Original Contribution

LOW-INTENSITY PULSED ULTRASOUND AFFECTS CHONDROCYTE EXTRACELLULAR MATRIX PRODUCTION VIA AN INTEGRIN-MEDIATED P38 MAPK SIGNALING PATHWAY

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Abstract—Although low-intensity pulsed ultrasound (LIPUS) regulates p38 mitogen-activated protein kinase (MAPK) and promotes cartilage repair in osteoarthritis, the role of integrin-mediated p38 MAPK in the effect of LIPUS on extracellular matrix (ECM) production of normal and OA chondrocytes remains unknown. The aim of this study was to investigate whether LIPUS affects ECM production in normal and OA rabbit chondrocytes through an integrin–p38 signaling pathway. A rabbit model of OA was established by anterior cruciate ligament transection, and chondrocytes were isolated from normal or OA cartilage and cultured in vitro. Chondrocytes were treated with LIPUS and then pre-incubated with the integrin inhibitor GRGDSP or the p38 inhibitor SB203580. Expression of type II collagen, MMP-13, integrin β1, p38 and phosphorylated p38 was assessed by Western blot analysis. We found that type II collagen and integrin β1 were upregulated (p < 0.05), whereas MMP-13 was downregulated (p < 0.05) in normal and OA chondrocytes. Furthermore, phosphorylated p38 was upregulated (p < 0.05) in normal chondrocytes, but downregulated (p < 0.05) in OA chondrocytes after LIPUS stimulation. Pre-incubation of chondrocytes with the integrin inhibitor disrupted the effects of LIPUS on normal and OA chondrocytes. Pre-incubation of chondrocytes with the p38 inhibitor reduced the effects of LIPUS on normal chondrocytes, but had no impact on OA chondrocytes. Our findings suggest that the integrin–p38 MAPK signaling pathway plays an important role in LIPUS-mediated ECM production in chondrocytes. (E-mail: lixueping6504@163.com) © 2015 World Federation for Ultrasound in Medicine & Biology.

Key Words: Low-intensity pulsed ultrasound, Osteoarthritis, Chondrocytes, Integrin, p38 MAPK.

INTRODUCTION

Osteoarthritis (OA), caused by articular cartilage degeneration (Lohmander et al. 2003), manifests as joint pain, stiffness, dysfunction and different degrees of deformity in the late stage of the disease (Pearle et al. 2005). The degradation of extracellular matrix (ECM) accelerates degeneration in OA cartilage (Sandell and Aigner 2001). The pathologic changes are specifically associated with the chondrocytes. Chondrocytes play a key role in OA and are very important to maintain the anabolic–catabolic balance of matrix maintenance and tissue function (Aigner et al. 2007).

Cartilage ECM is composed mainly of collagen and aggrecan. Type II collagen is the most abundant collagen in cartilage and is a typical marker for chondrocytes. The hydrolysis of cartilage ECM by proteinase is a key step in the pathogenesis of OA (Takaishi et al. 2008). Matrix metalloproteinases (MMPs) are the most important proteolytic enzymes in the degradation of ECM, and MMP-13 is the most effective enzyme for degrading type II collagen (Blain 2007). In pathologic conditions, chondrocytes produce MMP-13 to degrade type II collagen, resulting in damaged or defective cartilage and, ultimately, OA (Bramono et al. 2004; Mitchell et al. 1996).

Mechanical stress interactions within the ECM play a dominant role in cell regulation. Cells are able to sense forces to which they are exposed and generate forces on the substrate, which enables a rearrangement of matrix proteins (Guilak et al. 2009). Integrins are a family of cell surface stress receptors mediating cellular interactions with the ECM as well as cell–cell interactions (Hynes 1992; 2002). Integrin β1, the main integrin found on the chondrocyte membrane, regulates the proliferation and differentiation of chondrocytes (Kim et al. 2011). Stimulation of integrin signals by mechanical stress may transduce
signals across the cell membrane, leading to activation of downstream pathways, such as the mitogen-activated protein kinase (MAPK) and phosphatidylinositol 3-kinase (PI3K)/Akt pathway (Kim et al. 2011; MacKenna et al. 2000), which play a key role in regulating the production of MMPs in chondrocytes (Chun 2004). The MAPK signaling pathway consists of extracellular signal-regulated kinase (ERK), p38 MAPK (p38) and c-Jun N-terminal kinase (JNK), all of which are constitutively expressed in most cell types, including chondrocytes (Karsdal et al. 2008). The p38 MAPK signaling pathway mediates chondrocyte–perichondral communication (Stanton and Beier 2007), stabilizes chondrogenic transcription factor SOX 9 mRNA (Tew and Hardingham 2006) and is necessary for MMP expression and activity (Sondergaard et al. 2010). Therefore, the p38 MAPK signaling pathway plays a major role in chondrocyte differentiation (Zhen et al. 2001).

Low-intensity pulsed ultrasound (LIPUS) is a form of mechanical energy that is transmitted through and into tissues as pulsed acoustic pressure waves and has been clinically used as a non-invasive supplemental therapy to promote fracture and wound healing (Gebauer et al. 2005). LIPUS has been reported to stimulate chondrocyte proliferation and matrix production in vitro, suggesting an overall anabolic effect on cartilage formation. Therefore, LIPUS can promote the repair of cartilage after damage and can delay cartilage degeneration, leading to OA in the articular cartilage (Huang et al. 1999; Korstjens et al. 2008). Some studies have reported that LIPUS can activate the p38 MAPK signaling pathway (Ikeda et al. 2006; Zhou et al. 2008). Our previous studies also suggested that LIPUS can promote cartilage repair of OA chondrocytes through downregulation of MMP-13 and p38 MAPK (Li et al. 2011) and that the protective effect of LIPUS on chondrocytes may be mediated by the integrin–PI3K/Akt pathway (Cheng et al. 2014).

There has, however, been no study on the correlation of integrins and p38 MAPK with the effect of LIPUS on ECM production of normal and OA chondrocytes. In this study, our aim was to investigate whether LIPUS affects ECM production in normal and OA chondrocytes through the integrin–p38 MAPK pathway.

**METHODS**

**Reagents**

Phosphate-buffered saline (PBS), 0.25% trypsin, 0.2% type II collagenase, Dulbecco’s modified Eagle medium-high glucose (DMEM-HG), fetal bovine serum (FBS), an immunohistochemical kit, a total protein extraction kit, toluidine blue and other cell isolation- and culture medium-related supplies were purchased from KeyGEN (Nanjing, Jiangsu, China). Mouse anti-rabbit monoclonal antibodies against type II collagen, MMP-13, integrin β1, p38, phosphorylated p38 (p-p38) or β-actin were purchased from Acris (Herford, NRW, Germany), and goat antimouse (Fab)2 secondary antibody was purchased from Santa Cruz (Dallas, TX, USA). The integrin inhibitor GRGDSP (glycine–arginine–glycine–aspartic acid–serine–proline) was purchased from AnaSpec (Fremont, CA, USA), and the p38 inhibitor SB203580 was purchased from Selleck (Houston, TX, USA).

**Animal experiments**

Twenty-two 2.5- to 3.0-kg, 2-mo-old healthy male New Zealand white rabbits were purchased from the Qinglongshan Experimental Animal Center of Jiangsu Province, China. All experimental protocols were approved by the ethics committee of Nanjing Medical University Nanjing Hospital.

A rabbit OA model (right knee, n = 11) was generated by anterior cruciate ligament transection. Normal rabbits served as the control group (n = 11). Both normal and OA rabbits (4 wk after transection) were euthanized and subjected to histopathologic observation of femoral condylar cartilage. Chondrocytes from knee joints of normal and OA rabbits were isolated from the cartilage and cultured in vitro, after cartilage changes were evaluated using Mankin scores (Mankin et al. 1971; van der Sluijs et al. 1992).

**Surgical procedures**

Anterior cruciate ligament transection (ACLT) of the OA rabbit model was performed as described previously (Boulocher et al. 2008; Jean et al. 2008). Briefly, rabbits were intravenously anesthetized with 3% sodium pentobarbital (1 mL/kg). The knee joint skin was disinfected with iodine after the fur was shaved, and a parapatellar skin incision was made on the medial side of the joint. The ACLT was transected using eye scissors, and a positive anterior drawer test was used to ensure complete transection of the ligament. The patella was relocated and the wound closed with a vicryl 4/0 braided absorbable sutures. Penicillin and fentanyl were given to prevent bacterial infection and pain, respectively, after the incised skin was closed. The anterior cruciate ligaments of animals in the normal control (NC) group were not transected.

**Histopathology**

The femoral condylar articular cartilage collected from knee joints was fixed in neutral formalin, decalcified in ethylenediaminetetraacetic acid (EDTA) for 3 wk, embedded in paraffin and sectioned into 4-μm-thick sections using a microtome. Pathologic changes in knee joint cartilage samples, including surface irregularities and formation of cracks, were examined under a microscope.
Fibrosis, matrix distribution, cartilage loss and chondrocyte colonization were evaluated in a double-blind fashion by two independent experts using the Mankin score system (Table 1).

**Chondrocyte isolation and culture**

Articular cartilage slices obtained from the femoral condyle of knee joints from OA and normal (control) rabbits were cut into smaller pieces, washed with PBS and digested with 0.25% trypsin for 30 min. The resultant chondrocytes were washed again with PBS; incubated for approximately 10 h in DMEM supplemented with 0.2% type II collagenase, 10% heat-inactivated FBS and antibiotics; washed with culture medium; and collected by centrifugation at 1,000 rpm for 10 min.

The isolated chondrocytes were cultured in tissue culture dishes with complete DMEM in 5% CO₂/95% air at 37°C. The morphology of the chondrocytes was observed under a microscope.

**Immunocytochemistry**

Chondrocytes were fixed with 4% paraformaldehyde for 30 min, washed with PBS three times, incubated with 3% H₂O₂–methanol solution at room temperature for 10 min, washed with PBS three times again and blocked and incubated with goat serum (50–100 μL) at room temperature for 20 min. The cells were then incubated with type II collagen antibodies (50–100 μL, 1:200 dilution) at 37°C for 2 h, washed with PBS three times before addition of 50 μL of an intensifier and incubated at room temperature for 30 min. Then the cells were washed with PBS three times, incubated with horse-radish peroxidase-conjugated anti-mouse-(Fab)₂ antibodies (50 μL) at 37°C for 30 min, washed with PBS three times and then subjected to color development using dianinobenzidine and hematoxylin staining. Three culture dishes were read per condition, and the three areas with most positive staining cells were selected. Type II collagen expression was observed under a microscope, and images were acquired. The integrated option density (IOD) was obtained with Image-Pro Plus software (Cheng et al. 2014; Rizzardi et al. 2012).

**Low-intensity pulsed ultrasound stimulation**

Normal and OA chondrocytes were subdivided into the control group and the treated group, which was subdivided into four subgroups (1–4) treated with LIPUS (HT2009-1, Ito, Tokyo, Japan) at intensities of 20, 30, 40 and 50 mW/cm², respectively. A layer of coupling agent (<1 mm thick) was applied between the LIPUS probe and culture dish, and the probe was placed below the tissue culture dish (Fig. 1). Chondrocytes were treated once daily for 6 d at an on–off ratio of 20%, irradiation of 3 MHz, for 20 min in a 37°C incubator under a humidified atmosphere with 5% CO₂ in air (Cheng et al. 2014; Korstjens et al. 2008).

**Application of integrin and p38 MAPK inhibitors**

To investigate the role of integrin and p38 in the effects of LIPUS on normal and OA chondrocytes, the chondrocytes were pre-treated with a 10, 30 or 50 μM concentration of the integrin inhibitor GRGDSP or a 1, 5 or 10 μM concentration of the p38 inhibitor SB203580, or mock treated with dimethyl sulfoxide for 4 h before the appropriate intensity of LIPUS stimulation. The optimal concentrations of inhibitors were determined using Western blot analysis. Then chondrocytes were incubated with the optimal concentration of inhibitors, 50 μM for GRGDSP or 10 μM for SB203580, and exposed or not exposed to LIPUS for 6 d.

<table>
<thead>
<tr>
<th>Table 1. Mankin scoring scale*</th>
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<tr>
<td>Subgroup 1: Fibrillation</td>
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<tr>
<td>1. Easy surface</td>
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<tr>
<td>2. Uneven surface</td>
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<tr>
<td>3. Fibrillated and fissured within superficial zone only</td>
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<tr>
<td>4. Fissures and erosions extending below the surface zone, without extending beyond the radial zone</td>
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<tr>
<td>5. Fissures and erosions extending into the deeper zone</td>
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<td>Subgroup 2: Matrix distribution</td>
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<td>1. Normal staining</td>
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<tr>
<td>2. Moderate loss in staining</td>
</tr>
<tr>
<td>3. Severe loss in staining</td>
</tr>
<tr>
<td>4. No staining</td>
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| Subgroup 3: Chondrocyte loss |
| 1. Loss extending into superficial zone |
| 2. Loss extending into midzone |
| 3. Loss extending into radial zone |

| Subgroup 4: Chondrocyte cloning |
| 1. No clusters |
| 2. Chondrocyte clusters in superficial zone |
| 3. Chondrocyte clusters in superficial to midzone (fewer than four cells) |
| 4. Chondrocyte clusters of more than four cells located in superficial to midzone, or chondrocyte clusters in deeper zone |

* Grading was performed separately for the medial femoral condyle, lateral femoral condyle, medial tibial plateau and lateral tibial plateau. The minimum total score was 4, and the maximum total score was 16.  

Fig. 1. LIPUS irradiation of chondrocytes *in vitro*. A layer of coupling agent (<1 mm thick) was applied between the LIPUS probe and culture dish, and the probe was placed below the tissue culture dish. Normal and osteoarthritis chondrocytes were subdivided a control group that did not receive LIPUS stimulation and a treated group that was subdivided into four subgroups that received LIPUS at intensities of 20, 30, 40 and 50 mW/cm², respectively. Chondrocytes were treated once a day for 6 d at an on–off ratio of 20%, irradiation of 3 MHz, for 20 min. LIPUS = low-intensity pulsed ultrasound.
Western blot analysis

After LIPUS stimulation for 6 d, chondrocytes were collected, and expression of type II collagen, MMP-13, integrin β1, p38 and p-p38 was determined by Western blot analysis. Proteins were extracted from the chondrocytes using a total protein extraction kit, and 25 μg/μL was loaded onto sodium dodecyl sulfate–polyacrylamide gel for electrophoresis and electroblotted onto nitrocellulose membranes. The membranes were blocked with skim milk for 2 h and incubated with primary antibodies: anti-type II collagen (1:500 dilution), anti-MMP-13 (1:500 dilution), anti-integrin β1 (1:500 dilution), anti-p38 (1:1000 dilution), anti-p-p38 (1:1,000 dilution) and anti-β-actin (1:500 dilution) at 4°C overnight. Then the membranes were subsequently washed three times with Tween 20 in PBS and incubated with peroxidase-conjugated goat anti-mouse IgG secondary antibody (1:5,000 dilution) at 37°C for 2 h, followed by another three washes as described above. The membranes were developed with the ECL kit.

Statistical analysis

All data were expressed as the mean ± standard deviation (SD) and analyzed using SPSS 18.0 software (IBM, Armonk, NY, USA). Differences between groups were analyzed by single-factor analysis of variance (ANOVA). Modified Mankin scores were analyzed using the Wilcoxon signed rank test. A p-value, 0.05, was considered to indicate statistical significance.

RESULTS

General and histopathologic observations

Four weeks after ACLT, the articular surface of normal femoral condylar cartilage was smooth, had a bright luster and did not any cracks (Fig. 2a), whereas the articular surface of OA femoral condylar cartilage had become soft, lost its original luster and bore crannies (Fig. 2b). Femoral condylar cartilage from normal and OA rabbits was stained with hematoxylin and eosin and examined under a
microscope. The surface of articular cartilage from normal rabbits was smooth and evenly stained with the chondrocytes arranged in normal order (Fig. 2c); however, in articular cartilage from rabbits with OA, there were fissures extending deep from the surface with significant loss of staining (Fig. 2d). The chondrocytes from rabbits with OA were also disorganized and reduced in number (Fig. 2d).

**Modified Mankin scores**

Compared with those of the normal control group, the Mankin scores of the OA rabbit model group were overall significantly higher ($p < 0.05$). Mankin scores for subgroups 1–4 of the OA rabbit model group were also significantly higher ($p < 0.05$) than those of the normal control group (Fig. 2h).

**Identification of normal and OA chondrocytes**

The normal chondrocytes had abundant cytoplasm and a round nucleus, which grew to full confluence within 4 to 7 days (arrows in Fig. 2e). The OA chondrocytes had hypertrophic nuclei, which were mixed with some dendrite-like cells and grew to full confluence within 9
to 12 d (arrows in Fig. 2f). Immunohistochemical staining of type II collagen in normal chondrocytes (Fig. 2e) was much deeper than that in OA chondrocytes (Fig. 2f). The mean density of type II collagen was significantly higher in normal chondrocytes than in OA chondrocytes \((p < 0.05)\) (Fig. 2g).

**Expression of type II collagen and MMP-13 in normal and OA chondrocytes after 20 min of LIPUS stimulation at four different intensities**

To determine an appropriate LIPUS intensity for normal and OA rabbit chondrocytes, we examined the expression of type II collagen and MMP-13 in chondrocytes after LIPUS stimulation at four different intensities (20, 30, 40 and 50 mW/cm²). Western blot analysis revealed that in normal and OA rabbit chondrocytes, type II collagen expression was significantly increased \((p < 0.05)\) and MMP-13 expression was significantly decreased \((p < 0.05)\) after LIPUS stimulation at all four intensities. In normal chondrocytes, type II collagen expression was significantly increased \((p < 0.05)\) in the 40 mW/cm² intensity group compared with all other groups, whereas MMP-13 expression was significantly decreased \((p < 0.05)\) in the 40 mW/cm² intensity group compared with the 20 mW/cm² intensity \((p < 0.05)\), 50 mW/cm² intensity \((p < 0.05)\) and control \((p < 0.05)\) groups. However, no significant difference \((p > 0.05)\) was observed compared with the 30 mW/cm² intensity group. In OA chondrocytes, type II collagen expression was significantly increased \((p < 0.05)\) in the 30 mW/cm² intensity group compared with the 40 mW/cm² intensity group \((p < 0.05)\), 50 mW/cm² intensity group \((p < 0.05)\) and control group \((p < 0.05)\). However, no significant difference \((p > 0.05)\) was observed compared with the 20 mW/cm² intensity group. MMP-13 expression was also significantly decreased \((p < 0.05)\) in the 30 mW/cm² intensity group compared with all other groups (Fig. 3).

**Integrin β1 expression and p38 phosphorylation in normal and OA rabbit chondrocytes after 20 min of LIPUS stimulation**

We examined integrin β1 expression and p38 phosphorylation in normal and OA rabbit chondrocytes after LIPUS stimulation. LIPUS at 40 and 30 mW/cm² was used to stimulate normal and OA rabbit chondrocytes, respectively. Integrin β1 expression significantly increased after LIPUS stimulation \((p < 0.05)\) in both normal and OA chondrocytes, whereas expression in
OA chondrocytes was significantly higher than in normal chondrocytes \( (p < 0.05) \). The level of p38 phosphorylation increased significantly after LIPUS stimulation in normal chondrocytes \( (p < 0.05) \), but significantly decreased after LIPUS stimulation in OA chondrocytes \( (p < 0.05) \). The level of p38 phosphorylation was significantly higher in OA chondrocytes than normal chondrocytes \( (p < 0.05) \) (Fig. 4).

Expression of integrin \( \beta 1 \) and p-p38 in normal and OA rabbit chondrocytes after treatment with integrin and p38 inhibitors

The expression of integrin \( \beta 1 \) and p-p38 in normal and OA chondrocytes was examined after treatment with inhibitors. Integrin \( \beta 1 \) expression in normal and OA rabbit chondrocytes significantly decreased \( (p < 0.05) \) after treatment with 10, 30 or 50 \( \mu M \) GRGDSP, with the lowest expression in chondrocytes at 50 \( \mu M \) GRGDSP \( (p < 0.05) \). Expression of p-p38 in normal and OA rabbit chondrocytes significantly decreased \( (p < 0.05) \) after treatment with 1, 5 or 10 \( \mu M \) SB203580, with the lowest expression at 10 \( \mu M \) \( (p < 0.05) \) (Fig. 5).

Expression of type II collagen, MMP-13, p38 and p-p38 in normal and OA rabbit chondrocytes after integrin inhibitor treatment

Expression of type II collagen, MMP-13, p38 and p-p38 in normal and OA rabbit chondrocytes was examined after treatment with the integrin inhibitor GRGDSP at 50 \( \mu M \). After GRGDSP treatment, type II collagen expression significantly increased \( (p < 0.05) \), and MMP-13 expression significantly decreased \( (p < 0.05) \) in normal and OA chondrocytes. The phosphorylation level of p38 significantly increased \( (p < 0.05) \) in normal chondrocytes, but significantly decreased \( (p < 0.05) \) in OA chondrocytes. Expression of type II collagen expression significantly increased \( (p < 0.05) \) and that
of MMP-13 significantly decreased \( (p < 0.05) \) after LIPUS stimulation in normal and OA chondrocytes. When GRGDSP was used, there was no significant change in the expression of type II collagen and MMP-13 in normal and OA chondrocytes \( (p > 0.05) \) after LIPUS stimulation. The phosphorylation level of p38 did not significantly change \( (p > 0.05) \) in normal chondrocytes and OA chondrocytes after LIPUS stimulation (Figs. 6 and 7).

**Expression of type II collagen, MMP-13, p38 and p-p38 in normal and OA rabbit chondrocytes after p38 MAPK inhibitor treatment**

Expression of type II collagen, MMP-13, p38 and p-p38 was examined after treatment with the p38 MAPK inhibitor. Type II collagen expression significantly increased in normal and OA chondrocytes \( (p < 0.05) \). Type II collagen expression did not change significantly in normal chondrocytes \( (p > 0.05) \), but significantly decreased in OA chondrocytes \( (p < 0.05) \) after LIPUS stimulation following SB203580 treatment. MMP-13 expression significantly decreased \( (p < 0.05) \) in normal and OA chondrocytes after SB203580 treatment. On exposure to LIPUS, MMP-13 expression significantly decreased in OA chondrocytes \( (p < 0.05) \), but LIPUS had no significant effect on normal chondrocytes treated with SB203580 \( (p > 0.05) \). The phosphorylation level of p38 significantly decreased \( (p < 0.05) \) in normal and OA chondrocytes treated with SB203580. LIPUS stimulation significantly decreased the phosphorylation level of p38 in OA chondrocytes \( (p < 0.05) \), but had no significant effect in normal chondrocytes \( (p > 0.05) \) after LIPUS stimulation following SB203580 treatment (Figs. 6 and 7).

**DISCUSSION**

In this study, we sought to determine whether LIPUS affects ECM production through an integrin–p38 MAPK signaling pathway in normal and OA chondrocytes. We found that LIPUS significantly increases ECM production and decreases MMP-13 expression through an integrin–p38 MAPK signaling pathway.

We found that after LIPUS stimulation, type II collagen expression significantly increased \( (p < 0.05) \), but MMP-13 expression significantly decreased \( (p < 0.05) \). We suggested that the increased levels of MMP-13 degraded type II collagen in the ECM of chondrocytes to cause OA and that LIPUS prevented the type II collagen from being degraded by MMP-13 in both normal and OA chondrocytes.
We further observed that inhibition of integrin by GRGDSP suppressed MMP-13 expression, which may prevent the degradation of type II collagen and decrease the activity of p38 in both normal and OA chondrocytes. Therefore, we speculate that integrin may have a negative effect on chondrocytes without LIPUS stimulation. We also found that integrin β1 expression in both normal and OA chondrocytes significantly increased \((p < 0.05)\) after LIPUS stimulation. In fact, integrin plays an important role in mechanical signal transduction in chondrocytes by participating in the proliferation, differentiation and migration of chondrocytes (Huang et al. 2004; Humphries 2000; Loeser et al. 2000; Luo et al. 2013; Stamenovic and Wang 2000). Fibronectin fragment (Fn-f), on one hand, stimulates MMP production to cause chondrocyte degeneration; on the other hand, integrin binds to Fn-f (Guo et al. 2009; Yasuda 2010). Therefore, we deduced that the increased integrin expression caused by LIPUS in both normal and OA chondrocytes may be ascribed to the binding to Fn-f to eliminate the negative effect of Fn-f on chondrocyte degradation. In this case, integrins might have a beneficial effect on chondrocytes. To sum up, integrins played different roles under different situations.

We found that after LIPUS stimulation, phosphorylation levels of p38 increased significantly \((p < 0.05)\) in normal chondrocytes, but decreased significantly \((p < 0.05)\) in OA chondrocytes. This indicates that when integrin was activated by LIPUS, the downstream p38 MAPK signaling pathway was activated in normal chondrocytes, but inhibited in OA chondrocytes. In fact, p38 activity is much higher in OA chondrocytes, and inhibition of p38 can curb apoptosis and hypertrophic terminal differentiation of cultured OA chondrocytes (Reilly et al. 2005; Takebe et al. 2011), whereas p38 can be an effector kinase of mechanotransduction in various cell types and is essential to normal articular cartilage (Sondergaard et al. 2010). On the other hand, in adult articular tissues, p38 is known to play an important role in the inflammatory process (Joos et al. 2009), and some evidence suggests that p38 is a major signaling molecule in the induction of MMP-13 and the activity of p38 can have negative effects on cartilage matrix maintenance (Julovi et al. 2011; Mengshol et al. 2000; Pei et al. 2006). In addition, moderate mechanical stress such as knee loading reduces MMP-13 activity through the p38 MAPK signaling pathway in normal articular cartilage (Hamamura et al. 2013). Therefore, we speculated that LIPUS inhibited phosphorylation of p38 in OA chondrocytes, but facilitated phosphorylation of p38 in normal chondrocytes to promote adaptation to a moderate mechanical stress.

We found that after application of the integrin inhibitor GRGDSP, levels of type II collagen, MMP-13 and p38 phosphorylation did not significantly change...
Low-intensity pulsed ultrasound was found to have effects on the expression of type II collagen and MMP-13 through the integrin–p38 MAPK signaling pathway. Therefore, we deduced that the integrin–p38 MAPK signaling pathway plays an important role in LIPUS-mediated ECM production in chondrocytes. Our findings further revealed the mechanism underlying the effects of LIPUS on chondrocytes and provide some basis for the development of OA treatments using LIPUS.

CONCLUSIONS

Low-intensity pulsed ultrasound was found to have effects on the expression of type II collagen and MMP-13 through the integrin–p38 MAPK signaling pathway. Therefore, we deduced that the integrin–p38 MAPK signaling pathway plays an important role in LIPUS-mediated ECM production in chondrocytes. Our findings further revealed the mechanism underlying the effects of LIPUS on chondrocytes and provide some basis for the development of OA treatments using LIPUS.
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