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Research paper

Upregulation of lncRNA HOTAIR contributes to IL-1β-induced MMP overexpression and chondrocytes apoptosis in temporomandibular joint osteoarthritis

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ABSTRACT

Temporomandibular joint osteoarthritis (TMJ OA) is a common and heterogeneous disease that causes painful and progressive joint degeneration, which restricts daily activities, including talking and chewing. Long non-coding RNAs (lncRNAs) are an important class of genes involved in various physiological and pathological functions, including osteoarthritis (OA).The present study aimed to identify the lncRNAs that are important in TMJ OA and their potential functions. Here, we found that HOTAIR was significantly upregulated in the synovial fluid of TMJ OA patients compared with that of normal controls. Increased HOTAIR was similarly observed in the synovial fluid of TMJ OA rabbits as compared to control rabbits. Furthermore, in interleukin-1β (IL-1β)-induced TMJ OA in vitro model (primary rabbit condylar chondrocytes), the expressions of matrix metalloproteinase (MMP)-1, MMP3, MMP9 and HOTAIR were all dramatically increased. Most importantly, knockdown of HOTAIR in IL-1β-induced TMJ OA in vitro model could not only reverse the IL-1β-stimulated expressions of MMP1, MMP3 and MMP9, but also significantly decrease the apoptosis rate induced by IL-1β in primary rabbit condylar chondrocytes. Our data provides new insight into the mechanisms of chondrocytes destruction in TMJ OA.

1. Introduction

Osteoarthritis (OA) of the temporomandibular joint (TMJ) is a non-inflammatory degenerative disease of the jaw joint. It is mainly characterized by progressive destruction of the articular cartilage and decreased synovial fluid lubrication, which result in severe pain and loss of functional movements of the jaw joint (Haskin et al., 1995). Depending on different diagnostic criteria or methods of assessment that are adopted in the various epidemiological studies, the prevalence of TMJ OA could vary from 1% to 84% (de Souza et al., 2012). Although remarkable progress has been made in the diagnosis and therapeutics of TMJ OA in the past three decades, the pathogenesis and etiology of this disease remains uncertain (Guarda-Nardini et al., 2012). Several studies have reported that TMJ OA patients experience erosion of the articular cartilage predominantly resulting from the imbalance between chondrocyte-controlled catabolic and anabolic processes, in which members of matrix metalloproteinase (MMP) family play important roles (Hashimoto et al., 2008; Ge et al., 2009). Emerging evidence has indicated that proinflammatory cytokine interleukin 1β (IL-1β) induces cartilage destruction in vitro in several cell types, including TMJ condylar chondrocytes (Ge et al., 2011). In addition, inhibition of IL-1β frequently reduces the expression of MMP family members in cultured OA articular cartilage (Kobayashi et al., 2005), suggesting a potential connection between IL-1β and MMPs in promoting cartilage degradation in OA. However, the molecular mechanisms underlying the regulation of MMPs expression by IL-1β in chondrocytes are still not fully understood.

In human genome, only 1.5% generates protein-coding mRNAs, a large amount of sequence generates non-protein-coding RNAs that have crucial functions in translation, RNA splicing, and gene regulation (Kurth and Mochizuki, 2009). Specifically, long non-coding RNAs

Abbreviations: TMJ OA, temporomandibular joint osteoarthritis; lncRNAs, long noncoding RNAs; OA, osteoarthritis; IL-1β, interleukin-1β; MMP, matrix metalloproteinase; TMJ, temporomandibular joint; MEG3, maternally expressed gene 3; PCGEM1, prostate cancer gene expression marker 1; GAS5, growth arrest-specific 5; HOTAIR, HOX antisense intergenic RNA; TUNEL, TdT mediated dUTP nick end labeling; FACS, flow cytometry.

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(lncRNAs) are a class of non-coding RNAs that are longer than 200 nucleotides and take part in regulating key cellular processes, such as proliferation, apoptosis and differentiation (Mercer et al., 2009). Accumulating evidence has demonstrated that multiple lncRNAs are implicated in the pathogenesis of OA. For example, lncRNA H19 is shown to be an attractive marker for cell anabolism in cartilage and cultured chondrocytes (Steck et al., 2012); lncRNA maternally expressed gene 3 (MEG3) is significantly downregulated in OA cartilage samples compared to normal control and possibly contributes to OA development through regulating angiogenesis (Su et al., 2015); lncRNA prostate cancer gene expression marker 1 (PCGEM1) is dramatically elevated in osteoarthritic synoviocytes and its overexpression inhibits apoptosis and stimulates the proliferation of human synoviocytes (Kang et al., 2015); higher expression of lncRNA growth arrest-specific 5 (GAS5) is found in chondrocytes from OA patients compared to normal chondrocytes. Additionally, overexpression of GAS5 induces the expression of several MMPs family members, including MMP-2, MMP-3, MMP-9, and MMP-13 (Song et al., 2014). Furthermore, through microarray analysis, Xing et al. showed that expression of lncRNAs HOX antisense intergenic RNA (HOTAIR), GAS5, and H19 were all upregulated in OA cartilage compared with normal cartilage (Xing et al., 2014). Although TMJ OA shares many similarities with OA from other joints (Herb et al., 2006), knowledge of the involvement of lncRNAs in the development of TMJ OA is so far limited and requires further investigations.

In the current study, we compared the expression of lncRNAs in TMJ OA synovial fluid to that in normal synovial fluid to identify lncRNAs specifically expressed in TMJ OA. In addition, we established rabbit TMJ OA model and showed similar results as human samples in terms of differentially expressed lncRNAs between TMJ OA synovial fluid and normal synovial fluid. Importantly, the possible involvement and regulatory mechanisms of HOTAIR in TMJ OA were explored in IL-1β-induced TMJ OA primary rabbit condylar chondrocytes in vitro. We found that HOTAIR is involved in the upregulation of MMPs induced by IL-1β in TMJ OA and possibly contribute to the development of TMJ OA through regulating apoptosis in chondrocytes. These data may have important clinical implications and could be used to improve diagnosis and treatment strategies in TMJ OA.

2. Methods

2.1. Study patients

34 subjects of either gender were selected, including 21 patients with clinical signs and symptoms of TMJ OA, and 13 subjects with no clinical signs and symptoms of TMJ OA as control group. All the participants were between the age of 30 and 50 years. Selection of TMJ OA patients was based on the research diagnostic criteria for temporomandibular disorders (Manfredini et al., 2011). Full ethical consents were obtained from all participants before the experiments. Synovial fluid collection was carried out in accordance with the terms of the Medical Ethical Committee of the Yantai Yuhuangding Hospital and followed the guidelines of the Declaration of Helsinki.

2.2. Study animals

With the approval of the Institutional Animal Care and Use Committee of Yantai Yuhuangding Hospital, the purpose of this approach was to establish a genuine mechanical-stress-induced TMJ OA model in rabbit. A total of 15 rabbits were randomly assigned to either the experimental ($n = 10$) or control ($n = 5$) groups. The rabbits from both groups were anesthetized by intravenous injection of 0.5% pentobarbital sodium (0.8 mL/kg) (Weitonglihua, Beijing, China) in each experimental session. For experimental rabbits, adverse mechanical stress was applied to their TMJ by a repetitive and steady mouth-opening protocol 3 h per day for 5 continuous days. A jaw-opening device was utilized to hold the mandible in the maximal mouth-opening position with a steady 2-N interincisal expansion force (Fujisawa et al., 2003). While in the control rabbits, no forced jaw opening was applied. Food intake and animal weight were monitored each day during the experiment. The synovial fluid in rabbits' TMJ from both groups was obtained at day 5.

2.2.1. Chondrocyte cell culture

Chondrocytes were isolated from TMJ condylar cartilage from 2-week-old New Zealand white rabbits (lot no. SCXK2006-001; Baola, Beijing, China) by digestion with 0.25% trypsin (Sigma, St. Louis, MO) for 15 min, followed by digestion with 0.2% type II collagenase (Invitrogen, San Diego, CA) for 4–6 h, according to previously described methods with slight modifications (Cheng et al., 2003). Chondrocytes were resuspended in Dulbecco's modified Eagle's medium (DMEM; Gibco, Grand Island, NY) containing 10% fetal bovine serum (Hyclone, Logan, UT), 50 units of penicillin/streptomycin and 2 mM L-glutamine. Cells were then plated in 60-mm plates at a density of 1.5×10^6 cells/plate. After primary culture for 5 days, chondrocytes were harvested. Secondary cultures were placed in 6-well plates at a density of 5×10^5 cells/well for all further experiments. IL-1β induction was performed by adding IL-1β (10 ng/mL) (Sigma, USA) to the media for certain period of time prior to cell harvesting.

2.2.2. Real-time PCR

Purification of total RNA was performed with RNeasy Mini Kits (QIAGEN, Valencia, CA, USA) according to the manufacturer's instructions. RNA purity and concentration were determined spectrophotometrically. A reverse transcription and first strand cDNA synthesis was performed using an Omniscript RT kit (QIAGEN, USA) following the supplier's instructions. To detect relative mRNA level, real time PCR was conducted on an ABI 7500 Fluorescent Quantitative PCR system (Applied Biosystems, Bedford, MA, USA). Individual RT-PCR reactions were carried out in a final 25 μL reaction mixture contained 1 μL of cDNA from samples, 12.5 μL of 2× SYBR Green qPCR Master Mix, 1 μL primers (10 mM), and 10.5 μL of RNase/DNase free water. Amplification conditions included a pre-incubation at 95 °C for 1 min followed by 40 cycles of 10 s at 95 °C and 20 s at 60 °C. Melting curve analysis was performed at a linear temperature transition rate of 0.5 °C/s from 60 °C to 95 °C with continuous fluorescence acquisition. Relative expression levels for the tested genes were normalized by the Ct value of GAPDH (internal control) using a $2^{-\Delta\Delta Ct}$ relative quantification method. The sequences of the primers were listed below: 5′-CCATCTTTATGATG AGGCTTGT-3′ (HOTAIR forward); 5′-GCTGAGATAGAGGTGCTTGG-3′ (HOTAIR reverse); 5′-AAAATTACACGCCAGATTTGCC-3′ (MMP1 forward); 5′-GGTGTGACATTACTCCAGAGTTG-3′ (MMP1 reverse); 5′-CTGGACTCCG ACACTCTGGA-3′ (MMP3 forward); 5′-CAGGAAAGGTTCTGAAGTGACC-3′ (MMP3 reverse); 5′-AGACCTGGGCAGATTCCAAAC-3′ (MMP9 forward); 5′-CGGCAAGTCTTCCGAGTAGT-3′ (MMP9 reverse); 5′-CTGGGCTACA CTGAGCACC-3′ (GAPDH forward); and 5′-AAGTGGTCGTTGAGGGCA ATG-3′ (GAPDH reverse). All experiments were repeated at least three times for reproducibility.

2.3. RNA interference

Specific siRNA for HOTAIR and scrambled control siRNA were obtained from Ribo Company (Guangzhou, Guangdong, China). The sequences for HOTAIR siRNAs were: 5′-GGAGUACAGAGAGAAUAAUUU-3′ (sense) and 5′-AUUAUUCUCUCUGUACUCCUU-3′ (anti-sense); scramble siRNAs were 5′-AGAAUAAUGGAGUACAGAGUU-3′ (sense) and 5′-UCUGUACUCAUUAUUCUCCUU-3′ (anti-sense). Chondrocytes were seeded into 6-well plates at 6×10^5 cells/well and incubated for 24 h. The cells were then transfected with 100 nM siRNA using HiPerFect Transfection Reagent (QIAGEN, USA) according to the manufacturer's recommendations. The medium was changed 24 h later, and the

cells were incubated for an additional 24 h before stimulation with 10 ng/mL IL-1β (Sigma, USA).

2.4. Western blot

The cell lysates were extracted with lysis buffer. Equal amount of protein samples were mixed with 5× sample buffer (Beyotime, China) and boiled at 95 °C for 5 min. The samples were transferred on PVDF membrane (Millipore, USA) using the semi-dry transfer method after sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE). After blocking in 10% non-fat dried milk in TBST for 2 h, the blots were incubated with anti-MMP1, anti-MMP3, anti-MMP9 or anti-GAPDH (Santa Cruz, USA) overnight at 4 °C. GAPDH was used as an internal loading control. After washing with TBST, the blots were incubated with a horseradish peroxidase-conjugated secondary antibody (Santa Cruz, USA) at room temperature for 1 h. The blots were later visualized with Femto (Pierce, USA) following the manufacturer's instructions.

2.5. Immunofluorescence

Cultured chondrocytes cells on cover slides were rinsed in PBS and fixed with 4% paraformaldehyde for 15 min. After PBS wash, these samples were covered with 10% goat serum for 60 min and then incubated with anti-COL2A (Santa Cruz, USA) overnight at 4 °C. These samples were further incubated with goat anti-rabbit IgG conjugated TRITC (Invitrogen, USA) for 30 min at 37 °C. After rinsing in the dark, these samples were incubated with 0.1 μg/mL DAPI for 3 min. PBS was used to replace primary antibodies in negative controls. Images were captured with a laser confocal microscope LSM780NLO (Zeiss, Germany).

2.6. TdT mediated dUTP nick end labeling (TUNEL) assay

Apoptosis was examined in situ using a TUNEL assay according to the manufacturer's instructions (Roche, Mannheim, Germany). Briefly, cultured chondrocytes cells on cover slides were fixed with 4% paraformaldehyde for 25 min, pretreated with protease K (10 mg/mL, Sigma, USA) for 20 min, and blocked with 3% bovine serum albumin for 20 min at room temperature. The slides were then incubated with TUNEL reaction mixture for 1 h at 37 °C and covered with fluorescence mounting medium (Zhongshan-Golden-Bridge-Biotechnology, Beijing, China). Confocal microscope images were acquired using a Zeiss laser-scanning microscope (LSM 510, Jena, Germany).

2.7. Flow cytometry (FACS) assay for apoptosis

The procedure was conducted according to methodology provided in Annexin V-FITC Apoptosis Detection Kit (Sigma, USA). Cells at density of 1×10^6 cells/mL were incubated, harvested and washed twice with PBS before being transferred into sterile centrifuge tube. The cell pellet was then resuspended in binding buffer at a concentration of 1×10^6 cells/mL. Sample (500 μL) of this cell suspension was transferred to a 5 mL falcon tube and mixed with 5 μL of Annexin V-FITC conjugate and 10 μL of propidium iodide. The cells were then incubated in the dark for 10 min at room temperature. The fluorescence of the cells was determined by flow cytometry in the flow cytometry core facility in Yantai Yuhuangding Hospital.

2.8. Enzyme-linked immunosorbent assay (ELISA)

Synovial fluid samples were centrifuged at 1000 rpm for 5 min, levels of IL-1β was determined by ELISA kit according to the manufacturer's protocol (Sino Biological Inc., Beijing, China). All samples were detected in duplicate.

2.9. Analysis

Data are expressed as the mean ± S.E.M. Statistical comparisons were made between two groups with the two-tailed paired *t*-test. A value of $P < 0.05$ was considered significant unless otherwise described.

3. Results

3.1. Increased expression of HOTAIR in synovial fluid of TMJ OA

To assess the potential effect of lncRNAs in the development of TMJ OA, we compared the expression levels of several lncRNAs that have been previously implicated in OA, including HOTAIR, H19, MEG3, PCGEM1 and GAS5, between synovial fluid of TMJ OA patients and synovial fluid of normal participants using RT-PCR. The data showed that the expression of HOTAIR, but not other lncRNAs tested, was significantly upregulated in the synovial fluid of TMJ OA patients, as compared with normal controls (Fig. 1A). Next, we proceeded to confirm the above observations in a TMJ OA rabbit model. The NCBI database (http://www.ncbi.nlm.nih.gov/gene/) only contained the detailed sequence of HOTAIR in human, mouse, dog and chimpanzee, with no information for rabbit HOTAIR. Therefore, we aligned the sequence of human HOTAIR and mouse HOTAIR by rVista 2.0: evolutionary analysis of transcription factor binding sites (http://rvista.dcode.org) (Fig. 1B) and designed the appropriate primers for rabbit HOTAIR based on the conserved region between human and mouse HOTAIR. The TMJ OA rabbit model was established and no significant weight loss was observed in the TMJ OA rabbit model during the experiment (mean weight before the mechanical stress application, 2.70 ± 0.15 kg; after the mechanical stress application, 2.83 ± 0.16 kg). Consistent with our previous finding in human TMJ OA samples, HOTAIR expression was also significantly increased in the synovial fluid of TMJ OA rabbits when compared to that of control rabbits (Fig. 1C).

3.2. IL-1β induces upregulation of MMPs and HOTAIR in rabbit TMJ chondrocytes

It is well known that the expression of IL-1β is significantly increased in TMJ of patients affected by TMJ OA (Vernal et al., 2008). So, the concentration of IL-1β was detected via ELISA assay, though the synovial fluid volume was increased in TMJ OA patients and the rabbit model, the concentration of IL-1β was still significantly higher than the control group respectively (Supplementary material, Table 1, Table 2). Moreover, upregulation of IL-1β stimulates a series of destructive events in OA, including activation of cartilage catabolism, inhibition of cartilage anabolism and reduced synthesis of cartilage extracellular matrix (Daheshia and Yao, 2008). Therefore, we hypothesized that IL-1β might contribute to TMJ OA through regulating MMPs. Chondrocytes were isolated from the TMJ condylar cartilage of New Zealand white rabbits, and type II collagen (COL2A1) was used as a chondrocyte-specific surface marker for verification (Fig. 2A). Rabbit primary TMJ condylar chondrocytes stimulated by IL-1β showed significantly increased expressions of MMP1, MMP3 and MMP9 at both transcriptional (Fig. 2B) and translational levels (Fig. 2C), suggesting that IL-1β directly modulates the expression of MMPs in TMJ OA. Interestingly, we also demonstrated that IL-1β induction drastically upregulated the expression of HOTAIR in rabbit primary TMJ condylar chondrocytes. The maximum upregulation effect (nearly five folds increase) was seen at 2 h after IL-1β treatment and gradually returned to the basal level at 6 h after IL-1β treatment (Fig. 2D).

3.3. HOTAIR knockdown reversed IL-1β-induced upregulation of MMPs in rabbit TMJ chondrocytes

Next, we sought to understand whether HOTAIR is involved in the IL-1β-induced upregulation of MMPs in rabbit TMJ OA. Rabbit primary

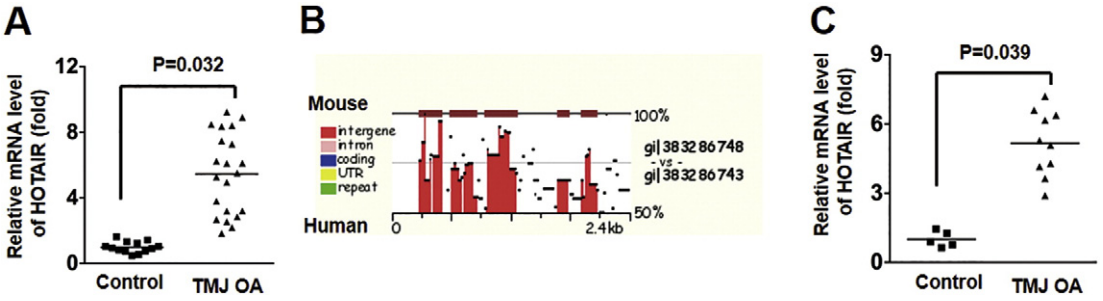

Fig. 1. Increased expression of HOTAIR in the synovial fluid of TMJ OA. (A) Expression of HOTAIR was determined by RT-PCR in the synovial fluid of TMJ OA patients (TMJ OA, n = 21) and the synovial fluid of normal participants (control, n = 13). (B) Conserved sequence of HOTAIR between human and mouse was aligned by rVista 2.0 (http://rvista.dcode.org). (C) Expression of HOTAIR was determined by RT-PCR in the synovial fluid of TMJ OA rabbits (TMJ OA, n = 10) and the synovial fluid of control rabbits (controls, n = 5).

condylar chondrocytes were maintained in monolayer culture and transfected with siRNA targeting HOTAIR (si-HOTAIR) or scramble siRNA (scramble). 48 h after siRNA transfection, HOTAIR expression was significantly downregulated in si-HOTAIR group, which indicated that the knockdown system was successful (Fig. 3A). 2 h after IL-1β induction in rabbit condylar chondrocytes following siRNA transfection, we found that HOTAIR knockdown markedly downregulated the IL-1β-induced overexpression of MMP1, MMP3 and MMP9 at mRNA (Fig. 3B) and protein levels (Fig. 3C) as compared with scramble controls. These data confirmed that HOTAIR mediated the IL-1β-induced upregulation of MMPs in rabbit TMJ OA.

3.4. HOTAIR knockdown decreased apoptosis rate in rabbit TMJ chondrocytes

Studies have shown that chondrocyte apoptosis might account for the condylar destruction, leading to subsequent OA (Hashimoto et al., 1998). Also, lncRNAs might be involved in the development of OA through modulation of apoptosis (Song et al., 2014). In the current study, we further explored whether HOTAIR had any effect on apoptosis in rabbit primary condylar chondrocytes. The rabbit primary condylar chondrocytes were transfected with si-HOTAIR or scramble siRNA, respectively, followed by stimulation with IL-1β for 2 h. Apoptosis rate was evaluated with both TUNEL assay and FACS assay. The

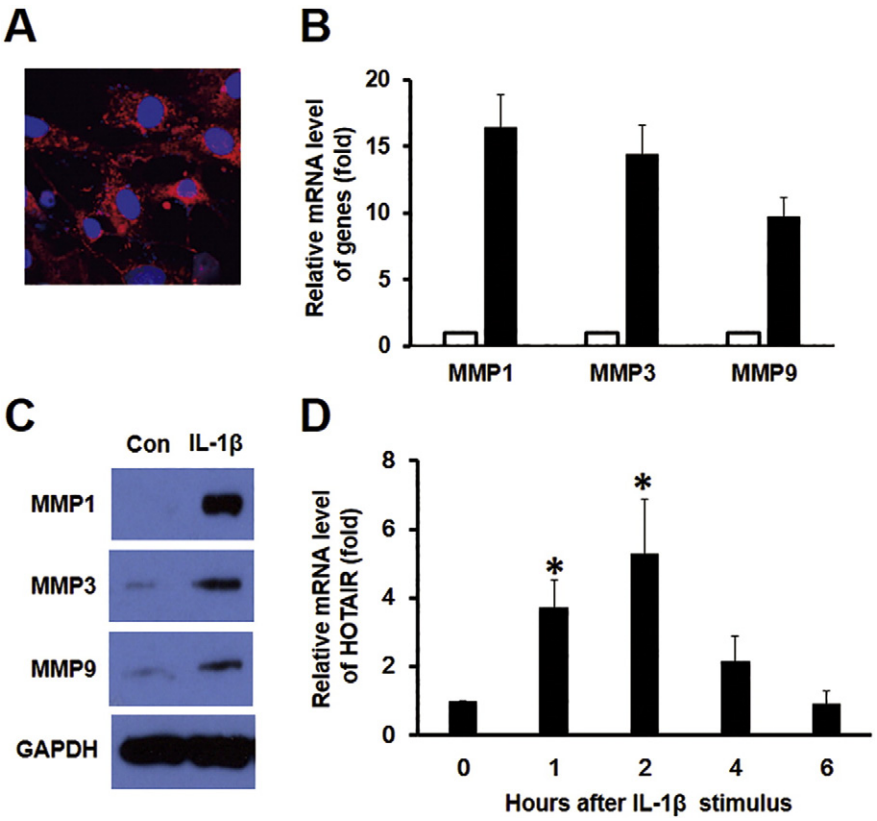

Fig. 2. IL-1β treatment induces upregulation of MMPs and HOTAIR in rabbit TMJ chondrocytes. (A) COL2A1 (red) was detected by immunofluorescence microscopy in chondrocytes from TMJ condyles obtained from 2-week-old rabbits. The nucleus was shown in blue (DAPI). (B) The mRNA levels of MMP1, MMP3 and MMP9 were determined in rabbit primary TMJ condylar chondrocytes treated with IL-1β for 12 h by RT-PCR. #, P < 0.01 vs. the control. (C) The protein levels of MMP1, MMP3 and MMP9 were determined in rabbit primary TMJ condylar chondrocytes treated with IL-1β for 12 h by western blot. (D) Expression of HOTAIR was determined by RT-PCR in rabbit primary TMJ condylar chondrocytes treated with IL-1β for 0, 1, 2, 4, 6 h, respectively. *, P < 0.05 vs. the control.

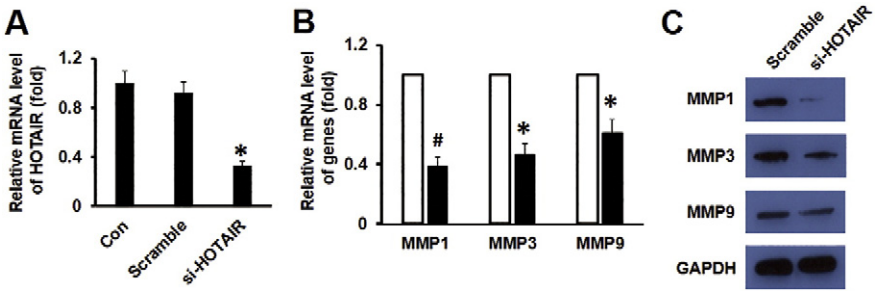

Fig. 3. HOTAIR knockdown reversed IL-1β-induced expression of MMPs in rabbit TMJ chondrocytes. (A) Expression of HOTAIR was determined by RT-PCR in rabbit primary TMJ condylar chondrocytes transfected with mock (control), scramble siRNA (scramble) or siRNA targeting HOTAIR (si-HOTAIR). *, $P < 0.05$ vs. the scramble siRNA. (B) The mRNA levels of MMP1, MMP3 and MMP9 were determined after IL-1β induction in rabbit primary TMJ condylar chondrocytes transfected with scramble siRNA or si-HOTAIR. *, $P < 0.05$ vs. the scramble siRNA, #, $P < 0.01$ vs. the scramble siRNA. (C) The protein levels of MMP1, MMP3 and MMP9 were determined after IL-1β induction in rabbit primary TMJ condylar chondrocytes transfected with scramble siRNA or si-HOTAIR. GAPDH was used as internal loading control.

representative pictures of TUNEL assay showed that the amount of apoptotic positive cells was greatly decreased when HOTAIR was knocked down (Fig. 4A). Consistent with the results obtained with TUNEL assay, the FACS assay also showed that knockdown of HOTAIR dramatically reduced the apoptosis incidence in IL-1β-induced rabbit TMJ condylar chondrocytes (Fig. 4B). Our observations indicated that lncRNA HOTAIR might play important role in the pathogenesis of TMJ OA by modulating apoptosis of chondrocytes.

4. Discussion

Osteoarthritis (OA) is a disease causing degenerative joint, which featured with synovitis, cartilage degeneration, subchondral bone sclerosis, and osteophyte formation (Heinegard and Saxne, 2011). The temporomandibular joint (TMJ), which is the only diarthrodial joint in the human jaw, plays an important role in craniofacial growth and function (Shen et al., 2015). TMJ can also be affected by OA (TMJ OA), which

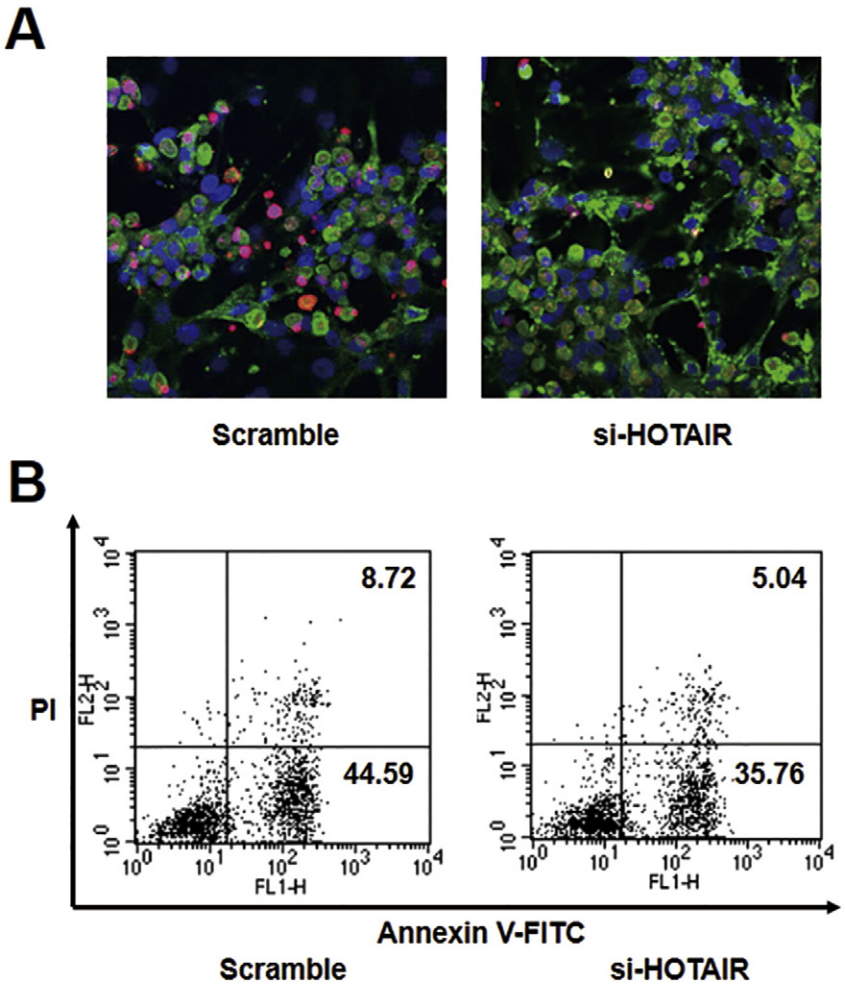

Fig. 4. HOTAIR knockdown decreased apoptosis rate in rabbit TMJ chondrocytes. (A) The rabbit primary condylar chondrocytes were transfected with si-HOTAIR or scramble siRNA, and then stimulated with IL-1β for 2 h. The apoptosis rate was detected by TUNEL assay. Apoptosis cells (red), COL2A (green) and nucleus (blue) were shown. (B) The rabbit primary condylar chondrocytes were transfected with si-HOTAIR or scramble siRNA, and then stimulated with IL-1β for 2 h. The apoptosis rate was detected by flow cytometry assay with Annexin V-FITC/PI double staining.

shows similar symptoms as OA of other joints. Therefore, effort to investigate the molecular mechanisms associated with TMJ OA is necessary for the development of faster and more effective diagnosis and treatment.

In recent years, lncRNAs has received increasing attention and research effort due to their involvement in a variety of biological processes, such as cell cycle control, immune response regulation, cell differentiation and maintenance of embryonic stem cell pluripotency (Khalil et al., 2009). Many lncRNAs, such as H19 (Steck et al., 2012), MEG3 (Su et al., 2015), PCGEM1 (Kang et al., 2015), GAS5 (Song et al., 2014), are found to function in cartilage degradation and considered as promising therapeutic targets of OA. However, knowledge about the feasibility of using lncRNA as a therapeutic target in the prevention or treatment of cartilage degradation in TMJ OA is very limited. In the current study, we detected the expression of several lncRNAs in synovial fluid obtained from TMJ OA patient, including HOTAIR, H19, MEG3, PCGEM1 and GAS5. We found that HOTAIR was significantly upregulated in the synovial fluid from TMJ OA patients compared with that in normal controls (Fig. 1A). Similar upregulation of HOTAIR was also observed in the synovial fluid of TMJ OA rabbit model (Fig. 1C). As we know, synovial fluid is secreted by the synovial cells to provide the chondrocytes with nourishment (Heinegard and Saxne, 2011). Therefore, the increased HOTAIR levels in synovial fluid might affect the functions of chondrocytes and the subsequent development of TMJ OA. Our results are also consistent with a previous report demonstrating upregulation of HOTAIR in OA tissues as validated by RT-PCR after microarray analysis (Xing et al., 2014). Further functional studies are critical to confirm the role of HOTAIR in TMJ OA and to explore potential targets for therapeutics of TMJ OA.

We next established an in vitro TMJ OA model with primary rabbit condylar chondrocyte and found that IL-1β induction upregulated the expressions of several MMP family members, including MMP1, MMP3 and MMP9, which is consistent with the well-recognized roles of IL-1β signaling pathway in the pathogenesis of OA (Daheshia and Yao, 2008). Intriguingly, we found that HOTAIR was upregulated in primary rabbit condylar chondrocytes at the beginning of IL-1β stimulation, and returned to basal level in condylar chondrocytes even though IL-1β stimulation continued (Fig. 2D). Hence, we believe that HOTAIR might be one of the immediate downstream effectors in response to IL-1β stimulation and that the expression of HOTAIR might be concurrently regulated by other signals. In addition, we further determined the potential functions of HOTAIR in TMJ OA. Our results showed that knockdown of HOTAIR could reverse the expressions of MMP1, MMP3 and MMP9 induced by IL-1β. Moreover, we found that knockdown of HOTAIR could decrease the apoptosis induced by IL-1β in primary rabbit condylar chondrocytes. Therefore, our data prove that HOTAIR possibly takes part in the chondrocyte destruction and development of TMJ OA through enhancing the expression of MMP family members and promoting chondrocytes apoptosis.

In conclusion, we have shown that upregulation of HOTAIR contributes to IL-1β-induced MMPs overexpression and apoptosis in rabbit TMJ condylar chondrocytes, suggesting an important pro-apoptotic role of HOTAIR in TMJ OA chondrocytes. This study provides new clue about the involvement of lncRNA HOTAIR in the destruction of chondrocytes in TMJ OA.

Contributors

ZCP, WP and JPF developed the study protocol and drafted the manuscript. LYB, DCX, DXY and TLX collected and analyzed the data. WZL analyzed the data and interpreted the results, and drafted the manuscript. All authors have read and approved the manuscript.

Competing interests

None declared.

Data sharing statement

No additional data are available.

Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.gene.2016.04.016.

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