



RESEARCH

Inhibition of miR-126 protects chondrocytes from IL-1 β induced inflammation via upregulation of Bcl-2

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doi: 10.1302/2046-3758.76.BJR-2017-0138.R1

Bone Joint Res 2018;7:414–421.

Objectives

The aim of this study was to investigate the role of miR-126 in the development of osteoarthritis, as well as the potential molecular mechanisms involved, in order to provide a theoretical basis for osteoarthritis treatment and a novel perspective for clinical therapy.

Methods

Human chondrocyte cell line CHON-001 was administrated by different doses of interleukin (IL)-1 β to simulate inflammation. Cell viability, migration, apoptosis, IL-6, IL-8, and tumour necrosis factor (TNF)- α expression, as well as expression of apoptosis-related factors, were measured to assess inflammation. miR-126 expression was measured by quantitative polymerase chain reaction (qPCR). Cells were then transfected with miR-126 inhibitor to assess the effect of miR-126 on IL-1 β -injured CHON-001 cells. Expression of B-cell lymphoma 2 (Bcl-2) and the activity of mitogen-activated protein kinase (MAPK) / Jun N-terminal kinase (JNK) signaling pathway were measured by Western blot to explore the underlying mechanism through which miR-126 affects IL-1 β -induced inflammation.

Results

After IL-1 β administration, cell viability and migration were suppressed while apoptosis was enhanced. Expression of IL-6, IL-8, and TNF- α were all increased, and miR-126 was upregulated. In IL-1 β -administrated CHON-001 cells, miR-126 inhibitor suppressed the effect of IL-1 β on cell viability, migration, apoptosis, and inflammatory response. Bcl-2 expression was negatively regulated with miR-126 in IL-1 β -administrated cells, and thus affected expressions of phosphorylated MAPK and JNK.

Conclusion

IL-1 β -induced inflammatory markers and miR-126 was upregulated. Inhibition of miR-126 decreased IL-1 β -induced inflammation and cell apoptosis, and upregulated Bcl-2 expression via inactivating the MAPK/JNK signalling pathway.

Cite this article: *Bone Joint Res* 2018;7:414–421.

Keywords: Chondrocyte, microRNA-126, Inflammation, Bcl-2, MAPK/JNK signal pathway

Article focus

- This study investigated the role of miR-126 in the development of osteoarthritis (OA) and the potential molecular mechanisms were involved.

Key messages

- Interleukin (IL)-1 β induces inflammation in chondrocytes.
- miR-126 is upregulated in IL-1 β -induced inflammatory chondrocytes *in vitro*.
- Inhibition of miR-126 reduces inflammation induced by IL-1 β and upregulates

B-cell lymphoma 2 (Bcl-2) via inactivation of the mitogen-activated protein kinase (MAPK) / Jun N-terminal kinase (JNK) signaling pathway.

Strengths and limitations

- These findings might provide a theoretical basis for osteoarthritis treatment.
- This study would have been strengthened by the use of a control cell line.
- Further animal and clinical research is necessary to establish a better understanding of the pathogenesis of OA.

Introduction

Osteoarthritis (OA) is a chronic joint disease characterized by degeneration of articular cartilage, degenerative joint inflammation, and secondary bone hyperplasia. It is common in middle-aged and elderly people, and affects more women than men.¹ The major pathological changes of OA patients are degeneration of articular cartilage, formation of osteophytes, and subsequent narrowing of the joint space, thus causing pain, rigidity, deformity, and dysfunction of the joint.² Due to the growing elderly population and rise in obesity, the incidence of OA is also likely to increase. It is therefore urgent that new, effective prevention and therapeutic methods for OA are developed.³

MicroRNA (miRNA), non-coding small molecule RNA, plays very important roles in regulation of gene expression, especially in post-transcriptional regulation during development.⁴ Regulation and expression of miRNA has become an emerging field in determining the mechanisms of inflammation-mediated diseases.⁵ It has been reported that many miRNAs are abnormally expressed in OA and act as important regulators in OA development, inflammatory response, osteogenic differentiation, and proliferation.^{6,7}

miRNA-126 (miR-126) has been reported to be associated with many physiological processes, including vascular integrity and angiogenesis,^{8,9} inflammation,¹⁰ and the development of many diseases, such as cancer.^{11,12} miR-126 has been described in several malignancies including lung, prostate, breast, renal cell, cervical, and thyroid cancer. Furthermore, it has been implicated in regulating processes of tumour development and progression.¹³ However, miR-126 expression in OA and its role in OA development remains unclear. Thus, the aim of this study was to investigate the roles of miR-126 in the development of OA, as well as the potential molecular mechanisms involved, in order to provide a theoretical basis for OA treatment and novel perspective for clinical therapy.

Materials and Methods

Cell culture and stimulation. The human chondrocyte cell line CHON-001, derived from normal human articular cartilage, was obtained from American Type Culture Collection (ATCC; Manassas, Virginia) and cultured in Dulbecco's Modified Eagle's Medium (Gibco, Grand Island, New York), supplemented with 0.1 mg/ml G-418 (Gibco) and 10% fetal bovine serum (FBS; Gibco), in a humidified atmosphere with 5% CO₂ at 37°C and passaged at a ratio of 1:5. The cell line was authenticated by Short Tandem Repeat (STR) profiling. The stable cultured CHON-001 cells were treated with recombinant human interleukin (IL)-1 β (R&D Systems, Minneapolis, Minnesota) for 12 hours to stimulate cell inflammation. The following concentrations of IL-1 β were used: 0.1 ng/ml, 2 ng/ml, 5 ng/ml, and 10 ng/ml.¹⁴⁻¹⁷

Cell transfection. The specific miR-126 inhibitor and scramble control, as well as specific small interfering RNA for B cell lymphoma 2 (si-Bcl-2) and siRNA negative control were synthesized by GenePharma Co. (Shanghai, China) and transfected into cells, respectively. The transfection was performed by using Lipofectamine 3000 reagent (Invitrogen, Waltham, Massachusetts), according to the manufacture's protocol. At 48 hours after transfection, cells were collected for further investigation.

Cell viability assay. Cell viability was assessed by a Cell Counting Kit-8 (CCK-8; Dojindo Molecular Technologies, Inc., Tokyo, Japan). Briefly, CHON-001 cells were seeded in a 96-well plate at a density of 5×10^3 . After administration, the CCK-8 solution was added into the culture medium, and cells were incubated for one hour at 37°C in humidified 95% air and 5% CO₂. The absorbance was measured at 450 nm using a Microplate Reader (Bio-Rad, Hercules, California).

Cell apoptosis assay. Cell apoptosis analysis was performed using an Annexin V fluorescein (Annexin V-FITC) and propidium iodide (PI) apoptosis detection kit (Beijing Biosea Biotechnology Co., Beijing, China) according to the manufacturer's recommendations. After treatment, cells were collected and washed in phosphate buffered saline (PBS). Cells were then suspended in binding buffer containing 10 μ l Annexin V-FITC and 5 μ l of PI in the presence of 50 μ g/ml RNase A (Sigma-Aldrich, St. Louis, Missouri). Cells were incubated for one hour at room temperature in the dark. Flow cytometry analysis was performed by using a FACScan (Beckman Coulter, Brea, California). Data were analyzed using FlowJo software (FlowJo, LLC, Ashland, Oregon).

Wound healing assay. Wound healing assay was performed by using a culture insert (ibidi GmbH, Martinsried, Germany). Briefly, CHON-001 cells were plated in 60 mm dishes with a culture insert and cultured until confluence. After pre-treatment with 50 μ m mytomicin C for three hours, a wound was created on a cell sheet by removing the culture insert. The culture medium was replaced with fresh medium with or without IL-1 β (5 ng/ml or 10 ng/ml). The pictures of the wound were taken by an inverted microscope (Leica, Wetzlar, Germany) at zero, six, 12, 24, and 48 hours. The wound width was measured and the relative width was calculated.

RNA extraction and quantitative polymerase chain reaction (qPCR). Total RNA was extracted by using Trizol (Invitrogen) and reverse transcription was performed by MultiScribe RT kit (Applied Biosystems, Foster City, California) according to the manufacturer's instructions. The qPCR was performed on Exicycler 96 Real-Time Quantitative Thermal Block (Bioneer, Daejeon, South Korea) with SYBR green Master Mix. U6 was used as internal control for miRNA expression analysis. The RT-PCR conditions were as follows: an initial ten minutes of incubation at 95°C, then 40 cycles consisting of 95°C for ten seconds, 60 °C for 20 seconds, and 72°C

for 30 seconds, followed by five minutes of incubation at 4°C. Relative quantification analysis was conducted using the $2^{-\Delta\Delta CT}$ method.¹⁸ All primers were synthesized from GenePharma (Shanghai, China). The data were analyzed with Real-Time StatMiner (Integromics, Granada, Spain). Each sample was analyzed in triplicate, and all experiments were carried out three times independently. The primer sequences were provided as follows: hsa-miR-126 forward, 5'-GGGTCGTACCGTGAGTAAT-3', hsa-miR-126 reverse, 5'-CAGTGGCTGTCGTGGAGT-3'; U6 forward 5'-CTCGCTTCGGCAGCAC-3', U6 reverse 5'-AACGCTTCACGAATTTGCGT-3'.

Western blot assay. The protein expression of IL-1 β and/or miR-126-inhibited cells was detected by Western blot analysis. Briefly, samples were extracted by using lysis buffer (Beyotime Biotechnology, Shanghai, China) supplemented with protease inhibitors (Roche, Guangzhou, China). Proteins were quantified by using the BCA Protein Assay Kit (Pierce, Appleton, Wisconsin). Western blot system was established by using a Bio-Red Bis-Tris Gel system according to the manufacturer's instructions. Primary antibodies (at dilution of 1:1000) were incubated with polyvinylidene fluoride (PVDF) membranes overnight at 4°C, followed by being washed and incubated with horseradish peroxidase (HRP)-marked secondary antibodies (at dilution of 1:5000; Santa Cruz Biotechnology, Dallas, Texas) for another one hour at room temperature. Then, binding proteins in the membranes were transfected into the Bio-Rad ChemiDoc XRS system (Bio-Rad), and covered by adding 200 μ l Immobilon Western Chemiluminescent HRP Substrate (Millipore, Burlington, Massachusetts). The signals were captured and quantified by Image La Software (Bio-Rad). The primary antibodies used in this experiment were as follows: p53 (sc-377567); pro-caspase 3 (sc-271759); cleaved caspase 3 (sc-22171); Cytochrome C (Cyto C; sc-13560); IL-6 (sc-130326); IL-8 (sc-32817); TNF- α (sc-130349); Bcl-2 (sc-7382); p38 (sc-33688); phosphorylated (p-) p38 (sc-166182); JNK (sc-7345); p-JNK(sc-293136) (Santa Cruz Biotechnology); and β -actin antibody (AA128, Beyotime Biotechnology).

Statistical analysis. Each experiment was repeated three times. Data are presented as the mean and standard deviation. Statistical analyses were performed using SPSS 19.0 statistical software (SPSS Inc., Chicago, Illinois). The statistical difference between groups was calculated by using Student's *t*-test. A *p*-value <0.05 was considered to be statistically significant.

Results

IL-1 β induced chondrocyte inflammation *in vitro*. Human chondrocyte cells CHON-001 were treated with different concentrations of IL-1 β (0.1 ng/ml, 2 ng/ml, 5 ng/ml, 10 ng/ml, respectively). Cell viability assay results suggested that after treatment with IL-1 β (5 ng/ml and

10 ng/ml), cell viability was decreased ($p < 0.01$, Fig. 1a). The 5 ng/ml and 10 ng/ml of IL-1 β were taken as effective concentrations for further detection of cell inflammatory response. The results of wound healing analysis, which was used to assess cell migration after IL-1 β treatment (5 ng/ml and 10 ng/ml), showed that relative wound widths of IL-1 β -administrated groups were higher than those of the control group without IL-1 β treatment, suggesting that IL-1 β inhibited cell migration of CHON-001 cells (Fig. 1b).

The apoptosis assay results showed that IL-1 β treatment significantly increased relative numbers of apoptotic cells ($p < 0.01$, Fig. 1c). Western blot analysis results also showed that Cyto C, Cleaved-Caspase 3, and p53 expression were increased in IL-1 β -administrated groups compared with the control group (Fig. 1d), suggesting that cell apoptosis was promoted by IL-1 β .

In order to confirm the inflammatory response of CHON-001 cells after IL-1 β administration, we examined inflammatory cytokine expression, including IL-6, IL-8, and TNF- α . Results showed that expressions of these three factors were all increased after being exposed to IL-1 β ($p < 0.01$, Figs 1e and 1f). All above results suggested that IL-1 β administration in CHON-001 cells could induce inflammatory reaction of CHON-0001 cells.

miR-126 was upregulated in chondrocyte cells CHON-001 administrated by IL-1 β . The qPCR analysis results showed that the expression of miR-126 was increased in CHON-001 cells after treatment with an increasing dosage of IL-1 β ($p < 0.01$ at the dosage of 5 ng/ml, $p < 0.01$ at the dosage of 10 ng/ml, Fig. 2a), suggesting that miR-126 was upregulated in IL-1 β -induced chondrocyte cells. miR-126 expression was then reduced by transfection of an miR-126 inhibitor in CHON-001 cells to assess the effect of miR-126 on IL-1 β -treated chondrocyte inflammation. The transfected efficiency was measured by qPCR, and results suggested that expression of miR-126 was significantly decreased after miR-126 inhibitor transfection compared with the negative control group, even in the presence of IL-1 β (10 ng/ml) ($p < 0.001$, Fig. 2b).

Inhibition of miR-126 reduced IL-1 β -induced-chondrocyte inflammation. We investigated the effect of miR-126 inhibition on IL-1 β -induced (10 ng/ml) cell apoptosis, the impairment of wound healing, and the release of inflammatory cytokines (IL-6, IL-8, and TNF- α) in CHON-001 cells. Results of cell viability in Figure 3a showed that, compared with IL-1 β administration alone, IL-1 β and miR-126 inhibitor transfection promoted cell viability of CHON-001 ($p < 0.05$). The wound healing assay results also showed that wound width was decreased by miR-126 inhibitor transfection compared with IL-1 β administration alone, suggesting that the miR-126 inhibitor transfection abrogated the effect of IL-1 β on cell migration (Fig. 3b). As shown in Figure 3c, the relative apoptotic cells were decreased by miR-126 inhibition transfection after IL-1 β

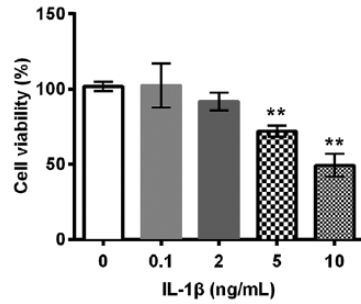


Fig. 1a

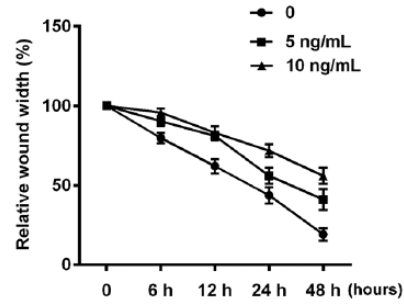


Fig. 1b

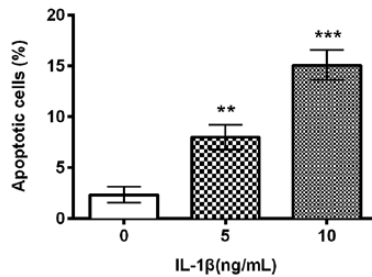


Fig. 1c

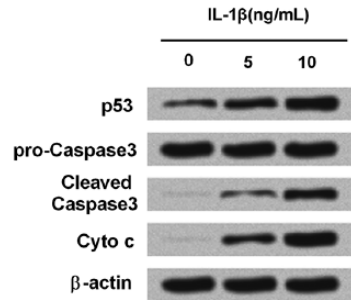


Fig. 1d

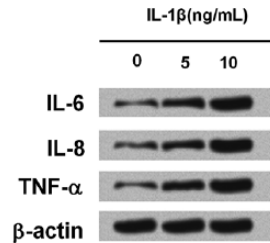


Fig. 1e

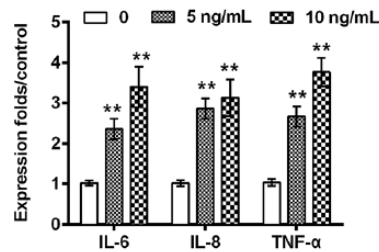


Fig. 1f

Interleukin (IL)-1 β induced chondrocyte inflammation *in vitro*. The human chondrocyte cell line CHON-001 was administrated by different concentrations of IL-1 β (0.1 ng/ml, 2 ng/ml, 5 ng/ml, and 10 ng/ml) to simulate inflammation. a) Graph showing the Cell Counting Kit-8 (CCK-8) analysis results of cell viability after IL-1 β administration. b) Graph showing the relative wound width at different time points after IL-1 β administration. c) Graph showing the relative apoptotic cells after IL-1 β administration. The protein immunoblots of: d) apoptosis-related factors, including Cytochrome C (Cyto C), Caspase 3, and p53, in CHON-001 cells; and e) pro-inflammatory cytokines (IL-6, IL-8, and tumour necrosis factor (TNF)- α) by Western blot assay. f) Graph showing the relative expression levels of IL-6, IL-8, and TNF- α . β -actin acted as an internal control (** p < 0.01; *** p < 0.001).

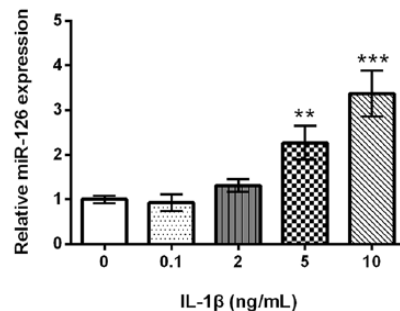


Fig. 2a

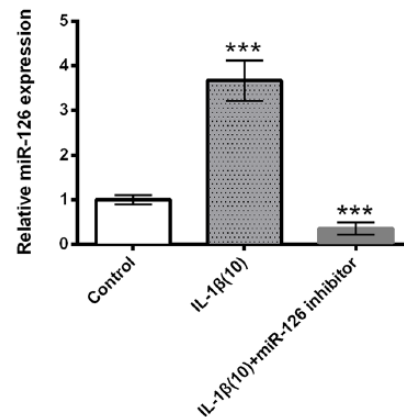


Fig. 2b

Graphs showing that miR-126 was upregulated in interleukin (IL)-1 β -administrated CHON-001 cells: a) relative miR-126 expression measured by quantitative polymerase chain reaction (qPCR) in IL-1 β administrated CHON-001 cells; b) relative miR-126 expression measured by qPCR after IL-1 β administration and/or miR-126 inhibitor transfection (** p < 0.01; *** p < 0.001).

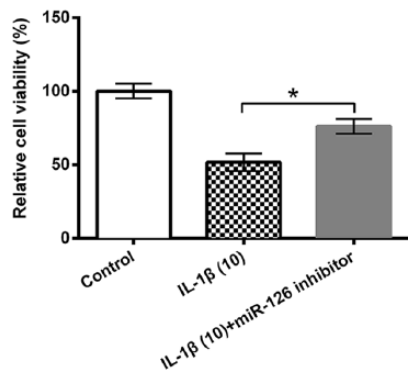


Fig. 3a

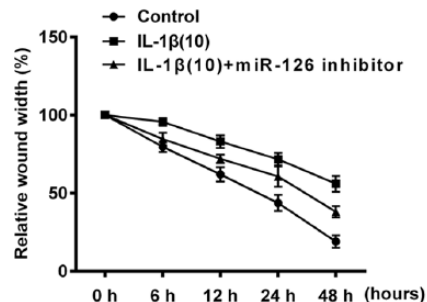


Fig. 3b

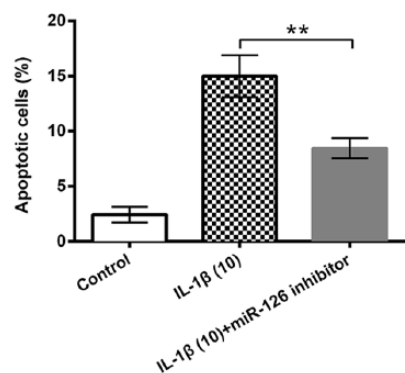


Fig. 3c

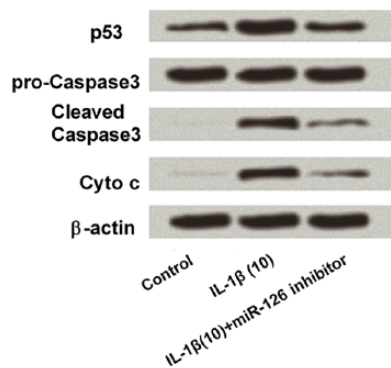


Fig. 3d

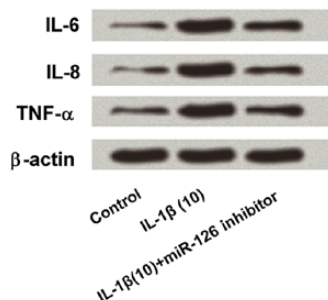


Fig. 3e

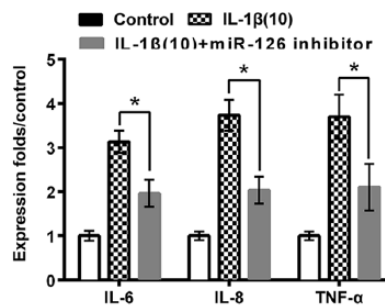


Fig. 3f

Inhibition of miR-126 reduced interleukin (IL)-1 β -induced chondrocyte inflammation. The human chondrocyte cell line CHON-001 was administrated by IL-1 β (10 ng/ml) and/or miR-126 inhibitor transfection: a) Graph showing the Cell Counting Kit-8 (CCK-8) analysis results of cell viability. b) Graph showing the relative wound width at different timepoints. c) Graph showing the relative apoptotic cells number of CHON-001 cells. The protein immunoblots of: d) apoptosis-related factors, including Cytochrome C (Cyto C), Caspase 3, and p53, in CHON-001 cells; and e) pro-inflammatory cytokines (IL-6, IL-8, and tumour necrosis factor (TNF)- α) by Western blot assay. f) Graph showing the relative expression levels of IL-6, IL-8, and TNF- α . β -actin acted as an internal control (* $p < 0.05$; ** $p < 0.01$).

administration ($p < 0.01$). Meanwhile, the expressions of Cyto C, Cleaved-Caspase 3, and p53 were all decreased by miR-126 inhibition transfection, as shown in Figure 3d. These results suggested that inhibition of miR-126 reduced IL-1 β -induced cell apoptosis of CHON-001 cells. We also measured the effect of miR-126 inhibitor on inflammatory cytokines, and results showed that miR-126 inhibitor transfection suppressed IL-1 β -induced expression of IL-6, IL-8, and TNF- α (Figs 3e and 3f).

To further confirm the effect of miR-126 inhibitor on IL-1 β -induced inflammatory response in CHON-001 cells,

we also employed different concentrations of IL-1 β (0.1 ng/ml, 2 ng/ml, 5 ng/ml) to stimulate CHON-001 cells and assess the expression levels of inflammatory factors with or without the presence of miR-126 inhibitor. As shown in Figure 4a and 4b, we found that the expression of pro-inflammatory cytokines was upregulated by the treatment of IL-1 β at the dosage of 0.1 ng/ml ($p < 0.05$). However, the upregulated pro-inflammatory factors were inhibited by the transfection of the miR-126 inhibitor ($p < 0.05$). Similar results were observed in the treatment of IL-1 β , with the dosage of 2 ng/ml and 5 ng/ml

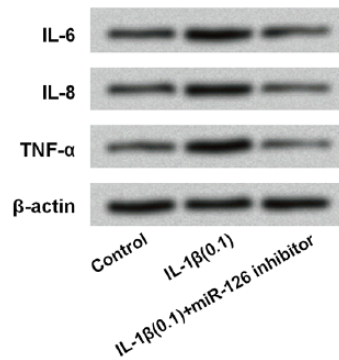


Fig. 4a

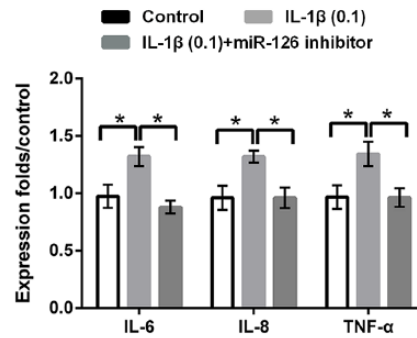


Fig. 4b

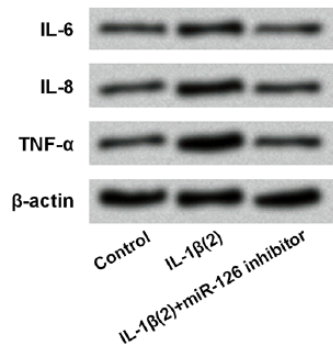


Fig. 4c

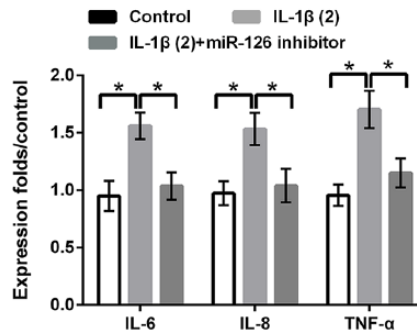


Fig. 4d

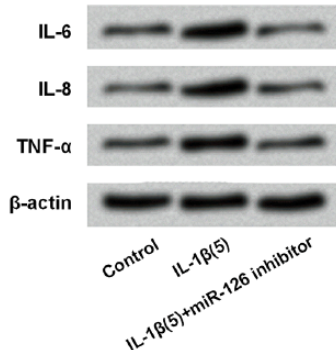


Fig. 4e

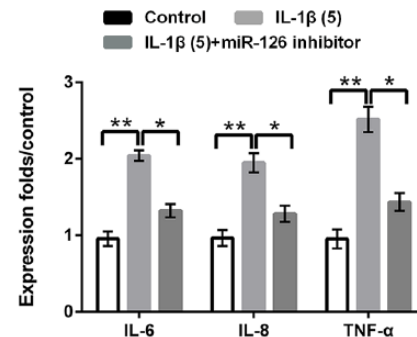


Fig. 4f

Inhibition of miR-126 reduced the expression levels of pro-inflammatory factors in interleukin (IL)-1 β -injured CHON-001 cells. The human chondrocyte cell line CHON-001 was administrated by IL-1 β (0.1 ng/ml, 2 ng/ml, 5 ng/ml, or 10 ng/ml) and/or miR-126 inhibitor transfection. a), c), and e) The protein immunoblots of pro-inflammatory cytokines (IL-6, IL-8, and TNF- α) by Western blot assay. b), d), and f) Graphs showing the relative expression levels of IL-6, IL-8, and TNF- α . β -actin acted as an internal control (*p < 0.05; **p < 0.01).

(Figs 4c to 4f; p < 0.05 or p < 0.01), which showed that miR-126 inhibitor decreased the promotive effect of IL-1 β (2 ng/ml and 5 ng/ml) on the expression of pro-inflammatory cytokines. These results suggest that even though IL-1 β did not significantly alter cell viability at lower concentrations, such as 0.1 ng/ml and 2 ng/ml, it is still induced inflammatory responses in CHON-001 cells. miR-126 inhibitor significantly reversed the effect of IL-1 β -induced cell inflammation on CHON-001 cells.

miR-126 negatively regulated Bcl-2 expression and regulated activity of MAPK/JNK signalling pathway. Western

blot was performed to assess the Bcl-2 expression in CHON-001 cells after IL-1 β administration. Results shown in Supplementary figure aa suggest that IL-1 β induction decreased Bcl-2 expression (p < 0.05 or p < 0.01). miR-126 inhibitor transfection in CHON-001 cells reversed the effect of IL-1 β on Bcl-2 expression. As shown in Supplementary figure ab, IL-1 β + miR-126 inhibitor transfection significantly increased Bcl-2 expression levels compared with IL-1 β alone (p < 0.05). Therefore, miR-126 may negatively regulate Bcl-2 expression in chondrocytes stimulated by IL-1 β .

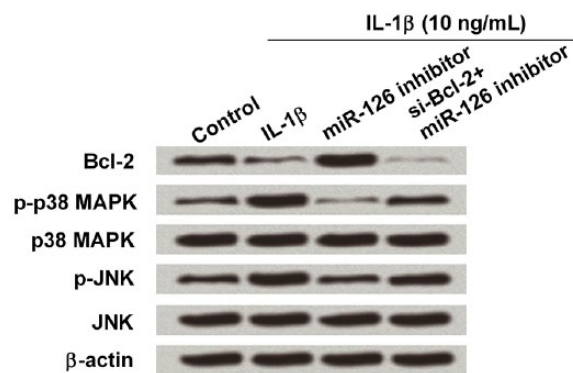


Fig. 5

miR-126 regulated Bcl-2 expression via inactivating the MAPK/JNK signaling pathway. The human chondrocyte cell line CHON-001 was exposed to IL-1 β (10 ng/ml), and then transfected with miR-126 inhibitor and/or Bcl-2 siRNA (si-Bcl-2). Expression of Bcl-2, phosphorylated (p)-p38 MAPK, p38 MAPK, p-JNK, and JNK were measured by Western blot. β -actin acted as an internal control.

Expressions of p38 mitogen-activated protein kinase (MAPK) and Jun N-terminal kinase (JNK) were measured to assess activity of MAPK/JNK signaling pathway in CHON-001 cells after administration with IL-1 β and/or miR-126 inhibitor. Results in Figure 5 showed that IL-1 β increased expression of phosphorylated (p)-MAPK and p-JNK, and that miR-126 inhibitor transfection decreased this expression, suggesting that IL-1 β induction might increase activity of MAPK/JNK signaling pathway while miR-126 inactivates the pathway. In IL-1 β -induced CHON-001 cells, miR-126 inhibitor + si-Bcl-2 transfection increased the expression of p-p38 MAPK and p-JNK, suggesting that miR-126 regulated activity of the MAPK/JNK signaling pathway occurs via negative regulation of Bcl-2 expression.

Discussion

Inflammation is an important pathogenic factor of OA. It has been proven that pro-inflammatory cytokine IL-1 β levels and osteoarthritis severity are positively correlated.¹⁹ In this study, IL-1 β was used to induce chondrocytes CHON-001 to simulate OA model *in vitro*. Results showed that IL-1 β (5 ng/ml and 10 ng/ml as effective concentration) could cause cell inflammation, including suppressing cell viability and migration, enhancing apoptosis and expression of apoptosis-related factors, as well as expressions of inflammatory factors.

Recent research has demonstrated the importance of miRNAs in cartilage function,²⁰ and many miRNAs were found to be differentially expressed in normal and OA chondrocytes.²¹ Abnormally expressed miR-126 has been found in many diseases and has also been proven to be relevant in many tumours.²² In this study, we found that miR-126 was upregulated in IL-1 β -administrated chondrocytes. This abnormal expression indicated that miR-126 might be involved in the regulation of inflammatory response in chondrocytes. miR-126 has been reported to

impair cancer progression through signaling pathways that control cell proliferation, migration, invasion, and survival.²³ This study also investigated the effect of miR-126 on chondrocytes after IL-1 β -induced inflammation. Our results suggest that miR-126 inhibitor suppressed IL-1 β -induced chondrocyte apoptosis and inflammatory response, suggesting that downregulation of miR-126 might be used as a treatment strategy for OA treatment.

In our study, we found that the Bcl-2 expression was decreased after IL-1 β administration, while inhibition of miR-126 reversed it and Bcl-2 expression was increased, suggesting that expression of Bcl-2 was negatively related with miR-126 in IL-1 β administrated chondrocytes. Bcl-2 plays an important role in the pathogenesis of disease by regulating apoptosis. Researchers suggested that Bcl-2 was a downstream target of miRNAs, which downregulated Bcl-2 expression via regulating Bcl-2 translation,²⁴ and that Bcl-2 suppression was related to cell apoptosis.²⁵⁻²⁷ In some cancer studies, it has also been found that ectopic expression of miR-126 was related to Bcl-2 expression.^{28,29} Our findings suggested that in inflammatory response of chondrocytes, miR-126 might also negatively regulate Bcl-2 expression and thus affect cell apoptosis.

The p38 MAPK and JNK signaling pathway, as important MAPK pathways, are involved in many important physiological processes, such as inflammatory responses, and contribute to the mediation of cell growth, development, differentiation, survival, and apoptosis.³⁰⁻³² It has been found that activation of the p38 MAPK/JNK signaling pathway is involved in the initiation of malignant tumour cell apoptosis caused by different stimuli.^{33,34} In this study, we found that p38 MAPK/JNK was activated after IL-1 β administration, while inhibition of miR-126 suppressed activity of this pathway, suggesting that upregulation of miR-126 and downregulation of Bcl-2 in IL-1 β -administrated chondrocytes was related to activity of the p38 MAPK/JNK pathway. Meanwhile, Bcl-2 knock-down released the activity inhibition effect caused by miR-126 inhibitor. Given the above results, we speculate that p38 MAPK/JNK was associated with downregulation of Bcl-2 mediated by miR-126 in human chondrocytes. A limitation of this study was that it lacked a control cell line, the use of which would strengthen these findings.

In conclusion, in this study, we simulated human inflammatory chondrocytes by using IL-1 β *in vitro*, and found that upregulated miR-126 might be involved in the regulation of inflammatory response. Suppression of miR-126 could increase Bcl-2 expression via inhibitory effect on MAPK/JNK signaling pathway activation.

Supplementary material



Supplementary graphs showing that expression of Bcl-2 and miR-126 was negatively relative in IL-1 β -administrated chondrocyte *in vitro*.

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Funding Statement

None declared

Author Contributions

- C. D. Yu: Study design, Data collection and analysis, Manuscript preparation.
- W. H. Miao: Data collection and analysis, Manuscript preparation.
- Y. Y. Zhang: Data collection and analysis, Manuscript preparation.
- M. J. Zou: Data collection, Manuscript preparation.
- X. F. Yan: Study design, Manuscript preparation, Final approval of paper.

Conflict of Interest Statement

None declared

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