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Long non-coding RNA THRIL promotes LPS-induced inflammatory injury by down-regulating microRNA-125b in ATDC5 cells

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ABSTRACT

Background: Osteoarthritis is an age-related disorder of bone-joint that causes pain and disability in middle and older people. This study aimed to investigate the potential effects of long non-coding RNA (lncRNA) THRIL on lipopolysaccharide (LPS)-induced osteoarthritis cell injury model (ATDC5 cell inflammatory injury), as well as the possible internal molecular mechanisms.

Methods: Cell viability and apoptosis were assessed using CCK-8 assay and Guava Nexin assay, respectively. Cell transfection was conducted to change the expression of THRIL and microRNA-125b (miR-125b) in ATDC5 cells. qRT-PCR was performed to detect the expression of THRIL, miR-125b and pro-inflammatory cytokines IL-6, TNF-α and monocyte chemotactic protein 1 (MCP-1) in ATDC5 cells. ELISA was used to measure the concentrations of IL-6, TNF-α and MCP-1 in culture supernatant of ATDC5 cells. Finally, the protein expression of key factors involved in cell apoptosis, inflammatory response, JAK1/STAT3 and NF-κB pathways were evaluated using western blotting.

Results: LPS significantly induced ATDC5 cell inflammatory injury and up-regulated the expression of THRIL. Overexpression of THRIL aggravated the LPS-induced ATDC5 cell inflammatory injury. Suppression of THRIL had opposite effects. Moreover, THRIL negatively regulated the expression of miR-125b in ATDC5 cells. miR-125b participated in the effects of THRIL overexpression on LPS-induced ATDC5 cell inflammatory injury. Furthermore, overexpression of THRIL enhanced the LPS-induced JAK1/STAT3 and NF-κB pathways activation by down-regulating miR-125b.

Conclusion: THRIL exerted pro-inflammatory roles in LPS-induced osteoarthritis cell injury model. Overexpression of THRIL promoted LPS-induced ATDC5 cell inflammatory injury by down-regulating miR-125b and then activating JAK1/STAT3 and NF-κB pathways.

1. Introduction

Osteoarthritis is an age-related disorder of bone-joint that causes pain and disability in middle and older people all over the world [1,2]. As a degenerative disease, degeneration of articular cartilage plays a key role in the pathogenesis of osteoarthritis [3]. Many factors contribute to the degeneration of articular cartilage, including aging, obesity, strain, trauma, congenital malformation and inflammation [4,5]. With the increasing prevalence of obesity and graying of the world's population, osteoarthritis has become a serious social and economic burden for government worldwide [6,7]. It is worthy believed that a more clear understanding of articular cartilage degeneration will be helpful for osteoarthritis prevention, diagnosis and treatment.

Long non-coding RNAs (lncRNAs) are a class of RNA transcripts in eukaryotic cells without protein-coding potential [8]. Increasing numbers of reports provide evidences that lncRNAs participate in the regulation of multiple genes expression [9,10]. Aberrant expressions of lncRNAs have been linked to the pathogenesis of many human diseases, including osteoarthritis [11,12]. Tumor necrosis factor (TNF) and hnRNPL related immune-regulatory lincRNA (THRIL) is a recently discovered lncRNA that plays a critical role in the regulation of TNF expression by interacting with hnRNPL [13]. Xia et al. proved that THRIL exerted pro-inflammatory effects and promoted hypoxia-induced myocardial infarction (MI) cell injury [14]. Until now, there is no any information available about the effects of THRIL on osteoarthritis. It's still unclear that whether THRIL has a similar pro-inflammatory role in osteoarthritis. More experimental and clinical researches are still needed.

MicroRNAs (miRNAs) are another class of non-coding RNA

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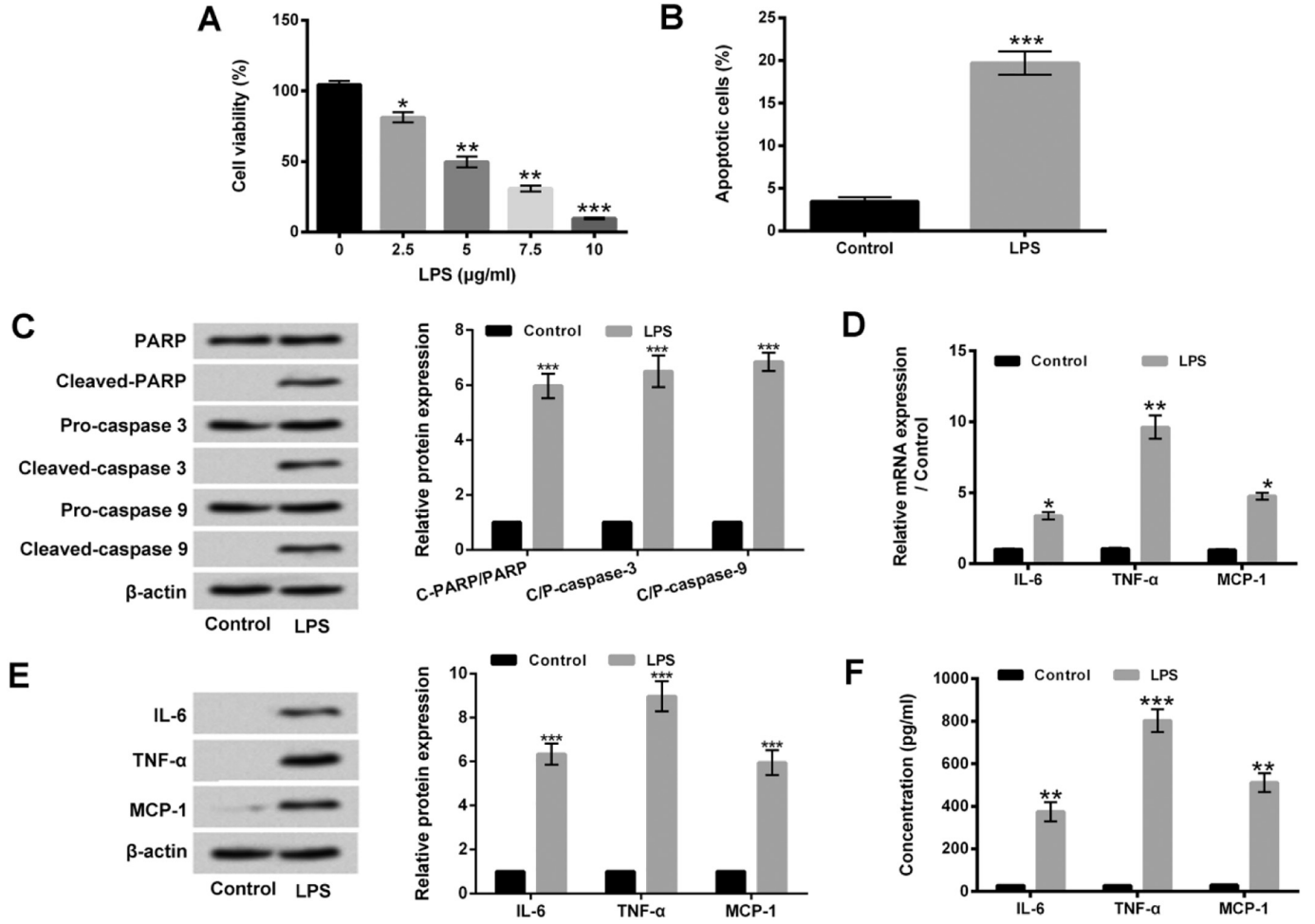

Fig. 1. LPS induced ATDC5 cell inflammatory injury.
(A) After 2.5, 5, 7.5 or 10 μg/ml LPS treatment, the viability of ATDC5 cells was detected using CCK-8 assay. After 5 μg/ml LPS treatment, (B) the apoptosis of ATDC5 cells, (C) the protein levels of PARP, Cleaved-PARP, Pro-caspase 3, Cleaved-caspase 3, Pro-caspase 9 and Cleaved-caspase 9 in ATDC5 cells, (D and E) the mRNA and protein levels of IL-6, TNF-α and MCP-1 in ATDC5 cells, and (F) the concentrations of IL-6, TNF-α and MCP-1 in culture supernatant of ATDC5 cells were assessed using Guava Nexin assay, qRT-PCR, ELISA and western blotting, respectively. LPS: lipopolysaccharide; PARP: poly ADP-ribose polymerase; IL-6: interleukin 6; TNF-α: tumor necrosis factor α; MCP-1: monocyte chemotactic protein 1. $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$.

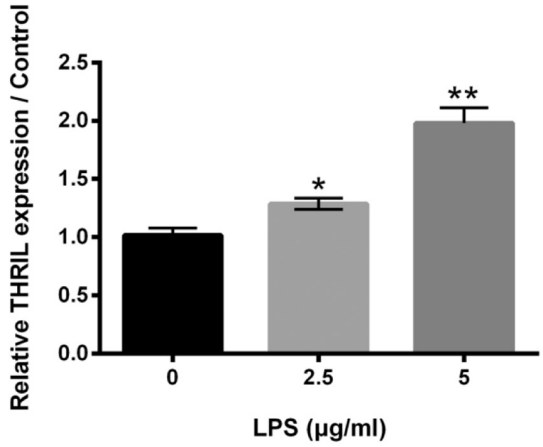

Fig. 2. LPS enhanced the expression of THRIL in ATDC5 cells.
The expression of THRIL in ATDC5 cells after 2.5 or 5 μg/ml LPS treatment was measured using qRT-PCR. LPS: lipopolysaccharide; THRIL: TNF and hnRNPL related immune-regulatory lincRNA. $^{*}P < 0.05$; $^{**}P < 0.01$.

transcripts in eukaryotic cells with 20–24 nucleotides (nt) in length [15]. Numerous lncRNAs, including THRIL, are implicated in the pathogenesis of human diseases by modulating the expression of miRNAs [8,14]. miRNA-125b (miR-125b) has been revealed to be involved in the pathogenesis of osteoarthritis by down-regulating synoviolin 1 [16]. Jia et al. demonstrated that overexpression of miR-125b suppressed lipopolysaccharide (LPS)-induced osteoarthritis cell inflammatory injury (ATDC5 cell inflammatory injury) by targeting macrophage inflammatory protein 1α (MIP-1α) [17].

Therefore, in this study, we focused our investigation on the potential pro-inflammatory effects of THRIL on osteoarthritis, exploring the chondrogenic cell viability, apoptosis and pro-inflammatory cytokines expression. Moreover, the possible internal molecular mechanisms involving in miR-125b-mediated protective activity were also analyzed. In our opinion, this study will be helpful for understanding the critical role of THRIL in the degeneration of articular cartilage and may provide possible diagnostic and therapeutic targets for osteoarthritis diagnosis and treatment.

2. Materials and methods

2.1. Cell culture and treatment

The murine chondrogenic ATDC5 cells were obtained from Riken Cell Bank (Tsukuba, Japan). Cells were cultured in Dulbecco's modified

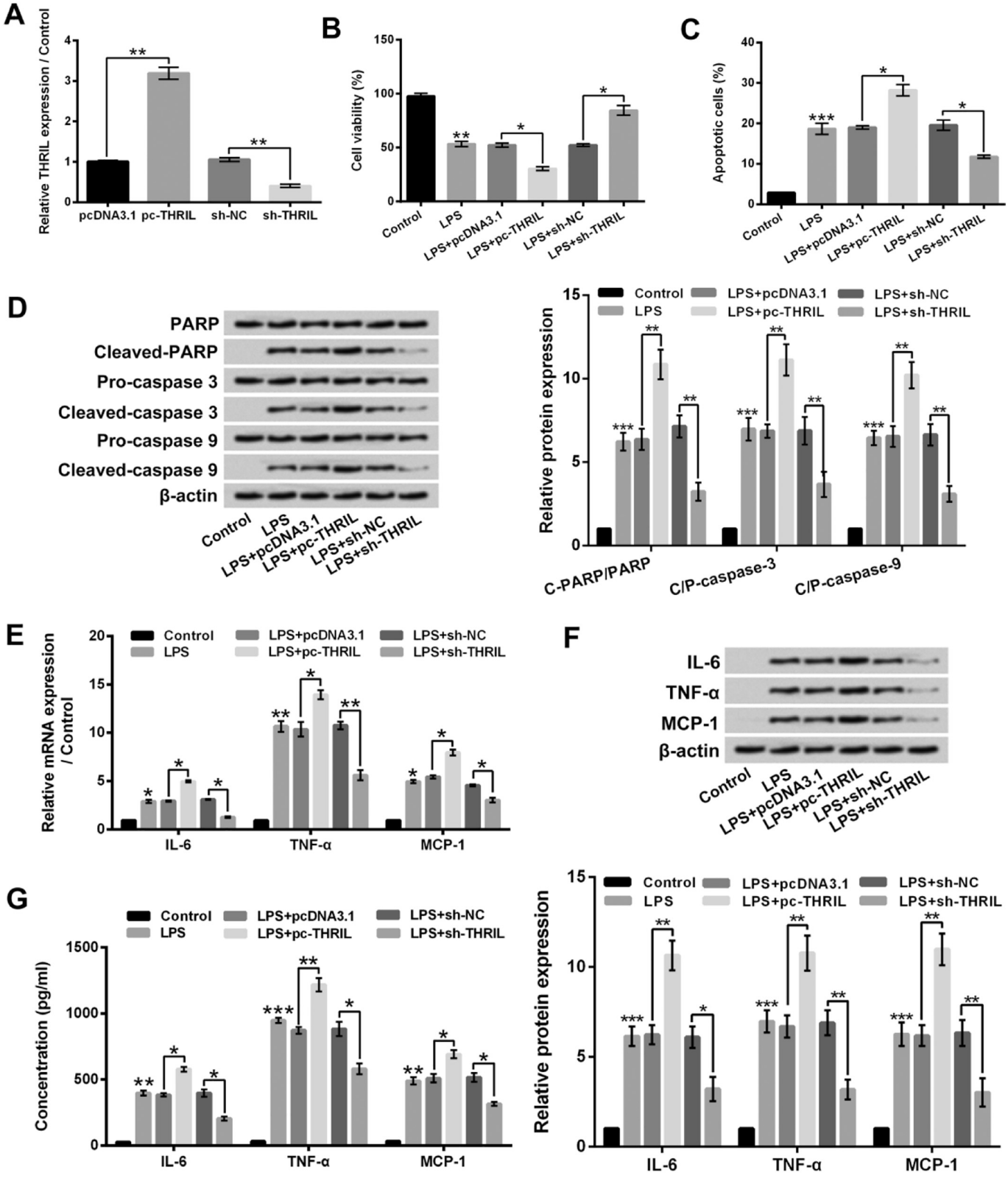

Fig. 3. THRIL was involved in the LPS-induced ATDC5 cell inflammatory injury.
(A) After pc-THRIL or sh-THRIL transfection, the expression of THRIL in ATDC5 cells was detected using qRT-PCR. After LPS treatment and/or pc-THRIL (or sh-THRIL) transfection, (B and C) the viability and apoptosis of ATDC5 cells, (D) the protein levels of PARP, Cleaved-PARP, Pro-caspase 3, Cleaved-caspase 3, Pro-caspase 9 and Cleaved-caspase 9 in ATDC5 cells, (E and F) the mRNA and protein levels of IL-6, TNF-α and MCP-1 in ATDC5 cells, and (G) the concentrations of IL-6, TNF-α and MCP-1 in culture supernatant of ATDC5 cells were assessed using CCK-8 assay, Guava Nexin assay, qRT-PCR, ELISA and western blotting, respectively. LPS: lipopolysaccharide; THRIL: TNF and hnRNPL related immune-regulatory lincRNA; PARP: poly ADP-ribose polymerase; IL-6: interleukin 6; TNF-α: tumor necrosis factor α; MCP-1: monocyte chemotactic protein 1. $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$.

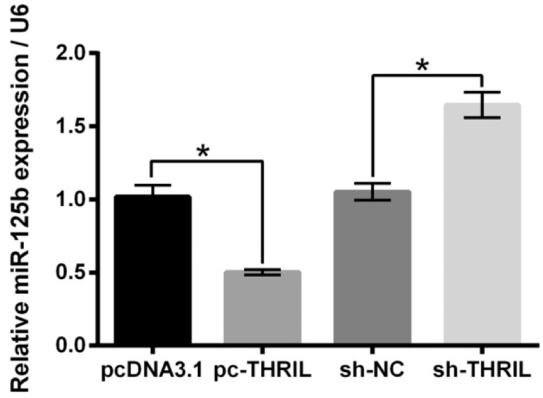

Fig. 4. THRIL negatively regulated the expression of miR-125b in ATDC5 cells. The expression of miR-125b in ATDC5 cells after pc-THRIL or sh-THRIL transfection was measured using qRT-PCR. THRIL: TNF and hnRNPL related immune-regulatory lincRNA; miR-125b: MicroRNA-125b. $*P < 0.05$.

Eagle's medium/Ham's Nutrient Mixture F12 (DMEM/F12, Gibco, Life Technologies, Carlsbad, CA, USA) supplemented with 10% (v/v) fetal bovine serum (FBS, Gibco, Life Technologies) and 1% (v/v) Penicillin-Streptomycin-Glutamine (100×, Gibco, Life Technologies) in 75 cm^2 tissue culture flask. Flask was placed at 37 °C in a humidity incubator (Thermo Fisher Scientific, Waltham, MA, USA) with 5% CO_2.

LPS was purchased from Sigma-Aldrich (St Louis, MO, USA) and dissolved in ultrapure water to a storage concentration of 5 mg/ml. The serum-free DMEM//F12 was used to dilute LPS solution to 2.5, 5, 7.5 or 10 μg/ml before experiments. ATDC5 cells were treated by 2.5, 5, 7.5 or 10 μg/ml LPS for 12 h in this research [18].

2.2. Cell transfection

The full-length of THRIL sequence was constructed into pcDNA3.1 plasmid (GenePharma Corporation, Shanghai, China), which was referred as pc-THRIL. Short-hairpin RNA directed against THRIL was ligated into the U6/GFP/Neo plasmid (GenePharma Corporation), which was referred as sh-THRIL. The unloaded pcDNA3.1 plasmid and the U6/GFP/Neo plasmid carrying non-targeting sequence were acted as negative control (NC), which were referred as pcDNA3.1 and sh-NC, respectively. miR-125b mimic and its NC were designed and synthesized by GenePharma Corporation. Cell transfection was conducted using Lipofectamine 3000 reagent (Invitrogen, Carlsbad, CA, USA) in line with the manufacturer's instruction. Transfection efficiency was assessed using quantitative reverse transcription PCR (qRT-PCR).

2.3. qRT-PCR

qRT-PCR was performed to detect the expression levels of THRIL, miR-125b, interleukin 6 (IL-6), TNF-α and monocyte chemotactic protein 1 (MCP-1) in ATDC5 cells after relevant treatment or transfection. Briefly, total RNAs in ATDC5 cells was extracted using TRIzol™ Plus RNA Purification kit (Invitrogen). cDNA was reversely transcribed using SuperScript™ Reverse Transcription Reagents (Invitrogen). Then, the expression level of THRIL was measured using TaqMan™ Non-coding RNA assay (Applied Biosystems, Foster City, CA, USA) and β-actin acted as endogenous control. The expression levels of IL-6, TNF-α and MCP-1 were measured using Fast SYBR™ Green Master Mix (Applied Biosystems) and β-actin acted as endogenous control. The expression level of miR-125b was measured using TaqMan™ MicroRNA Assay (Applied Biosystems) and the U6 acted as endogenous control. Data were quantified using classic $2^{-\triangle\triangle Ct}$ method [19].

2.4. Cell viability assay

Viability of ATDC5 cells was evaluated using cell counting kit-8 (CCK-8) assay (Beyotime Biotechnology, Shanghai, China) in this research. Briefly, after LPS treatment and/or pc-THRIL (or sh-THRIL) or miR-125b mimic transfection, 1×10^4 ATDC5 cells were seeded into 96-well plate and incubated at 37 °C in humidity incubator for 24 h. Then, 10 μl CCK-8 solution was added into the each well of the plate and the plate was incubated at 37 °C in humidity incubator for another 1 h. Subsequently, the absorbance of each well at 450 nm was recorded using Micro-plate Reader (Bio-Tek Instruments, Winooski, VT, USA). Cell viability (%) was calculated by average absorbance of treatment (transfection) group/average absorbance of control group × 100%.

2.5. Cell apoptosis assay

Apoptosis of ATDC5 cells was detected using Guava Nexin assay (Guava Technologies, Hayward, CA, USA). Briefly, after LPS treatment and/or pc-THRIL (or sh-THRIL) or miR-125b mimic transfection, 1×10^5 ATDC5 cells were seeded into 6-well plate and incubated at 37 °C in humidity incubator for 24 h. Then, cells in each group were collected, washed with phosphate buffered saline (PBS) for three times and stained using kit solution for 25 min at 37 °C in the dark. Followed by washed with PBS for three times, the rate of apoptotic cells in each group was recorded using Guava EasyCyte flow cytometer (Guava Technologies, Hayward, CA, USA). Data were analyzed using FCS Express software (De Novo software, Los Angeles, CA, USA).

2.6. Enzyme-linked immunosorbent assay (ELISA)

ELISA was conducted to measure the concentrations of IL-6, TNF-α and MCP-1 in culture supernatant of ATDC5 cells after relevant treatment or transfection. Briefly, after LPS treatment and/or pc-THRIL (or sh-THRIL) or miR-125b mimic transfection, 1×10^5 ATDC5 cells were seeded into 6-well plate and incubated at 37 °C in humidity incubator for 24 h. Then, culture supernatant of each group was collected and the concentrations of IL-6, TNF-α and MCP-1 were measured using Mouse IL-6 ELISA kit (Sigma-Aldrich, catalog number: RAB0308), Mouse TNF-α ELISA kit (Thermo Fisher Scientific, catalog number: BMS607-3FIVE) and Mouse MCP-1 ELISA kit (Sigma-Aldrich, catalog number: RAB0055), respectively.

2.7. Western blotting

After LPS treatment and/or pc-THRIL (or sh-THRIL) or miR-125b mimic transfection, total proteins in ATDC5 cells were isolated using RIPA Lysis Buffer (Beyotime Biotechnology) containing protease inhibitors (Roche, Basel, Switzerland). The concentration of total proteins was quantified using BCA Protein Assay kit (Beyotime Biotechnology). Then, proteins in equal concentration were electrophoresed using Bio-Rad Bis-Tris Gel system (Bio-Rad Laboratories, Hercules, CA, USA) and transferred onto polyvinylidene difluoride (PVDF) membranes. After blocking with 5% bovine serum albumin (BSA) solution for 1 h at 25 °C, the PVDF membranes were incubated with primary antibodies for 12 h at 4 °C. Anti-poly ADP-ribose polymerase (PARP) antibody (ab32138), Anti-Cleaved-PARP antibody (ab32064), Anti-Caspase 3 antibody (ab4051), Anti-Caspase 9 antibody (ab25758), Anti-IL-6 antibody (ab208113), Anti-TNF-α antibody (ab6671), Anti-MCP-1 antibody (ab25124), and Anti-β-actin antibody (ab8226) were obtained from Abcam Biotechnology (Cambridge, MA, USA). Anti-t-c-Jun N-terminal kinase 1 (JAK1) antibody (#3332), Anti-p-JAK1 antibody (#3331), Anti-t-signal transducer and activator of transcription 3 (STAT3) antibody (#9139), Anti-p-STAT3 antibody (#9145), Anti-t-inhibitor of nuclear factor kappa B (IκBα) antibody (#9242), Anti-p-IκBα antibody (#9246), Anti-t-p65 antibody (#4764) and Anti-p-p65 antibody (#3033) were obtained from Cell Signaling Technology (Beverly, MA,

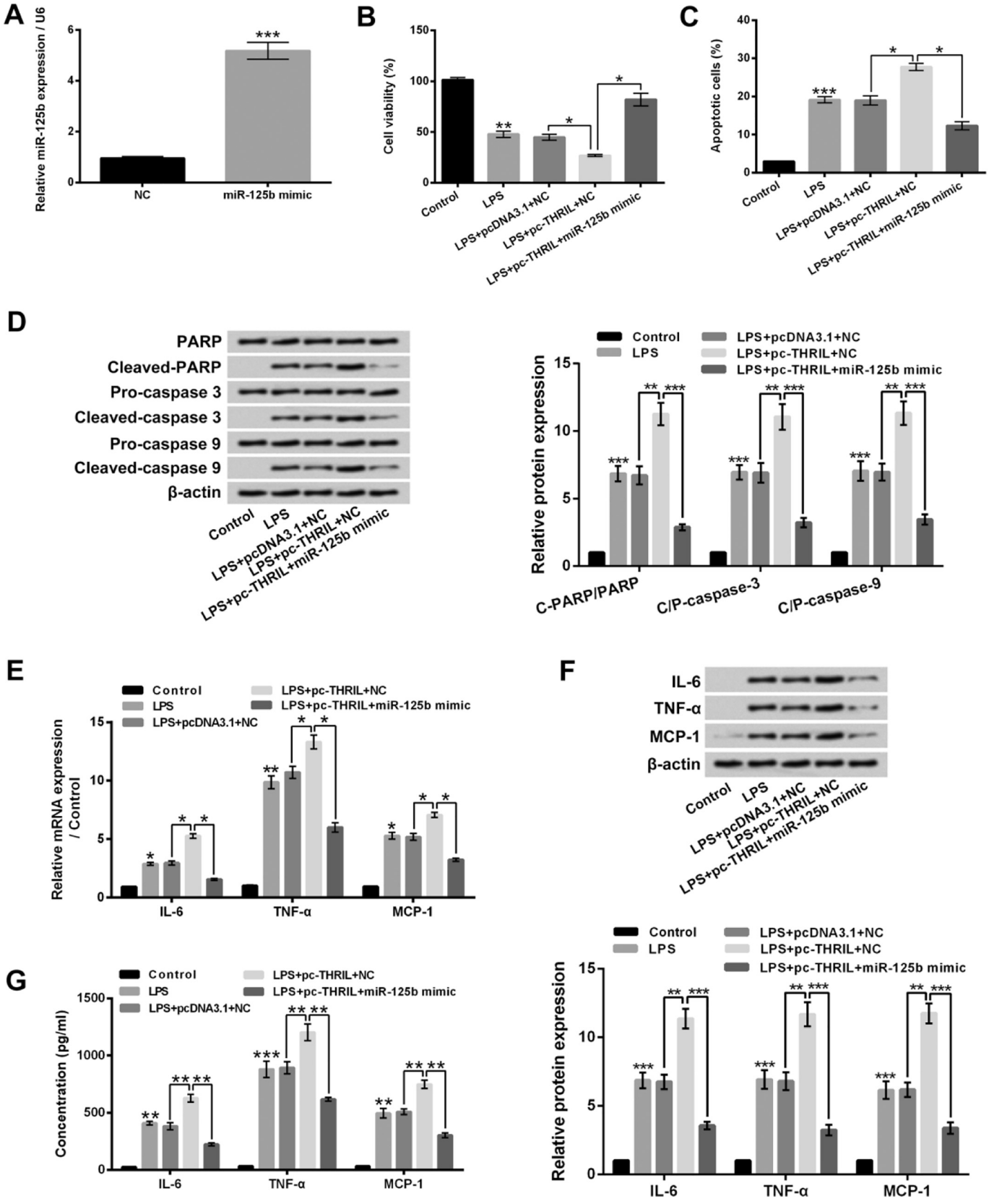

Fig. 5. miR-125b participated in the effects of THRIL overexpression on LPS-induced ATDC5 cell inflammatory injury.
(A) After miR-125b mimic transfection, the expression of miR-125b in ATDC5 cells was measured using qRT-PCR. After LPS treatment and/or pc-THRIL (or miR-125b mimic) transfection, (B and C) the viability and apoptosis of ATDC5 cells, (D) the protein levels of PARP, Cleaved-PARP, Pro-caspase 3, Cleaved-caspase 3, Pro-caspase 9 and Cleaved-caspase 9 in ATDC5 cells, (E and F) the mRNA and protein levels of IL-6, TNF-α and MCP-1 in ATDC5 cells, and (G) the concentrations of IL-6, TNF-α and MCP-1 in culture supernatant of ATDC5 cells were assessed using CCK-8 assay, Guava Nexin assay, qRT-PCR, ELISA and western blotting, respectively. LPS: lipopolysaccharide; THRIL: TNF and hnRNPL related immune-regulatory lincRNA; miR-125b: microRNA-125b; PARP: poly ADP-ribose polymerase; IL-6: interleukin 6; TNF-α: tumor necrosis factor α; MCP-1: monocyte chemotactic protein 1. $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$.

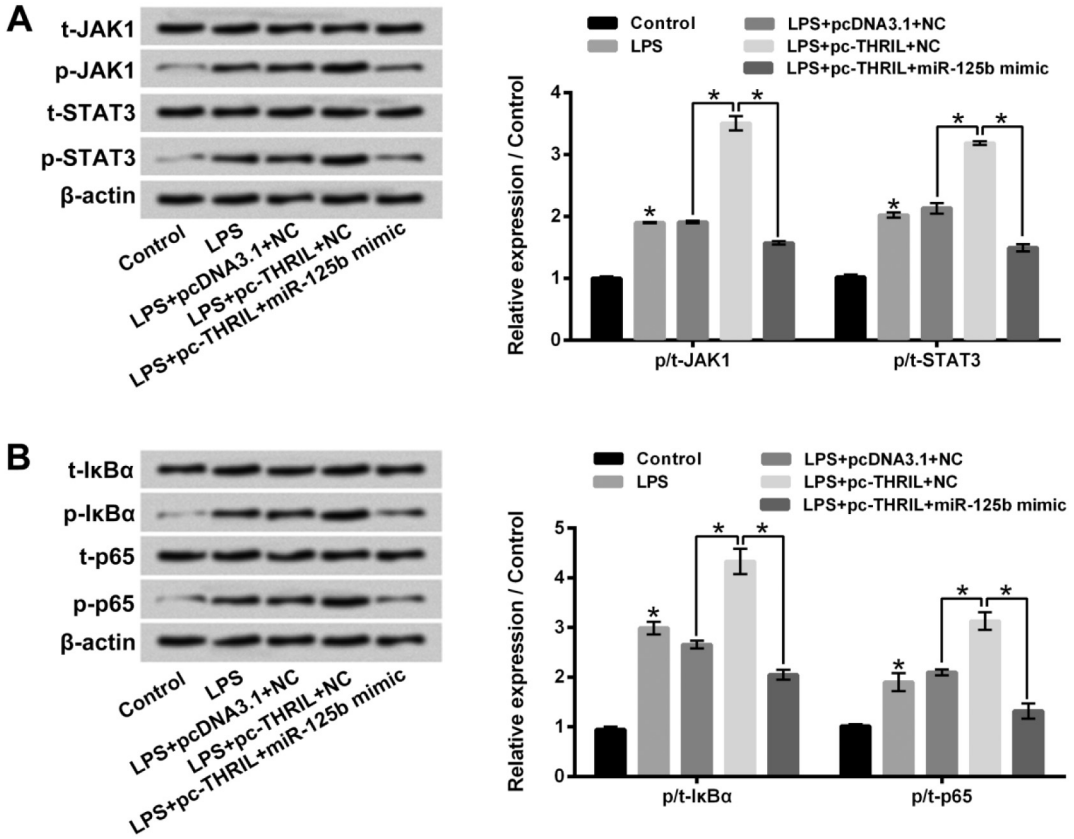

Fig. 6. Overexpression of THRIL aggravated LPS-induced JAK1/STAT3 and NF-κB pathways activation in ATDC5 cells by down-regulating miR-125b.
(A and B) After LPS treatment and/or pc-THRIL (or miR-125b mimic) transfection, the expression levels of t-JAK1, p-JAK1, t-STAT3, p-STAT3, t-IκBα, p-IκBα, t-p65 and p-p65 in ATDC5 cells were evaluated using western blotting. LPS: lipopolysaccharide; THRIL: TNF and hnRNPL related immune-regulatory lincRNA; miR-125b: MicroRNA-125b; JAK1: c-Jun N-terminal kinase 1; STAT3: signal transducer and activator of transcription 3; NF-κB: nuclear factor kappa B; IκBα: inhibitor of NF-κB. $^{*}P < 0.05$.

USA). Subsequently, the PVDF membranes were incubated with Goat Anti-Mouse (or Anti-Rabbit) IgG H&L DyLight™ 680 conjugate secondary antibodies (Cell Signaling Technology, catalog number: #5366 and #5470) for 1 h at 25 °C in the dark. After that, the signals of proteins were recorded using Odyssey System (Licor Biosciences, Bad Honburg, Germany). The intensities of bands were analyzed using Odyssey software (Licor Biosciences).

2.8. Statistical analysis

All experiments were repeated at least three times. The results of multiple experiments were presented as mean ± standard deviation (SD). Statistical analysis was conducted using Graphpad 6.0 statistical software (Graphpad, San Diego, CA, USA). Differences among groups were assessed using one-way analysis of variance (ANOVA). Statistically significance was set at $P < 0.05$.

3. Results

3.1. LPS induced ATDC5 cell inflammatory injury

Firstly, after LPS treatment, the viability, apoptosis and pro-inflammatory cytokines expression of ATDC5 cells were assessed, respectively. The results of Fig. 1A presented that LPS treatment significantly inhibited the viability of ATDC5 cells in a dose-dependent manner ($P < 0.05$, $P < 0.01$ or $P < 0.001$). The IC50 value was calculated as 4.87 ± 0.52 μg/ml. So, 5 μg/ml LPS treatment was chosen for further experiments. Fig. 1B showed that 5 μg/ml LPS exposure dramatically induced ATDC5 cell apoptosis ($P < 0.001$). The protein expression rates of C-PARP/PARP, C/P-caspase 3 and C/P-caspase 9 in ATDC5 cells were all increased after 5 μg/ml LPS exposure (Fig. 1C, $P < 0.001$). These results suggested that LPS inhibited ATDC5 cell viability and induced cell apoptosis. In addition, Fig. 1D and E displayed that the mRNA and protein expression levels of IL-6, TNF-α and MCP-1 in ATDC5 cells were all enhanced after 5 μg/ml LPS treatment ($P < 0.05$, $P < 0.01$ or $P < 0.001$). Similar results were found in Fig. 1F, which pointed out that 5 μg/ml LPS exposure notably increased the concentrations of IL-6, TNF-α and MCP-1 in culture supernatant of ATDC5 cells ($P < 0.01$ or $P < 0.001$). These above results indicated that LPS could induce ATDC5 cell inflammatory injury.

3.2. LPS up-regulated the expression of THRIL in ATDC5 cells

The expression level of THRIL in ATDC5 cells after 2.5 or 5 μg/ml LPS treatment was detected using qRT-PCR. As displayed in Fig. 2, LPS treatment obviously up-regulated the expression of THRIL in ATDC5 cells in a dose-dependent manner ($P < 0.05$ or $P < 0.01$), which suggested that THRIL might be involved in the LPS-induced ATDC5 cell inflammatory injury.

3.3. THRIL was involved in the LPS-induced ATDC5 cell inflammatory injury

Next, to analyze the effects of THRIL on LPS-induced ATDC5 cell inflammatory injury, pc-THRIL and sh-THRIL were transfected into ATDC5 cells, respectively. Fig. 3A showed that pc-THRIL transfection remarkably enhanced the expression level of THRIL ($P < 0.01$), while sh-THRIL transfection dramatically reduced the expression level of

THRIL in ATDC5 cells ($P < 0.01$). The results of Fig. 3B presented that LPS-induced ATDC5 cell viability inhibition was aggravated by overexpression of THRIL ($P < 0.05$) and inhibited by THRIL suppression ($P < 0.05$). Fig. 3C displayed that LPS-induced ATDC5 cell apoptosis was also aggravated by overexpression of THRIL ($P < 0.05$) and inhibited by THRIL suppression ($P < 0.05$). Western blotting pointed out that compared to LPS single treatment group, the protein expression rates of C-PARP/PARP, C/P-caspase 3 and C/P-caspase 9 in ATDC5 cells were increased in LPS + pc-THRIL group and decreased in LPS + sh-THRIL group (Fig. 3D, $P < 0.01$). Moreover, the results of Fig. 3E and F showed that LPS-induced up-regulations of IL-6, TNF-α and MCP-1 mRNA and protein expression levels were enhanced by overexpression of THRIL ($P < 0.05$ or $P < 0.01$) and reduced by THRIL suppression ($P < 0.05$ or $P < 0.01$). Compared to LPS single treatment group, the concentrations of IL-6, TNF-α and MCP-1 in culture supernatant of ATDC5 cells were also enhanced by overexpression of THRIL and reduced by THRIL suppression (Fig. 3G, $P < 0.05$ or $P < 0.01$). These above findings suggested that THRIL was involved in the LPS-induced ATDC5 cell inflammatory injury. Overexpression of THRIL aggravated LPS-induced ATDC5 cell inflammatory injury, while suppression of THRIL inhibited the LPS-induced ATDC5 cell inflammatory injury.

3.4. THRIL negatively regulated the expression of miR-125b in ATDC5 cells

The expression of miR-125b in ATDC5 cells after pc-THRIL or sh-THRIL transfection was measured using qRT-PCR. As presented in Fig. 4, pc-THRIL transfection reduced the expression level of miR-125b in ATDC5 cells ($P < 0.05$), while sh-THRIL transfection enhanced the expression of miR-125 b in ATDC5 cells ($P < 0.05$). These results indicated that THRIL negatively regulated the expression of miR-125b in ATDC5 cells and implied that miR-125b might participate in the effects of THRIL on LPS-induced ATDC5 cell inflammatory injury.

3.5. miR-125b participated in the effects of THRIL overexpression on LPS-induced ATDC5 cell inflammatory injury

Then, to explore the roles of miR-125b in LPS + pc-THRIL-induced ATDC5 cell viability inhibition, cell apoptosis and overproduction of pro-inflammatory cytokines, miR-125b mimic was transfected into ATDC5 cells. Fig. 5A presented that miR-125b mimic transfection dramatically up-regulated the expression level of miR-125b in ATDC5 cells ($P < 0.001$). The results of Fig. 5B and C showed that miR-125b mimic transfection obviously reversed the LPS + pc-THRIL-induced ATDC5 cell viability inhibition and cell apoptosis ($P < 0.05$). Compared to LPS + pc-THRIL group, the protein expression rates of C-PARP/PARP, C/P-caspase 3 and C/P-caspase 9 in ATDC5 cells were all decreased in LPS + pc-THRIL + miR-125b mimic group (Fig. 5D, $P < 0.001$). In addition, Fig. 5E and F displayed that miR-125b mimic transfection also reversed the LPS + pc-THRIL-induced up-regulations of IL-6, TNF-α and MCP-1 mRNA and protein expression levels in ATDC5 cells ($P < 0.05$ or $P < 0.001$). Similar results were found in Fig. 5G, which pointed out that compared to LPS + pc-THRIL group, the concentrations of IL-6, TNF-α and MCP-1 in culture supernatant of ATDC5 cells were also decreased in LPS + pc-THRIL + miR-125b mimic group ($P < 0.01$). These above findings indicated that miR-125b participated in the effects of THRIL on LPS-induced ATDC5 cell inflammatory injury. Overexpression of THRIL promoted LPS-induced ATDC5 cell inflammatory injury at least in part by down-regulating miR-125b.

3.6. Overexpression of THRIL aggravated LPS-induced JAK1/STAT3 and NF-κB pathways activation in ATDC5 cells by down-regulating miR-125b

Finally, the activation of JAK1/STAT3 and NF-κB pathways in ATDC5 cells after LPS treatment and/or pc-THRIL (or miR-125b mimic) transfection were evaluated using western blotting. Fig. 6A and B showed that LPS single treatment significantly activated JAK1/STAT3 and NF-κB pathways in ATDC5 cells by up-regulating the expression rates of p/t-JAK, p/t-STAT3, p/t-IκBα and p/t-p65 ($P < 0.05$). Overexpression of THRIL obviously enhanced the LPS-induced JAK1/STAT3 and NF-κB pathways activation in ATDC5 cells by further increasing the expression rates of p/t-JAK, p/t-STAT3, p/t-IκBα and p/t-p65 ($P < 0.05$). However, compared to LPS + pc-THRIL group, the expression rates of p/t-JAK, p/t-STAT3, p/t-IκBα and p/t-p65 in ATDC5 cells were all decreased in LPS + pc-THRIL + miR-125b mimic group ($P < 0.05$). These above findings suggested that overexpression of THRIL aggravated LPS-induced JAK1/STAT3 and NF-κB pathways activation in ATDC5 cells by down-regulating miR-125b.

4. Discussion

Osteoarthritis is a degenerative disease characterized by degeneration of articular cartilage [3,20]. In the present study, we used LPS to induce chondrogenic cell inflammatory injury model (ATDC5 cell inflammatory injury) and found that LPS treatment obviously inhibited ATDC5 cell viability, induced cell apoptosis and enhanced the expression of pro-inflammatory cytokines. Moreover, we revealed that LPS up-regulated the expression of THRIL in ATDC5 cells. Overexpression of THRIL promoted the LPS-induced ATDC5 cell viability inhibition, cell apoptosis and overproduction of pro-inflammatory cytokines; while suppression of THRIL had opposite effects. Furthermore, we pointed out that THRIL negatively regulated the expression of miR-125b in ATDC5 cells. miR-125b participated in the effects of THRIL overexpression on LPS-induced ATDC5 cell inflammatory injury. Finally, we indicated that overexpression of THRIL aggravated LPS-induced JAK1/STAT3 and NF-κB pathways activation in ATDC5 cells by down-regulating miR-125b.

As an ingredient of the cellular wall of Gram-negative bacteria, LPS can stimulate inflammatory response in a variety of cells through promoting pro-inflammatory cytokines expression [21,22]. Cell and animal models of osteoarthritis induced by LPS have been widely used for analyzing the pathogenesis of osteoarthritis and testing the new therapeutic medicine for osteoarthritis [17,23,24]. In this research, we found that LPS treatment significantly suppressed chondrogenic ATDC5 cell viability and induced cell apoptosis. The expression of pro-inflammatory cytokines IL-6, TNF-α and MCP-1 in ATDC5 cells and in culture supernatant of ATDC5 cells were all increased after LPS treatment. These results suggested that LPS notably induced ATDC5 cell inflammatory injury. Cell-based osteoarthritis inflammatory injury model was successfully established and could be used for further experiments.

Numerous studies have demonstrated that lncRNAs play critical regulatory roles in many cellular biological processes, including cell proliferation, differentiation, apoptosis and autophagy [25,26]. Abnormal lncRNAs expression and its association with pathogenesis of diseases have been reported in many types of human diseases, including osteoarthritis [12]. Previous study proved that THRIL exerted pro-inflammatory effects on hypoxia-induced MI cell injury model [14]. In consistent with the previous study, we found that LPS up-regulated the expression of THRIL in ATDC5 cells. Overexpression of THRIL remarkably exacerbated the LPS-induced ATDC5 cell inflammatory injury, as evidenced by the cell viability loss, cell apoptosis increase and pro-inflammatory cytokines overproduction. On the contrary, suppression of THRIL had opposite effects, which obviously attenuated the LPS-induced ATDC5 cell inflammatory injury. These above findings indicated that THRIL also exerted pro-inflammatory effects on LPS-induced osteoarthritis cell injury model. Considering that lncRNA maternally expressed gene 3 (MEG3) [3], lncRNA HOX antisense intergenic RNA (HOTAIR) [27], lncRNA reprogramming (ROR) [28] and lncRNA taurine upregulated gene 1 (TUG1) [29] are also related to pathogenesis of osteoarthritis, we could propose that many lncRNAs participate in the occurrence of osteoarthritis, which collectively form a very complex regulatory network.

miRNAs is often regulated by lncRNAs and involved in the gene

regulatory activity of lncNRAs [8,30]. miR-125b has been found to inhibit LPS-induced ATDC5 cell inflammatory injury [17]. In this research, we revealed that overexpression of THRIL down-regulated the expression of miR-125b, while suppression of THRIL up-regulated the expression of miR-125b in ATDC5 cells. More importantly, overexpression of miR-125b notably reversed the LPS + pc-THRIL-induced ATDC5 cell viability inhibition, cell apoptosis enhancement, and overproduction of pro-inflammatory cytokines. These findings indicated that THRIL negatively regulated the expression of miR-125b in ATDC5 cells and miR-125b was involved in the pro-inflammatory effects of THRIL on LPS-induced ATDC5 cell inflammatory injury.

JAK1/STAT3 and NF-κB signaling pathways are two important signaling pathways in eukaryotic cells that play key roles in the regulation of inflammation [31,32]. Previous studies reported that JAK1/STAT3 and NF-κB pathways are both activated in osteoarthritis and participated in the pathogenesis of osteoarthritis [33,34]. Therefore, in this research, we also explored the effects of LPS, THRIL and miR-125b on JAK/STAT and NF-κB pathways in ATDC5 cells. As results, we found that LPS notably activated JAK1/STAT3 and NF-κB pathways in ATDC5 cells. Overexpression of THRIL aggravated LPS-induced JAK1/STAT3 and NF-κB pathways activation in ATDC5 cells. In addition, overexpression of miR-125b noticeably alleviated the LPS + pc-THRIL-induced JAK1/STAT3 and NF-κB pathways activation. These above findings indicated that overexpression of THRIL exacerbated LPS-induced ATDC5 cell inflammatory injury might through down-regulating miR-125b and then activating JAK1/STAT3 and NF-κB pathways.

To sum up, our research verified that THRIL exerted pro-inflammatory effects on LPS-induced osteoarthritis cell injury model. Overexpression of THRIL promoted LPS-induced ATDC5 cell inflammatory injury by down-regulating miR-125b and then activating JAK1/STAT3 and NF-κB pathways. This study provides evidence for further understanding the pathogenesis of osteoarthritis. We proposed that THRIL and miR-125b might be as the potential diagnostic and therapeutic targets for osteoarthritis diagnosis and treatment.

Declarations of interest

None.

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