Original Paper

Knockdown of Long Non-Coding RNA RP11-445H22.4 Alleviates LPS-Induced Injuries by Regulation of MiR-301a in Osteoarthritis

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Key Words

LncRNA RP11-445H22.4 • MiR-301a • Cell viability • Apoptosis • Inflammatory injury • CXCR4

Abstract

Background/Aims: Several long non-coding RNAs (lncRNAs) play vital roles in osteoarthritis (OA), whereas the role of lncRNA RP11-445H22.4 in OA remains unclear. The study aimed to investigate the effect of lncRNA RP11-445H22.4 on lipopolysaccharide (LPS)-induced cell viability, apoptosis and inflammatory injury of OA. **Methods:** The expression of RP11-445H22.4, miR-301a and CXCR4 in human cartilage ATDC5 cells were altered by transfection, and then cells were exposed to 5 µg/ml LPS for 12 h. Then cell viability, apoptosis, apoptosis-related factors and inflammatory cytokines were analyzed by CCK-8, flow cytometry, western blot, RT-qPCR and ELISA, respectively. Dual-luciferase reporter assay was performed to assess the binding sites of RP11-445H22.4 and miR-301a. The signal pathways of NF-κB and MAPK/ERK were determined by western blot. **Results:** LPS reduced cell viability, increased apoptosis and stimulated release of IL-1β, IL-6, IL-8 and TNF-α. However, RP11-445H22.4 inhibition significantly rescued LPS-induced injuries by promoting cell viability, suppressing apoptosis and inflammatory cytokines secretions in ATDC5 cells. In addition, miR-301a directly bound to RP11-445H22.4, and suppression of miR-301a inversed the effects of RP11-445H22.4 inhibition. Furthermore, CXCR4 was a direct target of miR-301a, and CXCR4 silencing increased cell viability, decreased apoptosis and inflammatory cytokines secretions in LPS-treated ATDC5 cells. Besides, we found that CXCR4 silencing blocked LPS-activated NF-κB and MAPK/ERK pathways. **Conclusions:** The study indicated that lncRNA RP11-445H22.4-miR-301a-CXCR4 axis played an important role in cartilage ATDC5 cells and provided a theoretical basis of lncRNA RP11-445H22.4 in OA.

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Introduction

Osteoarthritis (OA) is a joint disease, that caused by destruction of articular cartilage and underlying bone [1, 2]. The common symptoms are joint pain, tenderness, swelling, stiffness, limitation of motion and deformity [3]. According to epidemiological analysis, approximately 190 million people are affected OA worldwide, and the most proportion in the elderly [4]. The treatment of OA is well accepted to be joint reconstruction via arthroplasty or used anti-inflammatory drugs to relieve OA [5, 6]. However, several side effects are always existed in these therapeutic methods. Therefore, to find a new effective therapeutic strategy is necessary.

Long non-coding RNAs (lncRNAs) is a non-coding RNA, length of more than 200 nt, exist in eukaryotic cells, and which is highly conserved among different species of mammals [7, 8]. Recently, increasing evidences demonstrated that lncRNAs are key elements of pathologic and physiologic processes, and are vital regulators of inflammatory response [9, 10]. Previous study reported that cartilage injury-related lncRNA (lncRNA-CIR) was higher expressed in chondrocytes induced by interleukin-1(IL-1), and inhibited the formation of collagen and aggrecan in OA [11]. In additional, Zhang et al. [12] exposed that lncRNA ubiquitin-fold modifier conjugating enzyme 1 (UFC1) enhanced chondrocyte cell proliferation and reduced apoptosis by targeting miR-34a in OA. However, whether lncRNA RP11-445H22.4 was related to pathogenesis of OA remains unclear.

lncRNA RP11-445H22.4 is a gene which located on human chromosome 20 with a length of 862 bp [13]. Recent study demonstrated that lncRNA RP11-445H22.4 was highly expressed in breast cancer cells, and it was related to the occurrence of tumor induced inflammatory response [13]. Furthermore, high expression lncRNA RP11-445H22.4 have been found in OA cartilage cells, and it might be associated with the pathogenesis of OA [14]. But, little is known about the function of lncRNA RP11-445H22.4 in OA and its potential targets for therapy.

In this study, we aimed to investigate the effect of lncRNA RP11-445H22.4 on lipopolysaccharide (LPS)-induced cell growth and inflammation injury of ATDC5 cells and the potential mechanism. Cell viability and apoptosis were examined by Cell Counting Kit-8 (CCK-8) and flow cytometry. The relative expressions of apoptosis-associated factors and different inflammation cytokines were detected by western blot and enzyme-linked immunosorbent assay (ELISA). Dual-luciferase reporter assays were performed to validate the function of the predicted binding sites of lncRNA RP11-445H22.4 and miR-301a. The signal pathways of nuclear factor (NF)- κ B and mitogen-activated protein kinase (MAPK)/extracellular regulated protein kinases (ERK) were analyzed by western blot. This current study will provide a theoretical basis for further study concerning OA diagnosis and treatment.

Materials and Methods

Cell culture and treatment

Human cartilage ATDC5 cells were purchased from the American Type Culture Collection (ATCC, Manassas, VA) and cultured in complete medium of Roswell Park Memorial Institute (RPMI)-1640 (Invitrogen, Grand Island, NY) supplemented with 10% fetal bovine serum (FBS, Gibco, Carlsbad, CA) in a humidified incubator containing 5% CO₂ at 37°C. All cells were maintained in growth medium in a 75 cm₂ flask. Fresh medium was added to cells every three days until confluence was achieved. Cells were treated with LPS at different concentrations of 0, 1, 5 and 10 μ g/ml for 12 h. Non-treated cells were taken as control group.

Cell transfection

To analyze the effects of lncRNA RP11-445H22.4 and C-X-C chemokine receptor type 4(CXCR4), the full-length of RP11-445H22.4 and CXCR4 sequences and short-hairpin RNA (shRNA) directed against RP11-

445H22.4 and CXCR4 were constructed in pEX-2 and U6/GFP/Neo plasmids (GenePharma, Shanghai, China), respectively. They were referred as to pEX-RP11-445H22.4, pEX-CXCR4, sh-RP11-445H22.4 and sh-CXCR4. To analyze the effects of miR-301a, miR-301a mimic, miR-301a inhibitor and their respective controls were synthesized (Life Technologies Corporation, MD, USA) and transfected into ATDC5 cells in the study. All cell transfections were performed by using Lipofectamine 3000 reagent (Life Technologies Corporation) according to the manufacturer's instructions.

Cell viability

The ATDC5 cells of logarithmic growth phase were seeded in 96-well plate with a density of 1×10^4 cells/well, and the transfection was carried out when the cell fusion reached to 90%. Then after transfection for 48 h, the 10 μ l of CCK-8 (Dojindo Molecular Technologies, Gaithersburg, MD) solution was added to the culture medium, and incubated for 4 h in humidified atmosphere with 5% CO₂ at 37°C. The optical density at 450 nm was analyzed by using a Microplate Reader (Bio-Rad, Hercules, CA).

Apoptosis assay

Cell apoptosis analysis was examined by Annexin V Apoptosis Detection Kit I (Univ-bio, Shanghai, China) according to the manufacturer's manual. In brief, ATDC5 cells (1×10^5) were washed twice with phosphate buffer saline (PBS), and then 10 μ l Annexin V-PE (20 μ g/ml) was added to the solution, and all cells were incubated in the dark for 30 min at room temperature. Subsequently, 100 μ l Binding Buffer was added and the apoptotic cells were measured using a BD FACSCanto II flow cytometer (BD Biosciences, San Jose, CA).

Real-Time quantitative PCR (RT-qPCR)

Total RNA of ATDC5 cells were isolated by using Trizol reagent (Life Technologies Corporation) according to the manufacturer's instructions. Synthetic of complementary DNA (cDNA) was performed by Transcriptor First Strand cDNA Synthesis Kit (Roche, USA). Real-Time PCR was conducted by using One Step SYBR® PrimeScript®PLUS RT-RNA PCR Kit (TaKaRa Biotechnology, Dalian, China). These data were normalized to GAPDH or U6 snRNA expression, and were tested by using the $2^{-\Delta\Delta Ct}$ method.

Luciferase reporter assay

lncRNA RP11-445H22.4 and 3'-untranslated region (3'-UTR) of CXCR4 were amplified by PCR and then cloned into the pmir-GLO dual-luciferase miRNA Target Expression Vector (Promega, Madison, WI, USA). Then the vectors were co-transfected with miR-301a mimic or mimic control into cells using Lipofectamine 3000 reagent (Life Technologies Corporation). After transfection for 48 h, the Dual-Luciferase reporter assay system (Promega, Madison, WI, USA) was used for testing the luciferase activity.

ELISA

Culture supernatant was collected from 24-well plates and concentrations of inflammatory cytokines of interleukin-1 beta (IL-1 β), interleukin-6 (IL-6), interleukin-8 (IL-8), and tumor necrosis factor-alpha (TNF- α) measured by ELISA using protocols supplied by the manufacturer (R&D Systems, Abingdon, UK) and normalized to cell protein concentrations.

Western blot

Cellular protein was extracted using radio immunoprecipitation assay (RIPA) lysis buffer (Beyotime Biotechnology, Shanghai, China) supplemented with protease inhibitors (Roche, Basle, Switzerland). The protein concentration was determined by using the BCA™ Protein Assay Kit (Pierce, Appleton, WI, USA). Protein samples were separated by the sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and transferred to nitrocellulose membranes (Maibio, Shanghai, China). After blocking with 5% nonfat milk for 1 h at room temperature, the membranes were incubated with specific primary antibodies against B-cell lymphoma 2 (Bcl-2; ab32124), Bcl-2-associated X (Bax, ab32503), pro-caspase-3 (ab32150), cleaved caspase-3 (ab13585), pro-caspase-9 (ab32068), CXCR4 (ab1670), MARK (ab60202), c-Jun N-terminal kinase (JNK, ab208035), phosphorylated JNK (p-JNK, ab76572), c-Jun (ab119944), p-c-Jun (ab81319), p-p65 (ab86299), p65 (ab16502), p-IkBa (ab92700), IkBa (ab32518) GAPDH (ab8245, Abcam, Cambridge, UK) and p-MARK (#4836), cleaved caspase-9 (#9501, Cell signaling Technology) (dilution 1:10, 00; Santa

Cruz Biotechnology, Santa Cruz, CA, USA) at 4°C overnight, followed by wash and incubation with secondary antibody marked by horseradish peroxidase for 1 h at room temperature. The signals were captured by using Enhanced chemiluminescence (ECL) reagent (GE Healthcare, Little Chalfont, UK).

Statistical analysis

All experiments were repeated three times. The results of multiple experiments are presented as the mean $\pm$ standard deviation (SD). Statistical analyses were performed using Graphpad 6.0 statistical software (GraphPad, San Diego, California, USA). The *P*-values were calculated using a one-way analysis of variance (ANOVA). A *P*-value of < 0.05 was considered to indicate a statistically significant result.

Results

LPS reduced cell viability, induced apoptosis and inflammatory response in ATGC5 cells

To investigate the effect of LPS on OA, ATGC5 cells were treated with different concentrations of LPS (0, 1, 5 and 10 $\mu\text{g/ml}$) for 12 h. Then cell viability and apoptosis were analyzed by CCK-8 and flow cytometry. As shown in Fig. 1A and 1B, LPS stimulation significantly suppressed cell viability and promoted apoptosis at 5 $\mu\text{g/ml}$ and 10 $\mu\text{g/ml}$ of LPS ($P < 0.01$ or $P < 0.001$). Western blot results displayed that LPS induced the up-regulation of Bax, cleaved-caspase-3 and cleaved-caspase-9 but down-regulation of Bcl-2. The expressions levels of pro-caspase-3 and pro-caspase-9 were not induced by LPS (Fig. 1C).

Next, RT-qPCR and ELISA were used to detect the different inflammatory cytokines expressions and secretions. RT-qPCR results showed that LPS enhanced IL-1 β , IL-6, IL-8 and TNF- α expression compared with control group (Fig. 1D, $P < 0.05$). ELISA results showed consistent result that LPS remarkably stimulated release IL-1 β , IL-6, IL-8 and TNF- α in ATGC5 cells (Fig. 1E, $P < 0.01$ or $P < 0.001$). Taken together, these data indicated that LPS could reduce cell viability, increase apoptosis and stimulate release of different inflammatory cytokines in ATDC5 cells.

Fig. 1. LPS caused ATGC5 cells injuries by decreasing cell viability, increasing apoptosis and inducing inflammatory cytokines secretion. ATGC5 cells were treated by 0, 1, 5, and 10 $\mu\text{g/ml}$ LPS for 12 h, and then (A) cell viability (B) cell apoptosis (C) apoptosis-related factor (D) mRNAs of IL-1 β , IL-6, IL-8 and TNF- α and (E) the concentrations of IL-1 β , IL-6, IL-8 and TNF- α were detected by CCK-8, flow cytometry, western blot, RT-qPCR and ELISA. LPS: lipopolysaccharide; IL-1 β : interleukin-1 beta; IL-6: interleukin-6; IL-8: interleukin-8; TNF- α : tumor necrosis factor-alpha; CCK-8: Cell Counting Kit-8; RT-qPCR: real-time quantitative PCR; ELISA: enzyme linked immunosorbent assay; *, $P < 0.05$, **, $P < 0.01$, ***, $P < 0.001$.

Fig. 2. LncRNA RP11-445H22.4 inhibition rescued LPS-induced cell injuries in ATGC5 cells. ATGC5 cells were transfected with pEX-RP11-445H22.4, sh-RP11-445H22.4 and corresponding controls. After stimulation by 5 μ g/ml LPS, (A) relative expression of RP11-445H22.4 in LPS-induced cells and (B) relative expression of RP11-445H22.4 in RP11-445H22.4 overexpressed or suppressed cells were examined by RT-qPCR; (C) cell viability (D) cell apoptosis (E) protein levels of apoptosis-related factors (F) mRNA of inflammatory cytokines (G) the concentration of inflammatory cytokines were detected by CCK-8, flow cytometry, western blot RT-qPCR, and ELISA, respectively. LPS: lipopolysaccharide; lncRNA: long non-coding RNA; RT-qPCR: real-time quantitative PCR; CCK-8: Cell Counting Kit-8; ELISA: enzyme linked immunosorbent assay; *, $P < 0.05$, **, $P < 0.01$.

Fig. 3. LncRNA RP11-445H22.4 acted as a molecular sponge of miR-301a. ATGC5 cells were transfected with pEX-RP11-445H22.4 and sh-RP11-445H22.4. (A) The expression level of RP11-445H22.4 was determined by RT-qPCR; (B) The activity of a luciferase reporter fused to RP11-445H22.4-Wt and RP11-445H22.4-Mt were examined by dual-luciferase assay. MiR-301a: microRNA-301a; lncRNA: long non-coding RNA; sh-RP11-445H22.4: short hairpin-RP11-445H22.4; RT-qPCR: real-time quantitative PCR; Wt: Wild-type; Mt: Mutated-type; *, $P < 0.05$.

LncRNA RP11-445H22.4 inhibition rescued LPS-induced cell injuries by promoting cell viability, suppressing apoptosis and inflammatory response

To explore the effect of LncRNA RP11-445H22.4 on LPS-induced cell injuries in OA, ATGC5 cells were transfected with pEX-RP11-445H22.4 and sh-RP11-445H22.4 to overexpress or suppress RP11-445H22.4 expression. Results showed that LPS significantly promoted RP11-445H22.4 expression (Fig. 2A, $P < 0.01$). Relative expression of RP11-445H22.4 was up-regulated by pEX-RP11-445H22.4 while down-regulated by sh-RP11-445H22.4 (Fig. 2B, $P < 0.01$). Cellular functional

results showed that RP11-445H22.4 inhibition enhanced cell viability, reduced apoptosis after treatment of LPS. Consistently, RP11-445H22.4 inhibition increased Bcl-2 expression and decreased Bax, cleaved-caspase-3 and cleaved-caspase-9 expressions in LPS-induced cells (Fig. 2C-2E, $P < 0.05$). The inverse results showed by RP11-445H22.4 overexpression. In addition, RP11-445H22.4 inhibition notably suppressed the expressions and concentrations of IL-1 β , IL-6, IL-8 and TNF- α after induced by LPS ($P < 0.05$ or $P < 0.01$). In a word, inhibition of RP11-445H22.4 exhibited a protective effect on LPS-induced cell injuries in ATGC5 cells.

LncRNA RP11-445H22.4 acted as a molecular sponge of miR-301a

To explore the relationship between lncRNA RP11-445H22.4 and miR-301a, pEX-RP11-445H22.4 and sh-RP11-445H22.4 were transfected into ATGC5 cells. The results showed in Fig. 3A, miR-301a expression was obviously inhibited by RP11-445H22.4 overexpression but promoted by RP11-445H22.4 silencing compared with their corresponding controls ($P < 0.05$). To further confirmation, the luciferase reporters containing RNA RP11-445H22.4 was constructed, which contains Wild-type (Wt) and Mutated-type (Mt) miR-301a binding sites for target investigation. Results showed that the binding RNA RP11-445H22.4-Wt construct suppressed miR-301a overexpression, while RNA RP11-445H22.4-Mt was no distinctly change, indicating that miR-301a directly bound to RP11-445H22.4 (Fig. 3B, $P < 0.05$). These data suggested that lncRNA RP11-445H22.4 acted as a molecular sponge of miR-301a and negatively regulated miR-301 expression.

Fig. 4. MiR-301a suppression abolished the protective functions of RP11-445H22.4 silencing on LPS-treated ATGC5 cells. MiR-301a mimic, miR-301a inhibitor, co-transfected with sh-RP11-445H22.4 and miR-301a inhibitor and corresponding controls were transfected into ATGC5 cells. (A) The expression level of miR-301a was detected by RT-qPCR. After stimulation with 5 μ g/ml LPS, (B) cell viability (C) cell apoptosis (D) protein levels of apoptosis-related factors (E) the mRNA of inflammatory cytokines (F) the concentration of inflammatory cytokines were detected by CCK-8, flow cytometry, western blot, RT-qPCR and ELISA, respectively. LncRNA: long non-coding RNA; miR-301a: microRNA-301a; LPS: lipopolysaccharide; RT-qPCR: real-time quantitative PCR; CCK-8: Cell Counting Kit-8; ELISA: enzyme linked immunosorbent assay; *, $P < 0.05$, **, $P < 0.01$, ***, $P < 0.001$.

MiR-301a suppression abolished the protective functions of RP11-445H22.4 silencing on LPS-treated ATGC5 cells

Further, we investigated whether miR-301a was involved in lncRNA RP11-445H22.4 regulated LPS-induced cell injuries in ATGC5 cells. To verify this suppose, ATGC5 cells were transfected with miR-301a mimic, inhibitor, sh-RP11-445H22.4 and corresponding controls. As demonstrated in Fig. 4A, the expression of miR-301a was visibly increased by miR-301a mimic, while decreased by miR-301a inhibitor ($P < 0.01$). Co-transfected with sh-RP11-445H22.4 and miR-301a inhibitor suppressed LPS-induced cell viability, meanwhile promoted apoptosis compared with sh-RP11-445H22.4 group (Fig. 4B and 4C, $P < 0.05$ or $P < 0.01$). Western blot results showed that co-transfected with sh-RP11-445H22.4 and miR-301a inhibitor down-regulated Bcl-2, up-regulated Bax cleaved-cadpase-3 and cleaved-cadpase-9 expressions (Fig. 4D). In terms of inflammatory cytokines, the expressions and concentrations of IL-1 β , IL-6, IL-8 and TNF- α were significantly increased by sh-RP11-445H22.4 together with miR-301a inhibitor compared with its control (Fig. 4E and 4F, $P < 0.05$, $P < 0.01$ or $P < 0.001$). In sum, these data indicated that miR-301a suppression abolished the protective functions of RP11-445H22.4 silencing on LPS-treated ATGC5 cells.

CXCR4 was a target of miR-301a

In ATGC5 cells with overexpression of miR-301a, we found a pronounced reduction in CXCR4 mRNA and protein expressions compared with their control. These opposite results explored by miR-301a suppression (Fig. 5A, $P < 0.05$ or $P < 0.01$). Moreover, miR-301a overexpression markedly decreased the activity of a luciferase reporter fused to CXCR4-Wt, while not the activity of a reporter binding to CXCR4-Mt (Fig. 5B, $P < 0.05$). Taken together, all results indicated that CXCR4 was a direct target of miR-301a, and was negatively regulated by miR-301a.

CXCR4 silencing promoted cell viability, inhibited apoptosis and inflammatory response in LPS-treated ATGC5 cells

To further clarify the effect of CXCR4 on OA, the stable pEX-CXCR4 and sh-CXCR4 ATGC5 cells were established. As displayed in Fig. 6A, CXCR4 overexpression prominently elevated CXCR4 expression whereas CXCR4 silencing reduced CXCR4 expression ($P < 0.01$). Western blot showed consistent changes in CXCR4 protein. Similar to RP11-445H22.4 inhibition, we found that CXCR4 silencing significantly promoted cell viability and inhibited apoptosis in ATGC5 cells (Fig. 6B and 6C, $P < 0.05$). Moreover, CXCR4 silencing remarkably up-regulated Bcl-2, down-regulated Bax, cleaved-cadpase-3 and cleaved-cadpase-9 expressions in LPS-

Fig. 5. CXCR4 was a target of miR-301a. ATGC5 cells were transfected with miR-301a mimic, miR-301a inhibitor and mimic or inhibitor controls. (A) The mRNA and protein levels of CXCR4 were measured by RT-qPCR and western blot; (B) The activity of a luciferase reporter fused to CXCR4-Wt and CXCR4-Mt were examined by dual-luciferase assay. CXCR4: C-X-C chemokine receptor type 4; miR-301a: microRNA-301a; RT-qPCR: real-time quantitative PCR; Wt: Wild-type; Mt: Mutated-type; *, $P < 0.05$, **, $P < 0.01$.

Fig. 6. CXCR4 silencing protected ATGC5 cells against LPS-induced injuries in ATGC5 cells. ATGC5 cells were transfected with pEX-CXCR4 and sh-CXCR4. (A) The mRNA and protein levels of CXCR4 were determined by RT-qPCR. After induction by 5 μ g/ml of LPS, (B) cell viability (C) cell apoptosis (D) protein levels of apoptosis-related factors (E) the mRNA of inflammatory cytokines (F) the concentration of inflammatory cytokines were detected by CCK-8, flow cytometry, western blot, RT-qPCR and ELISA, respectively. CXCR4: C-X-C chemokine receptor type 4; RT-qPCR: real-time quantitative PCR; LPS: lipopolysaccharide; CCK-8: Cell Counting Kit-8; ELISA: enzyme linked immunosorbent assay; *, $P < 0.05$, **, $P < 0.01$.

Fig. 7. CXCR4 silencing blocked LPS-activated NF- κ B and MAPK/ERK pathways. ATGC5 cells were transfected with pEX-CXCR4, sh-CXCR4 and their controls. After 5 μ g/ml of LPS stimulation, (A) the MAPK/ERK pathway factors protein levels and (B) the NF- κ B pathway factors protein levels were measured by western blot. GAPDH was used as an internal reference. CXCR4: C-X-C chemokine receptor type 4; LPS: lipopolysaccharide; NF- κ B: nuclear factor- κ B; MAPK: mitogen-activated protein kinase; ERK: extracellular regulated protein kinases.

induced cells (Fig. 6D). Simultaneously, the mRNA levels of IL-1 β , IL-6, IL-8 and TNF- α were inhibited by CXCR4 silencing (Fig. 6E, $P < 0.05$). The concentrates of these four inflammatory cytokines were also increased by CXCR4 overexpression (Fig. 6F, $P < 0.05$, or $P < 0.01$). The contrary results were confirmed by CXCR4 overexpression. In a word, CXCR4 silencing could promote cell viability, inhibit apoptosis and inflammatory response in LPS-treated ATGC5 cells.

CXCR4 silencing blocked LPS-activated NF- κ B and MAPK/ERK pathways

Several studies confirmed that CXCR4 regulated intracellular signaling through some divergent pathways, including NF- κ B, MAPK/ERK pathways [15, 16]. Based on the pathway analysis results, we examined the effect of CXCR4 on NF- κ B and MAPK/ERK pathways in ATGC5 cells. Cells were transfected with pEX-CXCR4, sh-CXCR4 and their controls, and the main protein levels of NF- κ B and MAPK/ERK pathways were detected by western blot. As results showed in Fig. 7A and 7B, the protein levels of p-MARK, p-JNK, p-c-Jun, p-I κ B α and p-p65 were significantly blocked by CXCR4 silencing. However, CXCR4 overexpression activated these five factors expressions. The expressions of MARK, JNK, c-Jun, I κ B α and p65 have no obviously changes. Thus, these data indicated that CXCR4 silencing blocked LPS-activated NF- κ B and MAPK/ERK pathways in ATGC5 cells.

Discussion

In present study, we found that LPS reduced cell viability, increased apoptosis and stimulated release IL-1 β , IL-6, IL-8 and TNF- α . However, lncRNA RP11-445H22.4 inhibition significantly recued LPS-induced cell injuries by promoting cell viability, suppressing apoptosis and inflammatory cytokines secretions in ATDC5 cells. In addition, miR-301a directly bound to RP11-445H22.4, and suppression of miR-301a inversed the effects of RP11-445H22.4 inhibition. Furthermore, we confirmed that CXCR4 was a direct target of miR-301a, and CXCR4 silencing exhibited the similarly protective effects of RP11-445H22.4 inhibition on LPS-induced cell injuries in ATDC5 cells. Besides, we found that CXCR4 silencing blocked LPS-activated NF- κ B and MAPK/ERK pathways.

As is known to all, LPS plays an important role in bacterial infection and disease evolution [17]. Moreover, LPS acts as endotoxin involving in induction of strong inflammatory responses [18, 19]. As Li et al. [20] reported that LPS induced gastric cancer cell proliferation, inhibited apoptosis and increased the secretion of IL-1 β , IL-6, IL-12 and TNF- α . Furthermore, Wang et al. [21] confirmed that LPS could induce pro-inflammatory cytokines of IL-1 β , IL-6, IL-8 and TNF- α productions in macrophage. Similar with previous research, our study showed that LPS significantly decreased cell viability, and increased apoptosis and induced IL-1 β , IL-6, IL-8 and TNF- α secretion. Overall, these data indicated that LPS could cause cell injuries in ATDC5 cells.

Several studies demonstrated that abnormal expression of lncRNA RP11-445H22.4 play an important role in biological processes and might be involved in variety kinds of cancers or diseases [22]. As Steck et al. [23] revealed that lncRNA H19 acted as a key regulator in chondrocyte metabolism process in OA. Xing et al. [14] reported that lncRNA RP11-445H22.4 was up-regulated in OA compared with normal cartilage tissues. Based on these previous studies, we further explored the effects of lncRNA RP11-445H22.4 on LPS-induced cell injuries in OA. Our results demonstrated that lncRNA RP11-445H22.4 inhibition as a protector rescued LPS-induced cell injuries in ATGC5 cells, as promoted cell viability, inhibited apoptosis and reduced inflammatory cytokines secretions.

Recently, miRNAs have been displayed in various research fields to have an important regulatory role [24, 25]. In terms of miR-301a, abnormal expression of miR-301a has been reported in various human diseases, such as breast cancer, hepatocellular carcinoma (HCC) and colorectal cancer (CRC) [26]. Moreover, accumulating evidences indicate that miR-301a has significant influence in diverse range of cellular processes, including cell proliferation, apoptosis, metastasis and immune response [27, 28]. As Liang et al. [29] displayed that miR-301a promoted cell proliferation and reduced apoptosis in multiple myeloma (MM). Furthermore, Huang et al. [28] demonstrated that down-regulated of miR-301a could inhibit pro-inflammatory cytokines in toll-like receptor-triggered macrophages. However, whether miR-301a was involved in LPS-induced cell injuries in OA remain not investigated. In present study, we confirmed that lncRNA RP11-445H22.4 acted as a molecular sponge of miR-301a, and miR-301a suppression significantly abolished the protective functions of

RP11-445H22.4 inhibition against LPS-induced injuries. Above all, these data suggested that lncRNA RP11-445H22.4 inhibition alleviated LPS-induced cell injuries by regulation of miR-301a in ATGC5 cells.

To further explore the possible effect of miR-301a on LPS-induced cell injuries in OA, a target of miR-301a was confirmed by luciferase reporter assay. We found that CXCR4 was a direct target of miR-301a, and negatively by miR-301a. Recently, CXCR4 has been verified as a direct target of many miRNAs, such as miR-221-3p, miR-9, miR-146a and miR-126 [30-33]. Moreover, CXCR4 play an important role in cell growth and development. As Duan et al. [34] demonstrated that CXCR4 was a bona fide target of miR-133b, and CXCR4 silencing promoted cell colonies and apoptosis. Our findings were partly similar with previous study that CXCR4 was a target of miR-301a and CXCR4 as a protector in LPS-induced cell injuries in ATGC5 cells.

NF- κ B and MAPK/ERK play vital roles in the inflammatory and immune response [35-37]. In terms of OA, the activated NF- κ B molecules trigger the expression of an array of genes which induce destruction of the articular joint, and leading to OA onset and progression [38]. Moreover, inhibition of the p38-MARK signaling pathway suppressed apoptosis and expression of pro-inflammatory cytokines of IL-1, IL-6 and TNF- α in osteoarthritis chondrocytes [39]. Besides, the inhibition of ERK was involved in PLC γ 1-mediated MMP-13 expression in human OA chondrocytes, with important implication for the understanding of OA pathogenesis [40]. In our study, we explored that CXCR4 silencing blocked LPS-activated NF- κ B and MAPK/ERK pathways in ATGC5 cells, further regulating cell viability, apoptosis and inflammatory injury in OA.

Conclusion

These results demonstrated that lncRNA RP11-445H22.4 silencing acted as a protector in LPS-induced cell injuries by regulation of miR-301a in ATGC5 cells. Our findings imply that lncRNA RP11-445H22.4 might serve as a potential therapeutic target in OA.

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Disclosure Statement

There was no conflict of interests.

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