinflammatory cytokines, mechanical damage and ageing, by expressing degradative enzymes and catabolic mediators. Published evidence indicates that inflammatory cytokines, such as IL-1β and tumor necrosis factor-α, induce the inflammatory process in the articular chondrocytes involved in OA pathology [1,2]. The inflammatory process involves excessive COX-2 expression and PGE2 production [3,4], which are found at high levels in the synovial fluid in patients with OA and in OA animal models [5,6]. The MAPK/ERK pathway plays an important role in IL-1β-induced proinflammatory mediator expression in chondrocytes, including COX-2 expression and PGE2 production [7,8] . Further understanding of the function of the specific isoforms of ERK on the regulation of joint inflammation-associated COX-2 expression and PGE2 production may be helpful for the selection of OA therapeutic targets. Few studies have examined the respective roles and relationship of ERK1 and ERK2 in regulating COX-2 expression and PGE2 production in human chondrocytes in response to IL-1β.

In our study, we confirmed that IL-1β induced COX-2 expression and PGE2 production in human chondrocytes. Activation of the MAPK/ERK pathway was involved in this process. Silencing of ERK1, ERK2 or both combined was then performed by small interfering RNA (siRNA) in human chondrocytes. Our results indicate that silencing of ERK1 or ERK2 may sufficiently reverse the effects of IL-1β on COX-2 expression and PGE2 production to a similar degree although the combined knockdown causes a synergistic effect.

## 2. Materials and methods

### 2.1. Chondrocyte isolation and culture

Human cartilage was obtained from OA patients undergoing knee replacement surgery, following patient consent and in accordance with the local ethics committee. It was sliced into 1 × 1 mm pieces in Dulbecco's modified Eagle's medium (DMEM, Invitrogen, Carlsbad, CA, USA). Cartilage pieces were washed with PBS and treated with 0.2 mg/ml collagenase (Sigma Chemical Co., Poole, UK) in serum-free DMEM overnight at 37 °C. The cells were collected by filtering through a 200 mesh nylon cell strainer,

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centrifugation at 1000*g* for 5 min and washing twice with PBS. Finally, the cells were resuspended and cultured in DMEM supplemented with 10% (vol/vol) fetal bovine serum (FBS, Invitrogen), plus 1% penicillin and streptomycin (GIBCO-BRL, San Diego, CA, USA). The culture medium was changed every second day.

### 2.2. Design of ERK1 and ERK2 siRNAs

The human ERK1 and ERK2 specific siRNA were screened and selected based on NCBI Reference Sequences (GenBank: ERK1: NM_002746.2 and ERK2: NM_002745.4). Three siRNAs oligomers were, respectively, chosen to target the ERK1 and ERK2 CDS region. A negative siRNA served as a control. The sequences of the siRNA oligomers and the corresponding oligonucleotide sequences, designated ERK1 siRNA1, 2, 3, ERK2 siRNA1, 2, 3 and negative siRNA, are shown in Table 1. Then, the corresponding oligonucleotide sequences for ERK1, ERK2 and negative siRNAs were synthesized (Invitrogen), annealed and subcloned into the pSIH1-H1-copGFP shRNA Vector (System Biosciences, CA, USA; GFP: green fluorescent protein). The 293 TN Producer Cell Line (System Biosciences, CA, USA) was seeded in 10 cm dishes in DMEM supplemented with 10% FBS. The medium was removed and DMEM containing 2.5% FBS was added the day before transfection. The pPACK Packaging Plasmid Mix (System Biosciences) and lentivectors containing the 7 siRNA sequences were transfected into 293 cells using Lipofectamine™ 2000 transfection reagent (Invitrogen). After 24 h of incubation, the transfection solution was removed and the medium was changed to DMEM containing 1% FBS. Lentiviral supernatant was collected after 48 h of incubation. Cell debris was eliminated by centrifugation at 5000*g* for 5 min. Subsequently, the lentiviral supernatant was filtered through 0.45-μm PVDF filters (Millipore, Watford, UK). The titers of lentivirus (LV) expressing the seven siRNAs were detected by infecting 293 cells with serial dilutions of concentrated LV. The lentiviral supernatant was adjusted to $1 \times 10^4$ ifu/μl using dPBS.

The human chondrocytes were infected with LV expressing the seven siRNAs at an optimum multiplicity of infection (MOI) of 30. For controls, cells were left untreated. Ninety-six hours after infection, quantitative real-time PCR and Western blotting revealed ERK1 siRNA3 and ERK2 siRNA2 were the most efficient siRNA for ERK1 and ERK2 silencing (data not shown) and were selected for the following experiments. The negative siRNA had no effect on ERK1 and ERK2 suppression.

### 2.3. Human chondrocyte infection and treatment

Human chondrocytes were infected with LV expressing negative siRNA, ERK1 siRNA3 or ERK2 siRNA2 at an MOI of 30. Other chondrocytes were co-infected with LVs expressing ERK1 siRNA3 and ERK2 siRNA2 at a MOI of 30 each. After 24 h, medium was removed. Subsequently, infected human chondrocytes were incubated with DMEM supplemented with 10% FBS. After 72 h, medium was removed and cells were washed once with PBS. Cells were then trypsinized and seeded in six well plates ($1 \times 10^5$/well) for 6 h. The medium was removed and cells maintained in serum-starved DMEM. The next day, the infected human chondrocytes were treated with 10 ng/ml IL-1β for 24 h. Non-infected human chondrocytes treated with 10 ng/ml IL-1β were used as controls. Great care was taken to compare cells that were seeded at the same densities. The mRNA and the protein levels of COX-2 were examined along with PGE2 levels in the cell culture medium. Phospho-ERK/2 and ERK1/2 was also examined in cell lysates from the same experiments.

### 2.4. Enzyme-linked immunosorbent assay (ELISA)

The cumulative production of PGE2 in the cell culture medium was measured in the undiluted medium using a commercially available ELISA kit (R&D Systems, Minneapolis, USA) according to the manufacturer's instructions.

### 2.5. Quantitative real-time PCR

Following the manufacturer's instructions, total RNA was extracted from the chondrocytes using TRIzol™ reagent (Invitrogen). First-strand complementary DNA (cDNA) was synthesized using the reverse transcriptase M-MLV cDNA synthesis Kit (Takara, Tokyo, Japan) according to the manufacturer's recommended conditions. Real-time PCR was carried out with a Detection System (GeneXpert® Operator manual, Cepheid, Sunnyvale, CA, USA), using a QuantiTect SYBR Green RT-PCR kit (Takara). The reaction mixtures contained 10 μl of QuantiTect SYBR Green RT-PCR master mix, 0.4 μM of each target-specific primer designed to amplify a part of each gene, 2 μl of cDNA template and nuclease free PCR grade water up to a total volume of 20 μl. The primer pairs' sequences were as follows: GAPDH: F: 5′-GCACCGTCAAGGCTGAG AAC-3′, R: 5′-ATGGTGGTGAAGACGCCAGT-3′; ERK1: F: 5′-CCTGC GACCTTAAGATTTGTGATT-3′, R: 5′-CAGGGAAGATGGGCCGG TTAG AGA-3′; ERK2 F: 5′-GCGCGGGCCCGGAGATGGTC-3′, R: 5′-TGAAGCG CAGTAAGATTTTT-3′. COX-2: F: 5′-AATGGGGTGATGAGCAGTTGT TC-3′, R: 5′-GGATGCCAGTGATAGAGGGTGTTA-3′. The real-time PCR was performed using 40 cycles at 95 °C for 10 s (denaturing), followed by 20 s at 60 °C (primer annealing), 20 s at 72 °C (elongation).The ERK1, ERK2 and COX-2 gene expression levels were normalized against the housekeeping gene GAPDH and relative gene expression was analyzed with the $2^{-\Delta\Delta Ct}$ method.

**Table 1**
siRNAs oligomer sequences and oligonucleotide sequences.

| siRNA | siRNA oligomers (5′–3′) | Oligonucleotide sequences (5′–3′) |
|---|---|---|
| ERK1 siRNA1 | GCAGCTGAG | Forward: GATCCGCAGCTGAGCAATGACCATCTTCCTGTCAGAATGGTCATTGCTCAGCTGCTTTTTG |
| | CAATGACCAT | Reverse: AATTCAAAAAGCAGCTGAGCAATGACCATTCTGACAGGAAGATGGTCATTGCTCAGCTGCG |
| ERK1 siRNA2 | GCTGAACTC | Forward: GATCCGCTGAACTCCAAGGGCTATCTTCCTGTCAGAATAGCCCTTGGAGTTCAGCTTTTTG |
| | CAAGGGCTAT | Reverse: AATTCAAAAAGCTGAACTCCAAGGGCTATTCTGACAGGAAGATAGCCCTTGGAGTTCAGCG |
| ERK1 siRNA3 | GTCCATCGA | Forward: GATCCGTCCATCGACATCTGGTCTCTTCCTGTCAGAAGACCAGATGTCGATGGACTTTTTG |
| | CATCTGGTCT | Reverse: AATTCAAAAAGTCCATCGACATCTGGTCTTCTGACAGGAAGAGACCAGATGTCGATGGACG |
| ERK2 siRNA1 | GGACCTCAT | Forward: GATCCGGACCTCATGGAAACAGATCTTCCTGTCAGAATCTGTTTCCATGAGGTCCTTTTTG |
| | GGAAACAGAT | Reverse: AATTCAAAAAGGACCTCATGGAAACAGATTCTGACAGGAAGATCTGTTTCCATGAGGTCCG |
| ERK2 siRNA2 | GCAATGACC | Forward: GATCCGCAATGACCATATCTGCTACTTCCTGTCAGATAGCAGATATGGTCATTGCTTTTTG |
| | ATATCTGCTA | Reverse: AATTCAAAAAGCAATGACCATATCTGCTATCTGACAGGAAGTAGCAGATATGGTCATTGCG |
| ERK2 siRNA3 | GATCTGTGA | Forward: GATCCGATCTGTGACTTTGGCCTGCTTCCTGTCAGACAGGCCAAAGTCACAGATCTTTTTG |
| | CTTTGGCCTG | Reverse: AATTCAAAAAGATCTGTGACTTTGGCCTGTCTGACAGGAAGCAGGCCAAAGTCACAGATCG |
| Negative | CGTTTAACT | Forward: GATCCCGTTTAACTCTCCCAATTACTTCCTGTCAGATGGTTGGGAGAGTTAAACGTTTTTG |
| siRNA | CTCCCAACCA | Reverse: AATTCAAAAACGTTTAACTCTCCCAATTATCTGACAGGAAGTGGTTGGGAGAGTTAACGG |

### 2.6. Protein extraction and analysis by Western blotting

Whole cell lysates were prepared using 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]. Equal amounts of protein were separated by 10% SDS–polyacrylamide gel electrophoresis and subsequently electrotransferred onto PVDF membranes. After the membranes were blocked with 3% skimmed milk, anti-human ERK1, anti-human ERK2, anti-human ERK1/2, anti-human phospho-ERK1/2 and anti-human COX-2 antibodies (Santa Cruz, CA, USA) were incubated with the membranes overnight at 4 °C. The membranes were then washed and incubated with horseradish peroxidase-conjugated secondary antibody (anti-mouse or anti-rabbit IgG, Cell Signaling Technology Inc., Danvers, MA, USA) at room temperature for 2 h. A monoclonal anti-GAPDH antibody (Cell Signaling Technology Inc., Danvers, MA, USA) was used as a control. Finally, the signal bands were detected with ECL reagent (Pierce Biotechnology, USA). Quantifications were done using the Total Lab 100 software (Nonlinear Dynamics, Durham, NC, USA).

### 2.7. Statistical analysis

All the experiments were performed three times and the results are expressed as the mean ± standard deviation. Statistical significance was determined using the Mann–Whitney *U* test or Kruskal–Wallis ANOVA test, when appropriate, using SPSS 13.0 statistical software. A $p$ value of <0.05 was considered statistically significant.

## 3. Results

### 3.1. ERK activation is involved in IL-1β-mediated COX-2 expression and PGE2 production in human chondrocytes

Human chondrocytes were seeded in six well plates ($1 \times 10^5$/well) and cultured with or without 10 ng/ml IL-1β for 24 h. In the presence of IL-1β, the mRNA levels of COX-2 were enhanced by 4.06-fold compared with controls ($p < 0.01$) (Fig. 1A). Western blotting for COX-2 also revealed 4.3-fold increase (Fig. 1B and E). PGE2 production was increased by 3.35-fold in the presence of IL-1β compared with controls ($p < 0.01$) (Fig. 1C). Simultaneously,

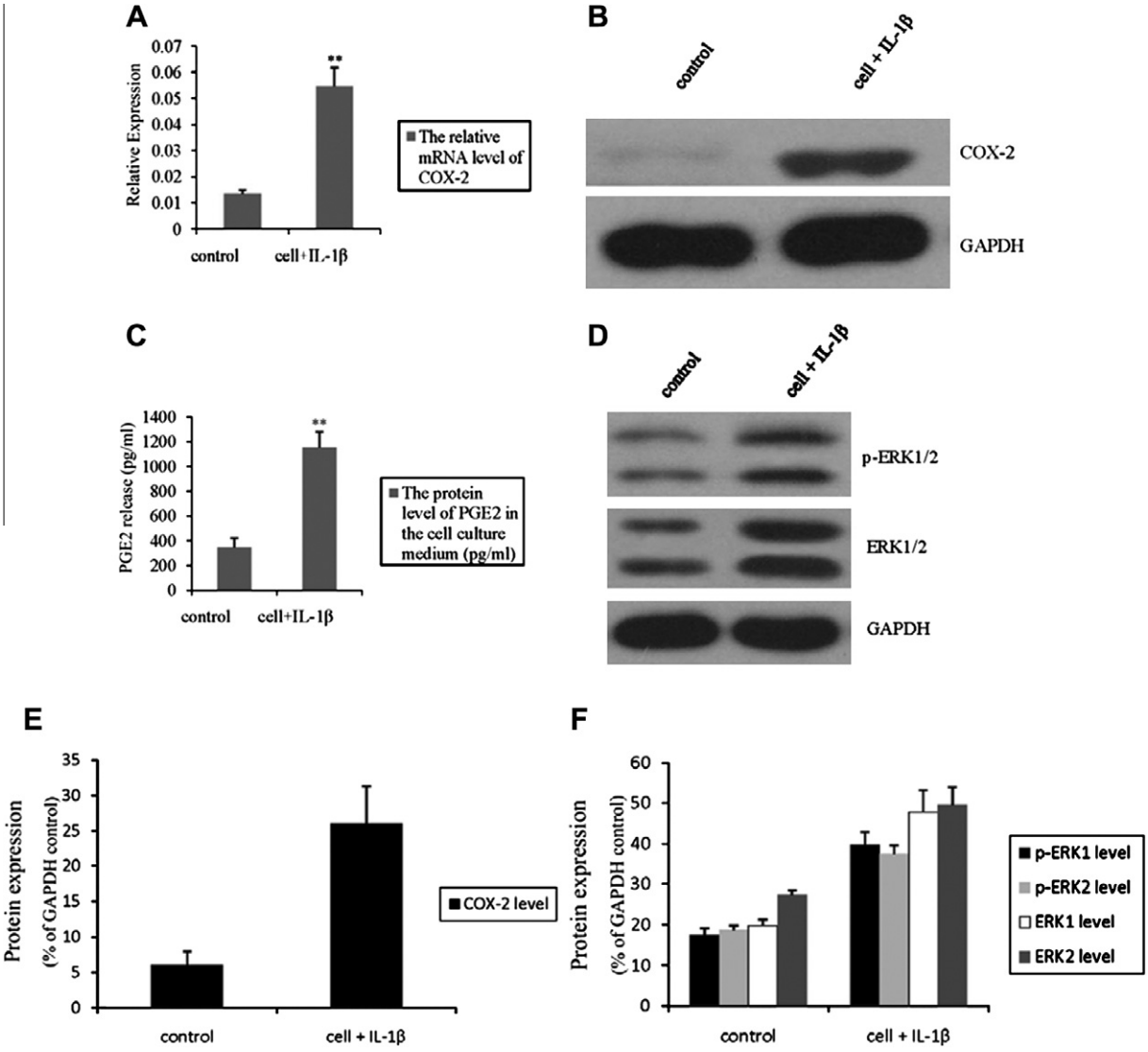


**Fig. 1.** Activation of ERK is involved in IL-1β-mediated COX-2 expression and PGE2 production. Human chondrocytes were seeded in the presence of IL-1β or left untreated (control) for 24 h. The mRNA (A) and protein (B) levels of COX-2 were assessed by quantitative real-time PCR and Western blotting. PGE2 production in cell culture medium was assessed by ELISA (C). The phospho-ERK1/2 and ERK1/2 was evaluated by Western blotting (D). Densitometric analyses of COX-2 (E) and phospho-ERK1/2 and ERK1/2 (F) proteins are presented as bar plots. Data are expressed as percent protein expression of GAPDH control, and are the mean ± SD from three experiments. The results were obtained from three independent experiments. The mRNA levels of COX-2 and PGE2 production are expressed as the mean ± SD (**$p < 0.01$).

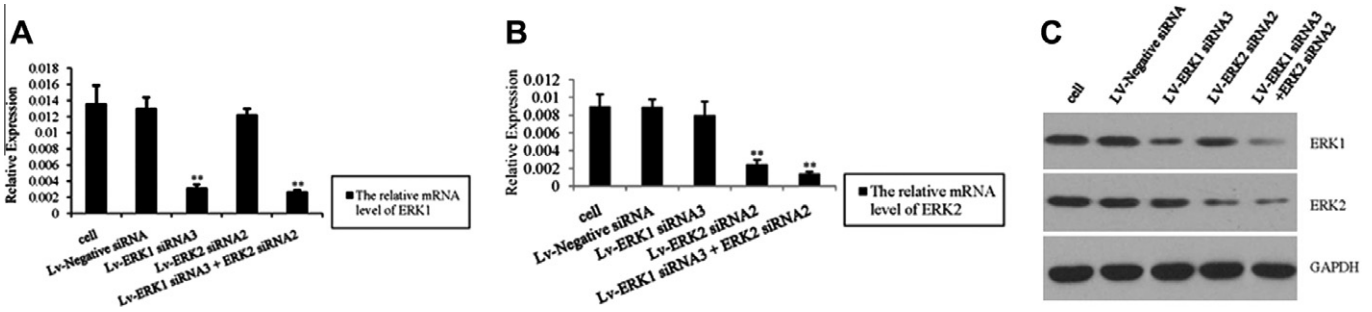


**Fig. 2.** LV expressing ERK1 siRNA3 and ERK2 siRNA2 specifically target ERK1 and ERK2 with high efficiency. Human chondrocytes were infected with LV expressing the negative siRNA, ERK1 siRNA3, ERK2 siRNA2 or ERK1 siRNA3 and ERK2 siRNA2. For controls, cells were left untreated. Ninety-six hours after transfection, the mRNA and protein levels of ERK1 and ERK2 were examined by quantitative real-time PCR (A and B) and Western blotting (C). All data were obtained from an individual experiment performed in triplicate. The mRNA levels of ERK1 and ERK2 are expressed as the mean ± SD (**$p < 0.01$).

the phospho-ERK1/2 level was evaluated by Western blotting (Fig. 1D and F). The result suggested that the level of phospho-ERK1 (2.3-fold), phospho-ERK2 (2.0-fold), ERK1 (2.5-fold) and ERK2 (1.8-fold) were increased following IL-1β treatment, indicating that IL-1β mediates COX-2 expression and PGE2 production through the MAPK/ERK pathway.

### 3.2. LV expressing ERK1 siRNA3 and ERK2 siRNA2 specifically target ERK1 and ERK2, respectively

Human chondrocytes were infected with LV expressing negative siRNA, ERK1 siRNA3, ERK2 siRNA2 or ERK1 siRNA3 and ERK2 siRNA2 as described in Section 2. For controls, cells were left untreated. Ninety-six hours later, quantitative real-time PCR and Western blotting revealed that the LV expressing ERK1 siRNA3 led to a 76.7% reduction in ERK1 expression relative to control levels ($p < 0.01$) without inducing marked changes in ERK2 and GAPDH (GAPDH data not shown) (Fig. 2A and C). Similarly, the LV expressing ERK2 siRNA2 resulted in a 72.7% reduction in ERK2 expression ($p < 0.01$) without marked changes in ERK1 and GAPDH (GAPDH data not shown) (Fig. 2B and C). The negative siRNA had negligible effects on ERK1 and ERK2. Infection with the LV expressing both ERK1 siRNA3 and ERK2 siRNA2 suppressed ERK1 and ERK2 gene expression levels by 81.2% and 84.3%, respectively. Therefore, they were used to study the function of the specific isoforms of ERK in this study.

### 3.3. LV expressing siRNAs exhibits high infection efficiency

As show in Fig. 3, the expression of copGFP 96 h after infection demonstrated the high infection efficiencies of LV expressing negative siRNA, ERK1 siRNA3, ERK2 siRNA2, or ERK1 siRNA3 and ERK2 siRNA2 in human chondrocytes.

### 3.4. The roles and relationship of ERK isoforms on IL-1β-mediated COX-2 expression and PGE2 production in human chondrocytes

As shown in Fig. 4A, the mRNA levels of COX-2 in cells that had ERK1, ERK2 or the combined knockdown were significantly decreased (0.59-fold, 0.53-fold or 0.37-fold, respectively, relative to cells that were treated with IL-1β alone, $p < 0.01$), with the protein level decreased (0.57-, 0.54- and 0.29-fold, respectively) (Fig. 4B and E). ERK1, ERK2 or the combined knockdown also decreased PGE2 production by 0.64-fold, 0.65-fold and 0.43-fold, respectively, compared with cells treated with IL-1β alone ($p < 0.01$) (Fig. 4C). There was no statistically significant difference between the ERK1 and ERK2 knockdowns on IL-1β-induced COX-2 expression and PGE2 production. In the cells infected with the LV expressing the negative siRNA, COX-2 expression and PGE2 production was unchanged relative to cells that were incubated with IL-1β. In addition, the mRNA levels of COX-2 and PGE2 production were significantly decreased by the combined knockdown compared with the knockdown of ERK1 or ERK2 alone ($p < 0.05$). Thus, our results

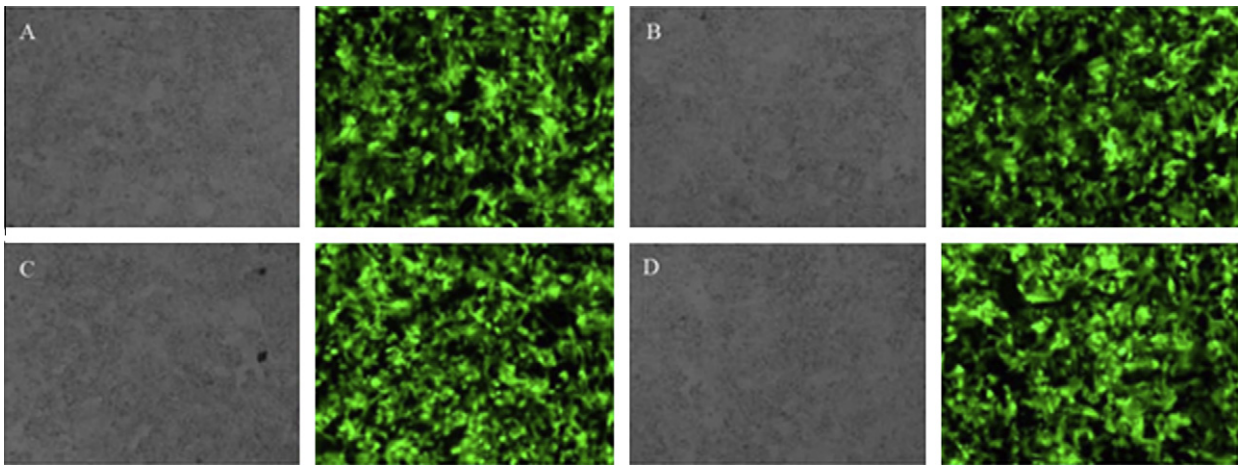


**Fig. 3.** LV expressing siRNAs exhibit high infection efficiencies. High infection efficiencies were indicated using fluorescence microscopy to examine the expression of copGFP 96 h after infection (magnification = 120×). Human chondrocytes were infected with LV expressing negative siRNA (A), ERK1 siRNA3 (B), ERK2 siRNA2 (C), or ERK1 siRNA3 and ERK2 siRNA2 (D), before observation by light microscopy (left) and fluorescence microscopy (right).

demonstrated that ERK1 and ERK2 knockdowns alone similarly inhibit IL-1β induced COX-2 expression and PGE2 production. Moreover, the results indicated that knockdown of ERK1 and ERK2 displayed a synergistic effect.

We simultaneously examined the effects of ERK1, ERK2 or the combined knockdowns on IL-1β induced phospho-ERK1/2 and ERK1/2 levels. As shown in Fig. 4D and F, knockdown of ERK1 or ERK2 down regulated phospho-ERK1 (0.55-fold) and ERK1 (0.53-fold) or phospho-ERK2 (0.48-fold) and ERK2 (0.54-fold) compared with cells that were treated with IL-1β, respectively. We also found that there was no compensation of one isoform for the loss of the other. In addition, the combined knockdown even further suppressed the phospho-ERK1/2 and ERK1/2 levels compared with the single knockdowns. Importantly, phospho-ERK1/2 and ERK1/2 were not affected by LV expressing the negative siRNA compared with cells that were treated with IL-1β.

## 4. Discussion

Increased COX-2 expression and abundant PGE2 production was found in human OA cartilage and synovial tissue [9,10]. Increased levels of inflammatory mediators, such as COX-2 and PGE2, results in the inflammatory process of OA and catabolic processes in articular cartilage [3,4,11]. Inhibition of cyclooxygenase can reduce *in vitro* collagen loss from canine cartilage explants, which correlates with a reduction in PGE2 production [12]. Furthermore, COX-2 protein was significantly increased in joint tissues from dogs with naturally occurring coxofemoral joint OA

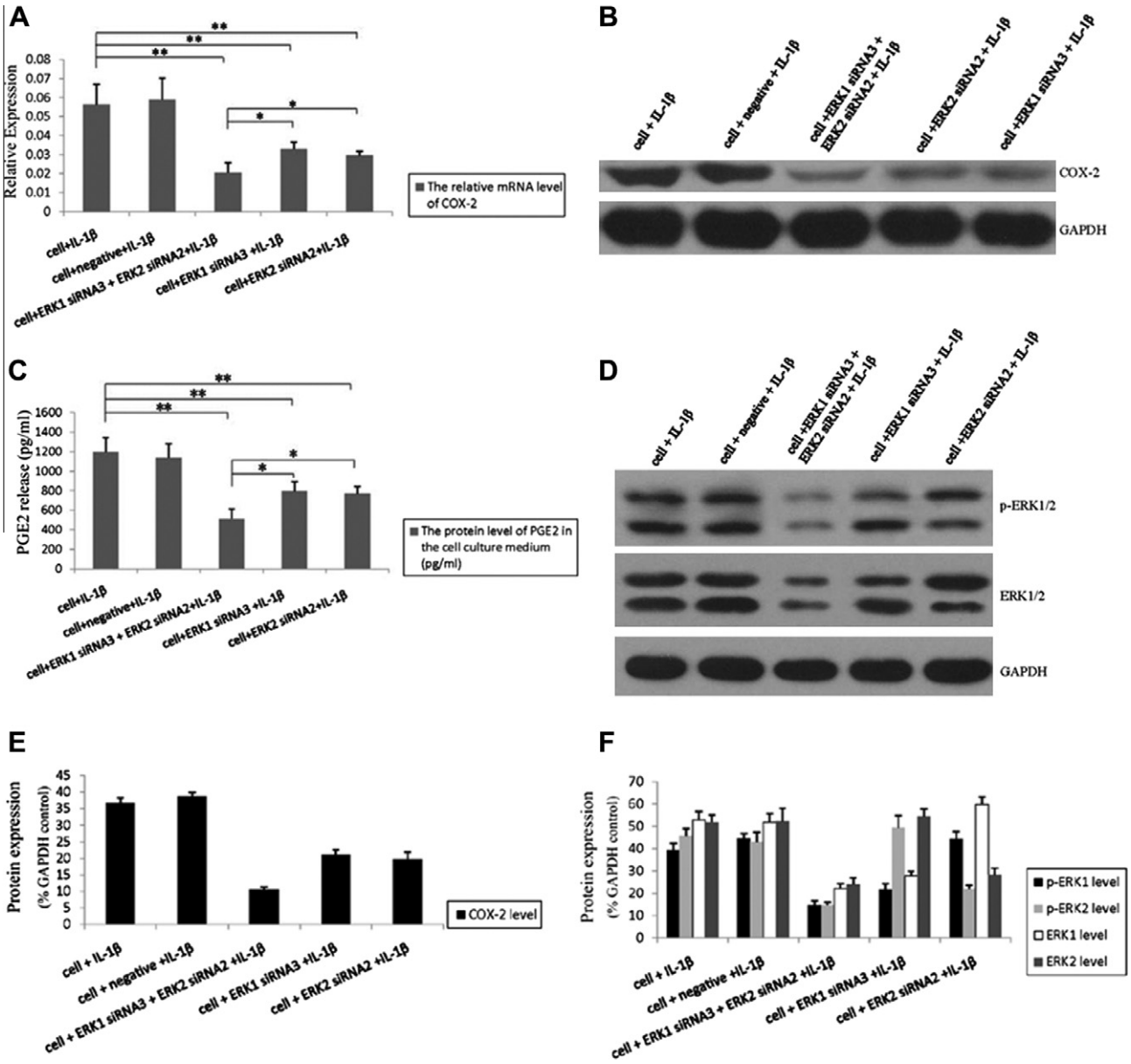


**Fig. 4.** The roles and relationship of ERK isoforms on IL-1β-mediated COX-2 expression and PGE2 production. Human chondrocytes infected with LV expressing negative siRNA, ERK1 siRNA3, ERK2 siRNA2 or ERK1 siRNA3 and ERK2 siRNA2 were incubated with IL-1β for 24 h. For controls, cells were treated with IL-1β. The mRNA (A) and protein (B) levels of COX-2 were assessed by quantitative real-time PCR and Western blotting. PGE2 production in cell culture medium was assessed by ELISA (C). The phospho-ERK1/2 and ERK1/2 was assessed by Western blot analysis (D). Densitometric analyses of COX-2 (E) and phospho-ERK1/2 and ERK1/2 (F) proteins are presented as bar plots. Data are expressed as percent protein expression of GAPDH control, and are the mean ± SD from three experiments. The results were obtained from three independent experiments. The mRNA levels of COX-2 and PGE2 production are expressed as the mean ± SD ($^{*}p < 0.05$; $^{**}p < 0.01$).

[13]. This suggests that COX-2 is an appropriate target for the management of OA. It has been shown that IL-1β induces COX-2 expression and PGE2 production in articular chondrocytes [14–16]. The synthesis of PGE2 from arachidonic acid requires the sequential action of COX-2 and prostaglandin E synthase-1 (mPGES-1). IL-1β markedly increased COX-2 expression and PGE2 production in human chondrocytes via the activation of ERK1/2, p38 and JNK [17]. Interestingly, Erk1/2 and p38 activation is also in the signaling cascades that mediate the upregulation of mPGES-1 and PGE2 production in human chondrocytes exposed to IL-1β [8]. In addition, Avocado-Soybean Unsaponifiables (ASU), one of the commonly used drugs to treat symptoms of OA, sufficiently suppressed IL-1β-induced PGE2 release solely by inhibiting ERK1/2 in chondrocytes [18]. Taken together, these results suggest that ERK plays an important role in IL-1β-induced COX-2 expression and PGE2 production. The present study confirmed that IL-1β significantly up-regulated COX-2 expression and PGE2 production in human chondrocytes, and that activation of the MAPK/ERK pathway was involved. The MAPK/ERK pathway may therefore be a regulatory element of the inflammatory process of OA.

The respective roles and relationship of ERK1 and ERK2 in the regulation of IL-1β-induced COX-2 expression and PGE2 production in human chondrocytes are not fully known. The specific functions of ERK1 and ERK2 remain elusive because of the similarity in their upstream activation pathways and that many of their known downstream targets are common. There are reports that suggest the knockdown of ERK1 alone is sufficient to decrease cell viability in ovarian cancer [19]. In contrast, recent research has shown that ERK2 plays a key role in hepatocyte replication and ERK1 cannot rescue the ERK2 deficiency [20]. It has been revealed that ERK1 attenuates the positive role of ERK2 in controlling normal and Ras-dependent cell proliferation [21]. A further study suggests that ERK1 and ERK2 target common and distinct gene sets in zebrafish embryogenesis [22]. Based on the above findings, we therefore speculate that ERK1 or ERK2 alone may be used to target the inflammatory process for the treatment of OA. To examine the respective roles and the relationship of ERK1 and ERK2 in COX-2 expression and PGE2 production in response to IL-1β, we used siRNA to target the ERK1 and ERK2 genes. In this study, we observed that ERK1 siRNA3 and ERK2 siRNA2 exhibit high interference efficiencies against their expected targets, the ERK1 and ERK2 genes, leading to specific suppression of ERK1 and ERK2, respectively. Our data indicated that the single knockdown of ERK1 or ERK2 significantly inhibited IL-1β-induced COX-2 expression and PGE2 production to similar degree. In addition, the data presented here provide support for the synergistic effect of the combined knockdown.

Simultaneously, Western blotting confirmed that phospho-ERK1/2 and ERK1/2 levels were increased in response to IL-1β. A recent study showed that single and combined silencing of ERK1 and ERK2 reveals their positive contribution to cell proliferation and immediate-early gene transcription in NIH 3T3 cells, a process dependent on total ERK activity, irrespective of isoform specificity [23]. In a mouse 1B6 T cell hybridoma, IL-2 production in response to TCR stimulation may be highly sensitive to total ERK1/2 activation levels [24]. It can be modulated by varying the level of cellular ERK protein via either isoform and without distinguishable contributions from ERK1 or ERK2. Our data suggest that ERK1 or ERK2 knockdown significantly inhibited IL-1β-induced COX-2 expression and PGE2 production to similar degrees through inhibition of phospho-ERK1 and ERK1 or phospho-ERK2 and ERK2 levels, respectively. In addition, no significant compensation occurs between the phospho-ERK and ERK isoforms due to knockdown of either isoform. These results are in contrast to a previous study that indicated that reducing the activation of one isoform increases the other ERK isoform via negative feedback regulation [25]. Moreover, the combined knockdown reduced phospho-ERK1/2 and ERK1/2 levels and displayed a synergistic effect on the inhibition of COX-2 expression and PGE2 production.

Taken together, inhibition of both ERK1 and ERK2 may therefore be a more effective therapeutic target for the inflammatory process of OA. However, the MAPK/ERK pathway responds to many diverse extracellular signals [26,27]. Inhibition of ERK may inevitably result in side-effect. Our findings suggest that ERK1 or ERK2 knockdowns alone may be sufficient to reverse the effect of IL-1β on COX-2 expression and PGE2 production. Although inhibition of ERK1 and ERK2 displayed synergistic effects, future studies should evaluate the role of each individual ERK isoform as a possible target for novel therapies or drugs to counteract the inflammatory process of OA with less toxicity.

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