

Down-regulation of microRNA-23b aggravates LPS-induced inflammatory injury in chondrogenic ATDC5 cells by targeting PDCD4

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ABSTRACT

Objective(s): Osteoarthritis (OA), characterized by degradation of articular cartilage, is a leading cause of disability. As the only cell type present in cartilage, chondrocytes play crucial roles in the progression of OA. In our study, we aimed to explore the roles of miR-23b in the lipopolysaccharide (LPS)-induced inflammatory injury.

Materials and Methods: LPS-induced cell injury of ATDC5 cells was evaluated by the loss of cell viability, enhancement of cell apoptosis, alteration of apoptosis-associated proteins, and release of inflammatory cytokines. Then, miR-23b level after LPS treatment was assessed by qRT-PCR. Next, the effects of aberrantly expressed miR-23b on the LPS-induced inflammatory injury were explored. The possible target genes of miR-23b were virtually screened by informatics and verified by luciferase assay. Subsequently, whether miR-23b functioned through regulating the target gene was validated. The involved signaling pathways were investigated finally.

Results: Cell viability was decreased but cell apoptosis, as well as release of inflammatory cytokines, was enhanced by LPS treatment. MiR-23b was down-regulated by LPS and its overexpression alleviated LPS-induced inflammatory injury. PDCD4, negatively regulated by miR-23b expression, was verified as a target gene of miR-23b. Following experiments showed miR-23b alleviated LPS-induced cell injury through down-regulating PDCD4 expression. Phosphorylated levels of key kinases in the NF- κ B pathway, as well as expressions of key kinases in the Notch pathways, were increased by PDCD4 overexpression.

Conclusion: MiR-23b was down-regulated after LPS treatment, and its overexpression ameliorated LPS-induced inflammatory injury in ATDC5 cells by targeting PDCD4, which could activate the NF- κ B/Notch pathways.

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Introduction

Osteoarthritis (OA), a common chronic arthritis, is a degenerative disease of cartilaginous tissues that affects approximately 10% of men and 20% of women over the age of 60, globally (1, 2). Large numbers of factors may affect OA, such as obesity, joint malalignment, trauma, muscle weakness, age, nutritional status, etc. (3). OA is a leading cause of pain and disability worldwide and is considered as an untreatable disease (4). Although total joint replacement has been widely used for treatment of OA, the surgery may lead to loss of joint function (5). In order to prevent progression of OA and relieve pain, understanding of the pathological mechanisms leading to joint degeneration and pain symptoms are urgently needed (6).

Currently, OA is not only considered as a process of wear and tear, but also identified as abnormal remodeling of joint tissues, which is caused by various inflammatory mediators (7, 8). Pro-inflammatory cytokines including interleukin (IL)-1 β and tumor necrosis factor- α (TNF- α) can promote cartilage catabolism. A meta-analysis has shown that TNF- α gene polymorphisms are related to increased risk of OA (9). In the pathophysiology of OA, IL-1 β is released and mediates the aberrant degenerative process (10). Moreover, these two factors stimulate their own production and also increase the productions of IL-6 and IL-8 in synovial cells and chondrocytes (11).

Thus, expressions of these four factors are meaningful for progression of OA.

MicroRNAs (miRNAs), with a length of 20–22 nucleotides, are small, non-coding RNAs that participate in a variety of biological processes (12). Accumulating evidence has revealed that a myriad of miRNAs is abnormally expressed during OA, suggesting the involvement of miRNAs in OA development (13, 14). MiR-23b (miR-23b) has been widely explored. For example, a previous study has demonstrated that miR-23b could inhibit epithelial-mesenchymal transition and metastasis in hepatocellular carcinoma (15). Down-regulation of miR-23b has been reported to be associated with tumor progression and poor prognosis in colon cancer (16). In addition, miR-23b is also reported to act as tumor suppressor in oral squamous cell carcinoma (17). However, effects of miR-23b on OA have not yet been well studied.

Herein, cell model of inflammatory injury in ATDC5 cells, a murine chondrogenic cell line, was constructed after stimulation of lipopolysaccharide (LPS). Cell viability, apoptosis, and release of inflammatory cytokines were assessed to evaluate cell injury. The functional roles of miR-23b, as well as the possible target gene in LPS-induced cell injury, were explored. Moreover, the involved signaling pathways were also investigated.

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Materials and Methods

Cell culture and treatment

ATDC5 cells (American Type Culture Collection, Manassas, VA, USA) were cultured in Dulbecco's Modified Eagle Medium/Ham's F-12 nutrient mixture (1:1, DMEM/F12; Invitrogen, Portland, OR, USA) supplemented with 2 mM Glutamine (Sigma-Aldrich, St. Louis, MO, USA) and 10% fetal bovine serum (FBS; Invitrogen) at 37 °C in a humidified incubator containing 5% CO₂. When the cells reached 75% confluence, cells were treated with 0.25% trypsin (Ameresco, Framingham, MA, USA) for cell passage. For LPS stimulation, cells were cultured in DMEM/F12 containing LPS (Sigma-Aldrich) in a series of concentrations (0, 1, 5, 10 µg/ml) for 5 hr.

Cell Counting Kit-8 (CCK-8) assay

Cell viability of ATDC5 cells was assessed using a CCK-8 assay kit (Dojindo Molecular Technologies, Gaithersburg, MD, USA). Briefly, ATDC5 cells with 5 × 10³ cells/well were seeded in 96-well plates and cultured until the confluence reached approximately 80%. After treatment for adequate times, CCK-8 solution (1 µl/10 µl DMEM/F12) was added to each well, followed by incubation for 1 hr at 37 °C. Afterwards, cell viability was reflected by the absorbance at 450 nm, which was measured by a Microplate Reader (Bio-Rad, Hercules, CA, USA).

FACScan flow analysis of apoptotic cells

Cell apoptosis of treated ATDC5 cells was evaluated by an Annexin V-FITC apoptosis detection kit (Sigma-Aldrich). In brief, after treatments, ATDC5 cells (1 × 10⁵ cells) were collected and washed with pre-cold PBS. Then, cells were suspended in binding buffer, followed by addition of Annexin V-FITC and propidium iodide according to the recommendation of the manufacturer. The apoptotic cells were identified by a FACScan flow cytometer (BD Biosciences, San Jose, CA, USA). Apoptotic cells were analyzed by the FlowJo software (Tree Star, San Carlos, CA, USA). The cell apoptosis was presented as the percentage of apoptotic cells to total cells.

Enzyme-linked immunosorbent assay (ELISA)

ATDC5 cells were seeded into 24-well plates and cultured until the confluence reached 80%. After stimulation with LPS or transfection followed by stimulation, the culture supernatant was collected for estimation of inflammatory cytokines. Accordingly, ELISA kits (R&D Systems, Abingdon, UK) were utilized according to the information of the supplier.

Cell transfection

Scramble miRNA, miR-23b mimic, miR-23b inhibitor, negative control of miR-23b inhibitor, programmed cell death 4 (PDCD4)-specific small interfering RNA (si-PDCD4), and its negative control (siNC) were all purchased from GenePharma Co. (Shanghai, China). The full-length PDCD4 was ligated into a pEX-2 plasmid (GenePharma Co.), and the recombined plasmid was referred to as pEX-PDCD4. Lipofectamine 3000 reagent (Invitrogen) was utilized for cell transfection in line with the protocol of the manufacturer. The empty pEX-2 plasmid was also transfected into ATDC5 cells, termed

as pEX group. Stable transfection was selected by G418 (0.5 mg/ml; Sigma-Aldrich). Cells transiently transfected with miRNAs were harvested at 72 hr post-transfection.

Quantitative reverse transcription PCR (qRT-PCR)

According to the protocol of the manufacturer's instructions, extraction of total RNA from treated ATDC5 cells was performed using Trizol reagent (Invitrogen). Then, the TaqMan MicroRNA Reverse Transcription Kit (Applied Biosystems, Foster City, CA, USA) and Multiscribe RT kit (Applied Biosystems) were used for reverse transcription of miRNAs and cDNAs, respectively, in accordance with the manufacturer's information. Then, TaqMan Universal Master Mix II (Applied Biosystems) was employed for estimation of miR-23b and mRNA levels following the manufacturer's protocol. The relative expression was calculated on the basis of the 2^{-ΔΔCt} method (18), normalized to U6 (for miR-23b level) and GAPDH (for mRNA levels of PDCD4 and inflammatory cytokines).

Dual-luciferase activity assay

The putative miR-23b-binding site in the 3'UTR of wild-type PDCD4 was amplified and sub-cloned into a pMiR-report vector (Promega, Madison, WI, USA) to generate PDCD4-WT. The mutant plasmid with mutations at the miR-23b-binding site (PDCD4-MUT) was obtained after mutation using the QuikChange site-directed mutagenesis kit (Stratagene, La Jolla, CA, USA). Then, scramble miRNA or miR-23b mimic, PDCD4-WT or PDCD4-MUT and pRL-TK vector (Promega) were co-transfected into ATDC5 cells using Lipofectamine 3000 reagent. Subsequently, luciferase activity was determined by dual-luciferase assay system (Promega) according to the supplier's suggestions.

Western blot analysis

The protein of treated ATDC5 cells was lysed using lysis buffer (Promega) supplemented with protease inhibitors (Roche, Mannheim, Germany). Each protein sample was quantified using the BCA™ Protein Assay Kit (Pierce, Appleton, WI, USA). Then, proteins were separated by SDS-PAGE and were blotted onto polyvinylidene difluoride membranes, followed by blocking with 5% non-fat milk. The blocked membranes were respectively incubated with anti-Bcl-2-associated X protein (Bax, ab182733), anti-B cell lymphoma-2 (Bcl-2, ab196495), anti-pro caspase-3 (ab90437), anti-cleaved caspase-3 (ab49822), anti-p65 (ab16502), anti-phosphorylated p65 (p-p65, ab86299), anti-inhibitor of nuclear factor κB α (IκBα, ab32518), anti-phosphorylated IκBα (p-IκBα, ab133462), anti-Notch1 (ab52627), anti-Notch2 (ab137665), anti-Notch3 (ab23426), anti-GAPDH (ab181603) (all Abcam, Cambridge, UK), anti-pro caspase-9 (9508), anti-cleaved caspase-9 (9509) (both Cell Signaling Technology, Beverly, MA, USA), or anti-PDCD4 (sc-376430; Santa Cruz, Santa Cruz, CA, USA) at 4 °C overnight. Then, after rinsing thrice, membranes were incubated with secondary antibodies marked by horseradish peroxidase for 1 hr at room temperature. After rinsing again, the bands on the membranes were visualized by ECL detection systems (Sigma-Aldrich).

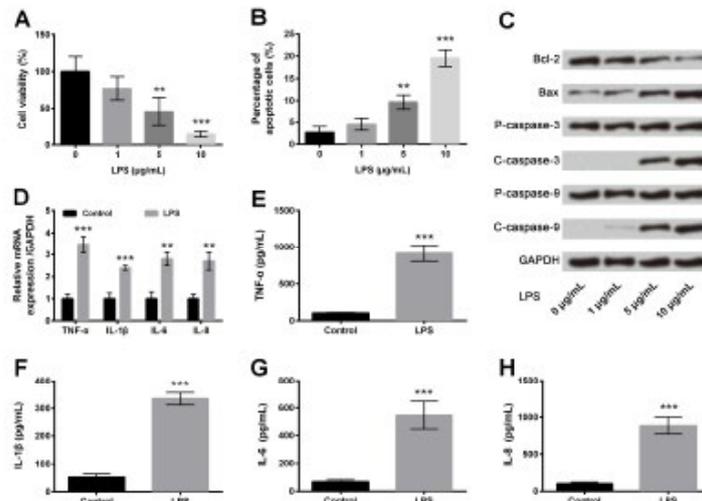


Figure 1. Lipopolysaccharide (LPS) induced inflammatory injury in ATDC5 cells. A. Cell viability was tested by Cell Counting Kit-8 assay. B. Cell apoptosis was measured by flowcytometry. C. Expression of apoptosis-associated proteins was determined by Western blot analysis. D. Relative mRNA expression levels of inflammatory cytokines were estimated by qRT-PCR. E-H. Protein expression levels of inflammatory cytokines were determined by enzyme-linked immunosorbent assay. The dose of LPS for D-H was 5 µg/ml and the duration of LPS treatment was 5 hr. Data are presented as the mean±SD. **, $P<0.01$; ***, $P<0.001$. P-, pro; C-, cleaved

Statistical analysis

Data were presented as the means±standard deviation (SD). Graphpad Prism 5 software (GraphPad, San Diego, CA, USA) was employed for statistical analysis. The student *t*-test or multiple *t*-tests was used for calculation of *P*-values. Statistically, significance was considered when $P<0.05$.

Results

LPS induced cell injury and up-regulation of inflammatory cytokines in ATDC5 cells

To construct the cell model of inflammation, ATDC5 cells were incubated with the presence of LPS (0, 1, 5, and 10 µg/ml). Results showed cell viability was significantly reduced while the percentage of apoptotic cells was markedly elevated with the increase of LPS (Figures 1A-1B). Compared with cells treated with 0 µg/ml LPS, the differences of cell viability and apoptosis were significant when the dose of LPS was 5 ($P<0.01$) or 10 µg/ml ($P<0.001$). Similarly, Bcl-2 was down-regulated and expressions of Bax, cleaved caspase-3 and cleaved caspase-9 were up-regulated, along with the increase of LPS (Figure 1C). Then, the release of inflammatory cytokines was evaluated. When compared to cells treated with 0 µg/mL LPS, mRNA and protein expressions of TNF-α, IL-1β, IL-6, and IL-8 were all remarkably up-regulated ($P<0.01$ or $P<0.001$) after stimulation of 5 µg/ml LPS (Figures 1D-1H). Results implied cell model of inflammation was successfully constructed after stimulation of LPS. The dose of LPS for subsequent experiments was 5 µg/ml.

MiR-23b was down-regulated by LPS in ATDC5 cells

After stimulation of LPS, the expression of miR-23b was estimated by qRT-PCR. As evidenced in Figure 2, the miR-23b level was significantly down-regulated compared with the control group ($P<0.05$).

MiR-23b level was aberrantly regulated in ATDC5 cells after cell transfection

MiR-23b mimic, miR-23b inhibitor or their negative

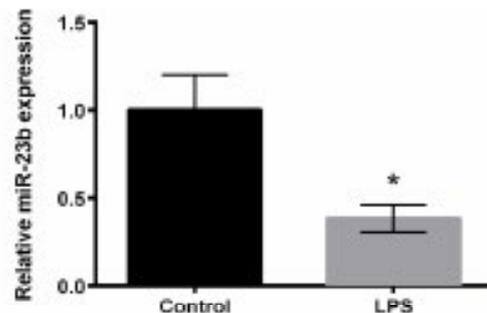


Figure 2. MicroRNA (miR)-23b was down-regulated by lipopolysaccharide (LPS). Level of miR-23b was measured by qRT-PCR. Data are presented as the mean±SD. *, $P<0.05$

controls were transfected into ATDC5 cells, and the results of qRT-PCR showed miR-23b level was markedly up-regulated by transfection with miR-23b mimic compared with the scramble group ($P<0.001$) but was down-regulated by transfection with miR-23b inhibitor compared with the NC group ($P<0.01$, Figure 3). Results stated that miR-23b was aberrantly expressed after transfection.

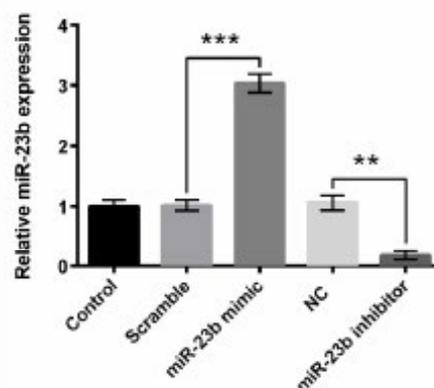


Figure 3. MicroRNA (miR)-23b was aberrantly expressed by cell transfection with different miRs. Level of miR-23b was measured by qRT-PCR. Data are presented as the mean±SD. **, $P<0.01$; ***, $P<0.001$. NC, negative control of miR-23b inhibitor

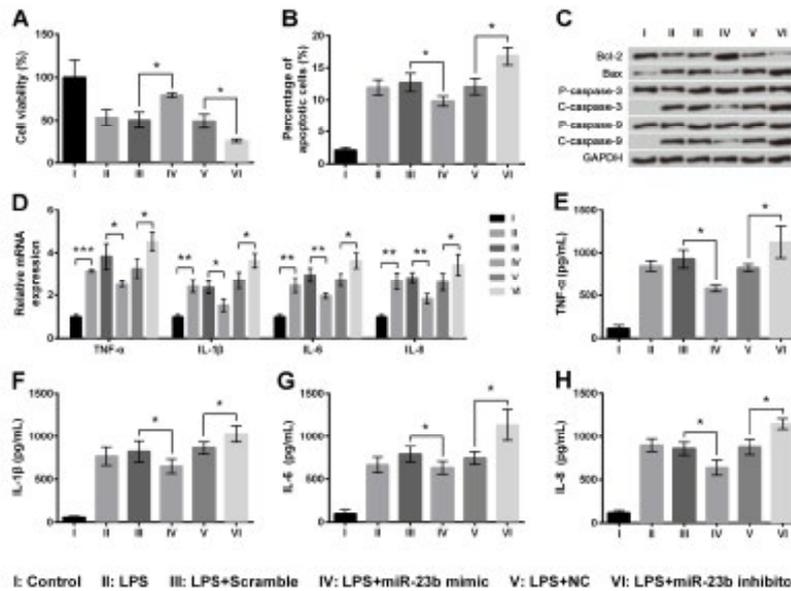


Figure 4. Lipopolysaccharide (LPS)-induced inflammatory injury was alleviated by microRNA (miR)-23b overexpression but was aggravated by miR-23b silence. A. Cell viability was measured by the Cell Counting Kit-8 assay. B. Cell apoptosis was tested by flowcytometry. C. Expression of apoptosis-associated proteins was determined by Western blot analysis. D. Relative mRNA expression levels of inflammatory cytokines were estimated by qRT-PCR. E-H. Protein expression levels of inflammatory cytokines were determined by enzyme-linked immunosorbent assay. The duration of LPS treatment (5 μ g/ml) was 5 hr. Data are presented as the mean \pm SD. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$. P-, pro; C-, cleaved

LPS-induced ATDC5 cell injury was exacerbated by miR-23b silence but was alleviated by miR-23b overexpression

To explore the effect of miR-23b on LPS-treated cells, ATDC5 cells were transfected with miR-23b mimic, miR-23b inhibitor or their negative controls and then treated with LPS. Results showed LPS-induced alterations of cell viability and apoptosis were both aggravated by miR-23b silence ($P < 0.05$) while they were alleviated by miR-23b overexpression ($P < 0.05$) compared with respective controls (Figures 4A-4B). Likewise, alterations of apoptosis-related proteins which were induced by LPS were further increased by miR-23b silence but were decreased by miR-23b overexpression (Figure 4C). The alteration of inflammatory cytokine expressions was consistent with cell apoptosis, showing that LPS-induced release of inflammatory cytokines was further increased by miR-23b silence ($P < 0.05$) while it was decreased by miR-23b overexpression ($P < 0.05$ or $P < 0.01$) compared with respective controls (Figures 4D-4H).

MiR-23b negatively regulated PDCD4 expression and PDCD4 was a target of miR-23b

The possible target genes of miR-23b were screened by TargetScan online software, and we focused on the interaction between miR-23b and PDCD4. Results in Figures 5A-5B showed PDCD4 was markedly down-regulated by miR-23b overexpression but was up-regulated by miR-23b silence (both $P < 0.01$) compared with respective controls at both mRNA and protein levels. The following assay showed, in cells co-transfected with PDCD4-WT, miR-23b overexpression significantly reduced luciferase activity compared with scramble miRNA-transfected cells ($P < 0.05$, Figure 5C). Together, there is no significant difference between groups co-transfected with PDCD4-MUT.

MiR-23b overexpression ameliorated LPS-induced injury through down-regulating PDCD4 expression

After cell transfection and LPS stimulation, cell viability, apoptosis, and release of inflammatory

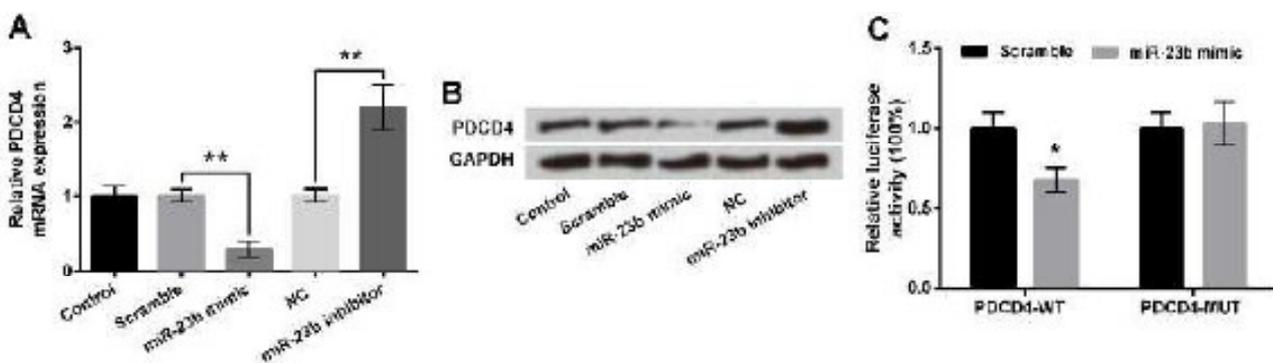


Figure 5. Programmed cell death 4 (PDCD4) was a target of microRNA (miR)-23b. Relative mRNA and protein expressions of PDCD4 were measured by qRT-PCR (A) and Western blot analysis (B), respectively. C. Luciferase activity was determined by luciferase assay. Data are presented as the mean \pm SD. *, $P < 0.05$; **, $P < 0.01$. NC, negative control of miR-23b inhibitor; PDCD4-WT, pmirG10 vector carrying the fragment from wild-type PDCD4 3'UTR containing the binding site; PDCD4-MUT, mutant PDCD4-WT

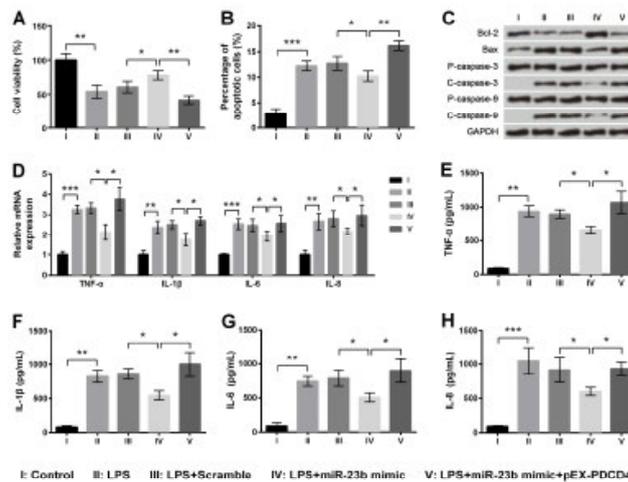


Figure 6. MicroRNA (miR)-23b overexpression functioned through down-regulating programmed cell death 4 (PDCD4). A. Cell viability was determined by Cell Counting Kit-8 assay. B. Cell apoptosis was measured by flowcytometry. C. Expression of apoptosis-associated proteins was tested by Western blot analysis. D. Relative mRNA expression levels of inflammatory cytokines were assessed by qRT-PCR. E-H. Protein expression levels of inflammatory cytokines were determined by enzyme-linked immunosorbent assay. The duration of LPS treatment (5 μ g/ml) was 5 hr. Data are presented as the mean \pm SD. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$. LPS, lipopolysaccharide; P-, pro; C-, cleaved; pEX-PDCD4, pEX-2 plasmid containing the full-length PDCD4 sequence

cytokines were all evaluated. In Figures 6A-6B, the LPS-induced alterations of cell viability and apoptosis were alleviated by miR-23b overexpression ($P < 0.05$), whereas the alleviations were reversed by PDCD4 overexpression ($P < 0.01$). Analogously, alterations of apoptosis-related proteins, induced by LPS, were all reversed by miR-23b overexpression, which was then reversed again by PDCD4 overexpression (Figure 6C). Subsequently, the effects of miR-23b overexpression on release of inflammatory cytokines in LPS-treated cells were all reversed by PDCD4 overexpression compared with the LPS+miR-23b mimic group ($P < 0.05$, Figures 6D-6H).

LPS-induced cell injury was aggravated by activation of the NF- κ B and Notch signaling pathways

The effects of abnormally expressed PDCD4 on the NF- κ B and Notch signaling pathways were finally assessed by Western blot analysis. Results in the Figures 7A-7B showed phosphorylated levels of p65 and I κ B α as well as expressions of Notch1, Notch2 and Notch3 were all increased by LPS stimulation (Figures 7A-7B). Then, these increases were further elevated by PDCD4 overexpression compared with the LPS+pEX group while they were reversed by PDCD4 knockdown compared with the LPS+siNC group.

Discussion

With the increase of life expectancy and aging population, OA is predicted to be the fourth leading cause of disability all over the world by the year 2020 (19). Hence, more details on the progression of OA should be well studied. In our study, cell model of inflammatory injury was induced by LPS. MiR-23b was identified to be down-regulated after LPS treatment, and the LPS-induced cell injury could be alleviated by miR-23b overexpression. PDCD4 is predicted to be a target of miR-23b and miR-23b overexpression functioned through down-regulating PDCD4. Moreover, PDCD4 knockdown inhibited the LPS-induced activation of the NF- κ B and Notch signaling pathways.

Commonly, OA is characterized by the degradation of articular cartilage. Cartilage extracellular matrix, produced and maintained by chondrocytes, can mediate cartilage function and compensate mechanical stress to avoid structural damage (20). As the sole cell types in the cartilage, chondrocytes play a crucial role in OA development. ATDC5 cells, derived from mouse teratocarcinoma cells, are an excellent *in vitro* model cell line which is characterized as a chondrogenic cell line (21). LPS-stimulated chondrocytes have been used for investigations focused on OA. One reason is that various inflammatory cytokines participate in the abnormal

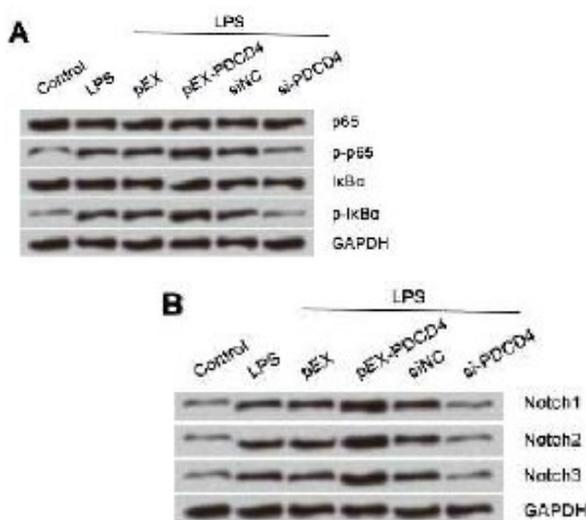


Figure 7. Programmed cell death 4 (PDCD4) overexpression activated the NF- κ B and Notch signaling pathways in ATDC5 cells with an LPS-induced inflammatory injury. ATDC5 cells were transfected with pEX-2 (pEX), pEX-PDCD4, siNC or si-PDCD4 and then stimulated with 5 μ g/ml LPS for 5 hr. Protein expressions of key kinases were assessed by Western blot analysis. LPS, lipopolysaccharide; pEX-PDCD4, pEX containing full-length PDCD4 sequence; si-PDCD4, PDCD4-specific small interfering RNA; siNC, negative control of si-PDCD4; p-, phosphorylated; I κ B α , inhibitor of nuclear factor κ B α

remodeling of joint tissues which is a character of OA. Another reason comes from the fact that LPS induces secretion of pro-inflammatory cytokines through activation of several signal transduction cascades (22). The underlying mechanism of calcitonin in OA treatments has been studied in LPS treated chondrocytes (23). In addition, the potential role of miR-1246 in OA has been studied by *in vitro* experiments using LPS-treated ATDC5 cells (24). Hence, in our study, we simulated the progression of OA in LPS-treated ATDC5 cells. Chondrocyte apoptosis and release of inflammatory cytokines have been reported as two major pathogenic mechanisms of OA (25). Thus, LPS-induced cell injury was evaluated through loss of cell viability, enhancement of cell apoptosis, alteration of apoptosis-associated proteins, and release of inflammatory cytokines. Ratios of Bax/Bcl-2, cleaved/procaspase-3, and cleaved/procaspase-9 were all increased by LPS, suggesting that LPS induced cell injury through intrinsic mitochondria-mediated and caspase pathways.

Next, we explored the expression of miR-23b and interestingly found it was down-regulated by LPS, implying the possible involvement of miR-23b in LPS-induced cell injury. Accordingly, miR-23b was aberrantly expressed through cell transfection with miRNAs in ATDC5 cells, followed by LPS treatment. Results in our study illustrated that miR-23b overexpression alleviated the LPS-induced cell injury whereas miR-23b knockdown exacerbated the cell injury induced by LPS. The influence of miR-23b on cell viability and apoptosis were consistent with a previous study, in which miR-23b was an oncogenic miRNA in renal cell carcinoma (26). Meanwhile, the suppressive effect of miR-23b on inflammation was coincident with a previous literature report (27).

miRNAs are considered as post-transcriptional regulators through binding to the 3'UTR of target genes (28). Currently, large numbers of genes have been reported as target genes of miR-23b, which participate in the biological function of miR-23b, including autophagy-related gene 12, high-mobility group box 2, Smad3, etc. (29, 30). Herein, the possible target genes of miR-23b were virtually screened by the TargetScan online software. PDCD4 is recognized as a tumor suppressor that binds to the RNA helicase eukaryotic initiation factor 4A, which plays a crucial role in the unwinding of mRNA and scanning during translation initiation, thereby inhibiting protein expression (31, 32). Moreover, PDCD4 is also reported to play pro-inflammatory roles in LPS signaling and is required for the lethality of LPS (33). Therefore, the interaction between miR-23b and PDCD4 as well as the role of PDCD4 in miR-23b-associated regulation were further studied. Firstly, PDCD4 was proved to be negatively correlated with miR-23b expression at both mRNA and protein levels. Then luciferase assay verified the direct binding of miR-23b to PDCD4 3'UTR. Finally, the alleviation of LPS-induced cell injury, induced by miR-23b overexpression, was proved to be realized through down-regulating PDCD4 expression.

The NF- κ B signaling pathway is identified to be the central regulator of the inflammatory responses in synovium and chondrocytes and is reported to promote chondrocyte apoptosis (34, 35). Under the

normal state, NF- κ B dimers (p65/p50) are stayed in the cytoplasm along with I κ B α . After LPS treatment, I κ B α is phosphorylated and release active NF- κ B dimers, which translocate to the nucleus to regulate downstream genes (33, 36). Notch signaling pathway is a highly conserved pathway that is responsible for the regulation of cell proliferation and apoptosis in OA (37). Hence, we finally explored the alterations of these two pathways during the modulation of miR-23b in LPS-induced cell injury. Results showed these two pathways were both activated by LPS, which was consistent with previous studies (38, 39). Then, the activations were ameliorated by PDCD4 knockdown but were further enhanced by PDCD4 overexpression, suggesting that aberrantly expressed PDCD4, which could be regulated by miR-23b, affected the activations of the NF- κ B and Notch pathways.

Conclusion

Taken together, miR-23b was down-regulated in LPS-treated ATDC5 cells, and its overexpression alleviated LPS-induced cell injury through down-regulating PDCD4, involving the NF- κ B and Notch pathways. The investigations might provide novel therapeutic targets for OA. More validations should be done *in vivo* in the future.

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