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Salidroside protects ATDC5 cells against lipopolysaccharide-induced injury through up-regulation of microRNA-145 in osteoarthritis

Meihan Liu[a], Jingzhe Zhang[b], Wanguo Liu[b], Wenjun Wang[b,*]

[a] Department of Ultrasonography, China-Japan Union Hospital of Jilin University, Changchun 130033, Jilin, China
[b] Department of Orthopedics, China-Japan Union Hospital of Jilin University, Changchun 130033, Jilin, China

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ABSTRACT

Background: Osteoarthritis (OA) is a kind of degenerative disease characterized by the degeneration of the articular cartilage. Salidroside (SAL) is an active component of *Rhodiola rosea* L., which exhibits diverse pharmacological effects in different diseases. However, the effects of SAL on OA remain largely unclear. The study aimed to investigate the roles of SAL in lipopolysaccharides (LPS)-induced inflammatory injury in murine ATDC5 chondrocyte cells.

Methods: LPS induced ATDC5 cell injury model was constructed by determining cell viability, apoptosis, apoptosis-associated factors as well as inflammatory cytokines expressions and concentrations. Then, the various concentrations of SAL were used to treat ATDC5 cells, and the effect of SAL on LPS-induce inflammatory injury was detected. After treatment with SAL, the expression level of miR-145 was measured by qRT-PCR. Subsequently, miR-145 inhibitor and corresponding control were transfected into ATDC5 cells to explore the influences of miR-145 in LPS-induce inflammatory injury. Besides, the key signaling pathways of NF-κB and p38MAPK were analyzed by using western blot.

Results: LPS inhibited cell viability, induced apoptosis, activated cleaved-caspase-3/-9 expression, as well as increased IL-6, MCP-1 and TNF-α expressions and secretions in ATDC5 cells. SAL significantly alleviated LPS-induced inflammatory injury. Meanwhile, the expression of miR-145 was up-regulated by SAL. The protective effect of SAL on LPS-induced injury was obviously reversed by miR-145 inhibition. Furthermore, SAL inactivated NF-κB and p38MAPK signaling pathways by regulating miR-145.

Conclusions: These findings suggested that SAL could protect ATDC5 cells against LPS-induced injury *via* up-regulation of miR-145 in ATDC5 chondrocyte cells.

1. Introduction

Osteoarthritis (OA) is a degenerative joint disease, which can lead to the progressive loss of articular cartilage [1,2]. This disease is usually associated with diverse factors, such as age, heredity, trauma and imbalance of physiological processes induced by inflammatory cytokines [3,4]. Under the normal circumstances, the anabolism and catabolism of articular cartilage always maintain a dynamic balance [5]. In OA, the balance between chondrocyte synthesis and catabolism is disturbed, resulting in the loss of the cartilage matrix and cartilage degeneration and exfoliation [6,7]. For the present guidelines from American College of Rheumatology (ACR) 2012 and 2014 OA Research Society International (OARSI), the recommended treatments for OA include oral and topical non-steroidal anti-inflammatory drugs (NSAIDs), biomechanical interventions, corticosteroid injection, exercise, power training and weight management [8,9]. Drug conservative treatment is usually used in the early stage of OA. Recent studies demonstrate that NSAIDs and cyclooxygenase-2 (COX-2) inhibitor are preferred treatments for symptomatic relief of OA [10,11]. Additionally, VA694, Naproxcinod and Naproxen have been reported to exert anti-inflammatory and chondroprotective effects on OA chondrocytes [12]. However, the joint replacement surgery has to be carried out when the drug treatment is not effective [13]. Therefore, to find novel and effective drugs for the treatment of OA are extremely needed.

Salidroside (SAL, chemical formation: $C_{14}H_{20}O_7$, molecular weight: 300.12) is a mainly biologically active compound of *Rhodiola rosea* L., which originates from the root and rhizome of *Rhodiola rosea* L. [14]. The extensive pharmacologic effects of SAL have been reported in different diseases, including Alzheimer's disease [15], cerebrovascular diseases [16] and various cancers [17,18]. In terms of bone diseases,

* Corresponding author at: Department of Orthopedics, China-Japan Union Hospital of Jilin University, No.126, Xiantai Street, Changchun 130033, Jilin, China.
E-mail address: wangwenjun0053@sina.com (W. Wang).

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recent study revealed that SAL could alleviate bone loss through inhibiting the bone-resorbing mediators release and oxidative stress [19]. Moreover, SAL could promote osteoblast differentiation and mediate bone metabolism by regulation of the bone morphogenetic protein (BMP) signaling pathway [20]. Furthermore, SAL could accelerate fracture healing *via* cell-autonomous and non-autonomous effects on osteoblasts [21]. Whereas, there is no evidence fully explained whether SAL affects OA.

It is generally known that lipopolysaccharide (LPS) is closely associated with inflammatory diseases [22]. In many OA studies, LPS has been widely used to construct cell injury model *in vitro* [23,24]. In this study, the different concentrations of LPS were used to treat murine ATDC5 chondrocyte cells to construct an inflammatory injury model. Additionally, the effect of SAL on LPS-induced inflammatory injury was investigated by testing cell viability, apoptosis, apoptosis-associated factors and inflammatory cytokines expressions in ATDC5 cells. The crucial effect of miRNAs on OA has confirmed in recent researches [25,26]. It has been reported that miRNAs act as key regulators to control cartilage homeostasis, catabolism and repair [27]. As an important miRNA, miR-145 has been studied in various cancers [28,29]. However, the role of miR-145 in OA remains not fully investigated. Therefore, miR-145 inhibitor and negative control (NC) were transfected into ATDC5 cells to explore the effect of miR-145 on LPS-induce inflammatory injury in OA. Finally, the key signaling pathways of nuclear factor kappa B (NF-κB) and p38 mitogen-activated protein kinase (p38MAPK) were analyzed. The study will exhibit a novel strategy for the treatment of OA.

2. Materials and methods

2.1. Cell culture

The murine ATDC5 chondrocyte cell line was purchased from the RIKEN cell bank (RIKEN Bioresource Center, Ibaraki, Japan). Cells were cultured in the complete medium of Roswell Park Memorial Institute (RPMI)-1640 (Thermo Fisher Scientific, Waltham, USA) containing 10% fetal bovine serum (FBS, Invitrogen, Carlsbad, CA, USA) in a humidified incubator with 5% CO_2 at 37 °C. All these ATDC5 cells were used between the fifth and tenth passages after thawing, and were maintained in growth medium in a 75 cm^2 flask. The culture medium was changed every 3 days until confluence was achieved 80–90%.

2.2. Cell treatment

The different concentrations of LPS (0, 2, 4, 8 and 10 μg/ml, Sigma, St. Louis, MO, USA) was used to treat ATDC5 cells for 12 h. SAL (purity > 98%) was purchased from Sigma (SMB00072), which was configured to different concentration solutions (0, 0.5, 1, 1.5 and 2 μM). Then, ATDC5 cells were pre-treated with these different concentrations of SAL for 1 h before stimulation of LPS.

2.3. Cell viability assay

The viability of ATDC5 cells was examined by Cell Counting Kit-8 (CCK-8, Dojindo Molecular Technologies, Gaithersburg, MD, USA) assay according to the instructions of manufacturer. Briefly, ATDC5 cells (5×10^3 cells/well) were seeded in 96-well plate. After treatment of LPS and SAL, 10 μl CCK-8 solution was added to the culture medium, and were incubated for another 2 h at 37 °C in humidified 95% air and 5% CO_2. The absorbance was measured at 450 nm using a Microplate Reader (Bio-Rad, Hercules, CA, USA).

2.4. Apoptosis assay

The percentage of ATDC5 apoptotic cells was analyzed by using Annexin V-Phycoerythrin (PE) apoptosis detection kit (Beijing Biosea Biotechnology, Beijing, China). In brief, ATDC5 cells were seeded in 6 well-plates, and were treated with SAL and LPS for 12 h. These treated cells were then washed twice with cold PBS and re-suspended in buffer. After this, 10 μl Annexin V-PE was added into the suspensions for staining cells for 15 min at room temperature in the dark. Finally, the stained cells were subjected into a FACS can (Beckman Coulter, Fullerton, CA, USA) for flow cytometry analysis. A total of 1×10^5 cells were collected for each sample for analysis, and the data were analyzed by the FlowJo software (Treestar, Ashland, OR, USA).

2.5. Enzyme-linked immunosorbent assay (ELISA)

ATDC5 cells were cultured in 24-well plates at the conventional culture conditions. After treatment with SAL and LPS, culture supernatant was collected, as well as the concentrations of inflammatory cytokines of interleukin-6 (IL-6), monocyte chemoattractant protein-1 (MCP-1) and tumor necrosis factor-α (TNF-α) were measured by ELISA kit (R&D Systems, Abingdon, UK) according to the protocols supplied by the manufacturer.

2.6. MiRNAs transfection

The expression plasmids of miR-145 inhibitor and its control (NC) were synthesized by GenePharma Co. (Shanghai, China). In brief, ATDC5 cells were grown in 96-well plate, and incubated for 24 h. Subsequently, miR-145 inhibitor and NC were transfected into ATDC5 cells by using Lipofectamine 3000 reagent (Invitrogen) following the manufacturer's instruction.

2.7. Quantitative real-time polymerase chain reaction (qRT-PCR)

The cellular total RNA was isolated from cells by using TRIzol reagent (Invitrogen) according to the manufacturer's protocol. Reverse transcription was performed by using Transcriptor First Strand cDNA Synthesis Kit (Roche, USA). qRT-PCR was performed by using One Step SYBR® PrimeScript®PLUS RT-RNA PCR Kit (TaKaRa Biotechnology, Dalian, China). Relative expression levels were calculated by using the $2^{-\Delta\Delta Ct}$ method [30].

2.8. Western blot assay

After treatment, ATDC5 cells were washed with PBS and lysed in RIPA buffer (Beyotime Biotechnology, Shanghai, China). The concentration of the protein was detected by Bicinchoninic Acid (BCA) kit (Pierce, Appleton, WI, USA). The western blot system was established using a Bio-Rad Bis-Tris Gel system according to the manufacturer's instructions. Protein samples were subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), and transferred to polyvinylidenedifluoride (PVDF) membranes (Millipore, Billerica, MA, USA). Afterward, these membranes were incubated with primary antibodies of pro-caspase-3 (ab32150, 1:1000), cleaved-caspase-3 (ab32042, 1:500), pro-caspase-9 (ab135544, 1:500), cleaved-caspase-9 (ab2324, 1:1000), IL-6 (ab6672, 1:500), MCP-1 (ab151538, 1:5000), TNF-α (ab9739,1:1000), phosphorylated (p)-p65 (ab28856, 1:1000), t-p65 (ab16502, 1:1000), p-IκBα (ab133462, 1:10000), IκBα (ab178846, 1:500), p-p38MAPK (ab47363, 1:1000), t-p38MAPK (ab170099, 1:1000) and β-actin (ab6276, 1:5000) (all from Abcam, Cambridge, UK) and at 4 °C overnight. The membranes were washed three times with PBS, and then the secondary antibody of horseradish peroxidase (HRP)-conjugated goat anti-rabbit IgG (ab205718, 1:2000, Abcam) was added and incubated for another 1 h at room temperature. The signals were exposed by ECL reagents (MultiSciences Biotech, Hangzhou, China).

2.9. Statistical analysis

The data of multiple experiments are presented as the mean ±

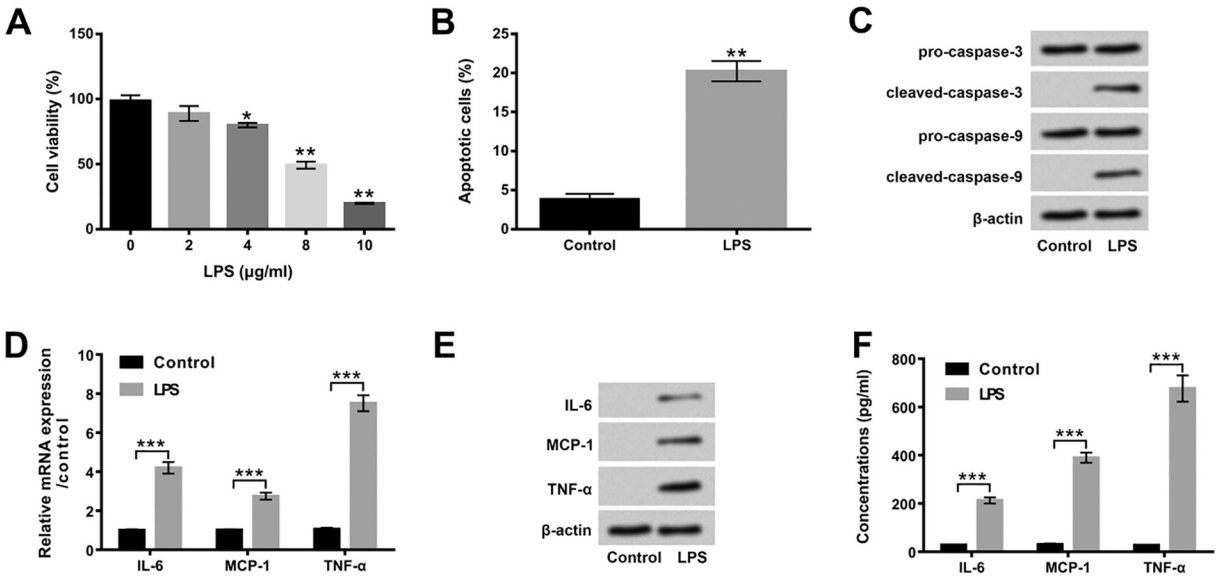

Fig. 1. LPS induced ATDC5 cells inflammatory injury.
(A) The different concentrations of LPS (0, 2, 4, 8 and 10 μg/ml) were used to treat ATDC5 cells, and cell viability was examined by CCK-8. After this, 8 μg/ml of LPS was selected as the appropriate concentration for the following experiments. (B) Cell apoptosis and (C) the protein levels of cleaved-caspase-3/-9 were analyzed by flow cytometry and western blot; (D) The mRNA expression levels, (E) the protein levels and (F) the concentrations of inflammatory cytokines (IL-6, MCP-1 and TNF-α) were detected by qRT-PCR, western blot and ELISA assays.
LPS: lipopolysaccharide; CCK-8: Cell Counting Kit-8; IL-6: interleukin-6; MCP-1: monocyte chemoattractant protein-1; TNF-α: tumor necrosis factor-α; qRT-PCR: quantitative reverse transcription PCR; ELISA: enzyme-linked immunosorbent assay; $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$.

standard deviation (SD). SPSS 19.0 statistical software (SPSS, Inc., Chicago, IL, USA) was used to calculate the statistical analyses. The *P*-values were calculated using one-way or two-way analysis of variance (ANOVA). A *P*-value of < 0.05 was considered to indicate a statistically significant result. All these experiments were repeated three times.

3. Results

3.1. LPS-induced inflammatory injury model was successfully constructed

In the study, LPS was used to treat ATDC5 cells to induce the inflammatory injury. To choose the appropriate concentration of LPS, ATDC5 cells were treated with a series of LPS (0, 2, 4, 8 and 10 μg/ml), and cell viability was then examined by CCK-8 assay. As shown in Fig. 1A, the viability of ATDC5 cells was significantly reduced by LPS at the concentrations of 4 μg/ml, 8 μg/ml and 10 μg/ml compared with untreated cells ($P < 0.05$ or $P < 0.01$). These data indicated that LPS inhibited cell viability at a dose-dependent manner with an IC50 value of 7.87 μg/ml. Thus, the concentration of 8 μg/ml LPS was selected as the appropriate concentration for the following experiments.

Next, cell apoptosis and apoptosis-associated factors were detected by flow cytometry and western blot. The results showed that the percentage of apoptotic cells was gradually induced by LPS compared with control group ($P < 0.01$, Fig. 1B). Likewise, the protein levels of cleaved-caspase-3 and cleaved-caspase-9 were notably activated by LPS stimulation (Fig. 1C). Furthermore, the mRNA and protein levels of interleukin IL-6, MCP-1 and TNF-α were significantly increased by LPS compared with control group ($P < 0.001$, Fig. 1D–E). Simultaneously, ELISA assay revealed that the concentrations of IL-6, MCP-1 and TNF-α were also enhanced by LPS compared with control group ($P < 0.001$, Fig. 1F). Above all, the model of LPS-induced ATDC5 cells inflammatory injury was successfully constructed.

3.2. SAL alleviated LPS-induced inflammatory injury in ATDC5 cells

To explore the effect of SAL on LPS-induced inflammatory injury in ATDC5 cells, we selected different concentrations of SAL (0, 0.5, 1, 1.5 and 2 μM) to expose ATDC5 cells. In Fig. 2A, the results displayed that cell viability was significantly declined at the concentration of 1.5 and 2 μM SAL. There was no effect of SAL on cell viability at the concentrations of 0.5 and 1 μM. Thus, we selected 1 μM of SAL to treat cells in the subsequent experiments. The results in Fig. 2B and C displayed that SAL significantly promoted cell viability and inhibited apoptosis in LPS-stimulated ATDC5 cells ($P < 0.05$). Western blot result revealed that SAL remarkably suppressed the activation of cleaved-caspase-3 and cleaved-caspase-9 (Fig. 2D). The expression levels and concentrations of IL-6, MCP-1 and TNF-α were all decreased by SAL in LPS-stimulated ATDC5 cells ($P < 0.05$, $P < 0.01$ or $P < 0.001$, Fig. 2E–G). However, SAL treatment alone had no effect on ATDC5 cell viability, apoptosis and the inflammatory factors as relative to control group (Fig. 2B–G). Taken together, all these data indicated that SAL alleviated LPS-induced inflammatory injury in ATDC5 cells.

3.3. MiR-145 was up-regulated by SAL in ATDC5 cells

To uncover the relationship between miR-145 and SAL, ATDC5 cells were treated with different doses of SAL (0, 0.5 and 1 μM), and the expression of miR-145 was examined by qRT-PCR. As shown in Fig. 3, the expression of miR-145 was significantly up-regulated by SAL at the concentrations of 0.5 and 1 μM compared with untreated cells ($P < 0.05$). The results suggested that miR-145 might be involved in regulation of LPS-induced inflammatory injury in ATDC5 cells.

3.4. SAL functioned in LPS-induced injury by regulation of miR-145

To further explore the effect of miR-145 on LPS-induced inflammatory injury, miR-145 inhibitor and its corresponding control were transfected into ATDC5 cells. After transfection, the expression level of miR-145 was significantly down-regulated by miR-145 suppression compared with NC group ($P < 0.01$, Fig. 4A). Therefore, the transfection efficiency of miR-145 inhibitor was well and could be successfully suppressed miR-145 expression. Then, the effect of miR-145 on cell viability, apoptosis and inflammatory factors were assessed. In Fig. 4B, the results showed that miR-145 suppression significantly

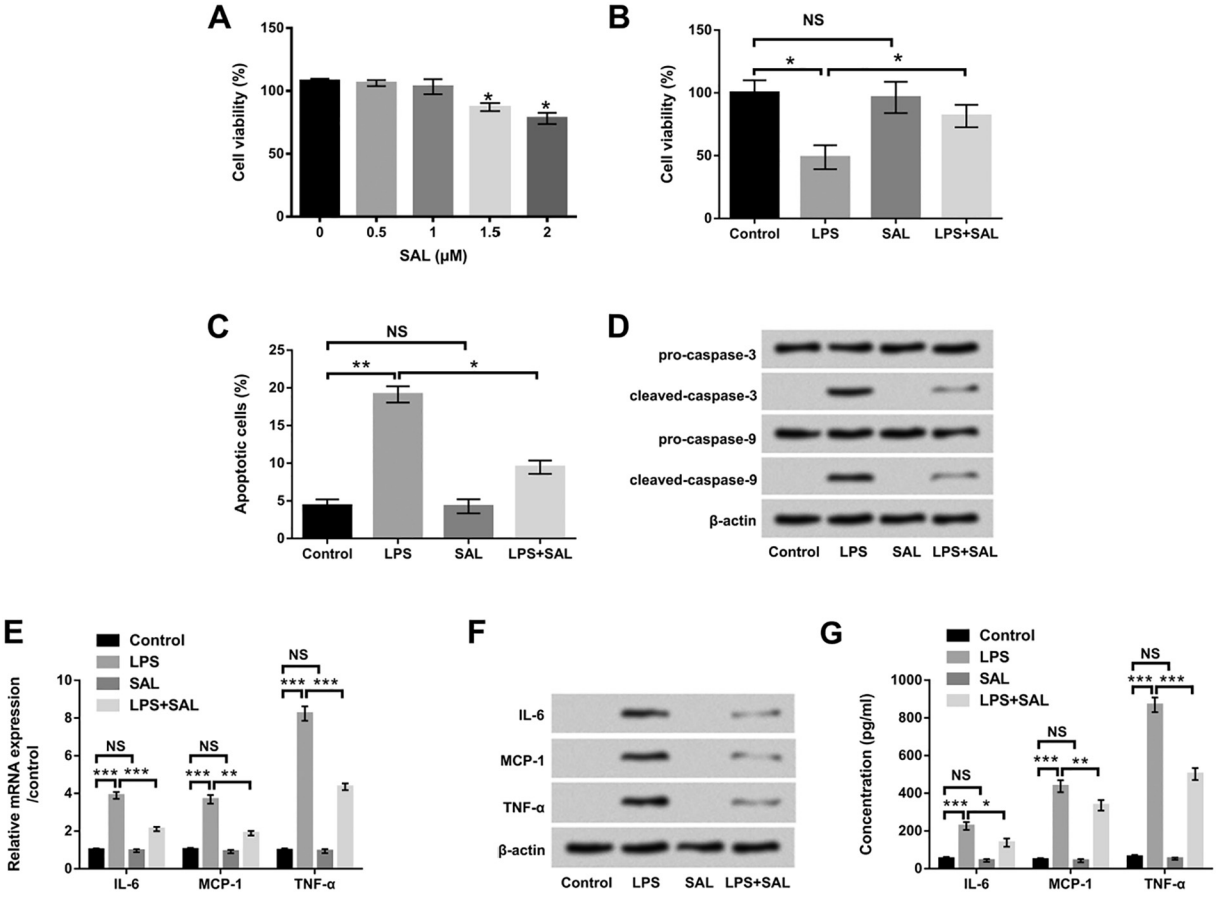

Fig. 2. SAL alleviated LPS-induced inflammatory injury in ATDC5 cells.
(A) The different doses of SAL (0, 0.5, 1, 1.5 and 2 μM) were used to treat ATDC5 cells, and cell viability was examined by CCK-8. Then, 1 μM of SAL was selected as the appropriate concentration for the subsequently experiments. (B) cell viability (B) apoptosis and (C) apoptosis-associated factors were analyzed by CCK-8, flow cytometry and western blot; (D) The mRNA expression levels, (E) the protein levels and (F) the concentrations of inflammatory cytokines (IL-6, MCP-1 and TNF-α) were detected by qRT-PCR, western blot and ELISA.
SAL: salidroside; LPS: lipopolysaccharide; CCK-8: Cell Counting Kit-8; IL-6: interleukin-6; MCP-1: monocyte chemoattractant protein-1; TNF-α: tumor necrosis factor-α; qRT-PCR: quantitative reverse transcription PCR; ELISA: enzyme-linked immunosorbent assay; *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$; NS: no significance.

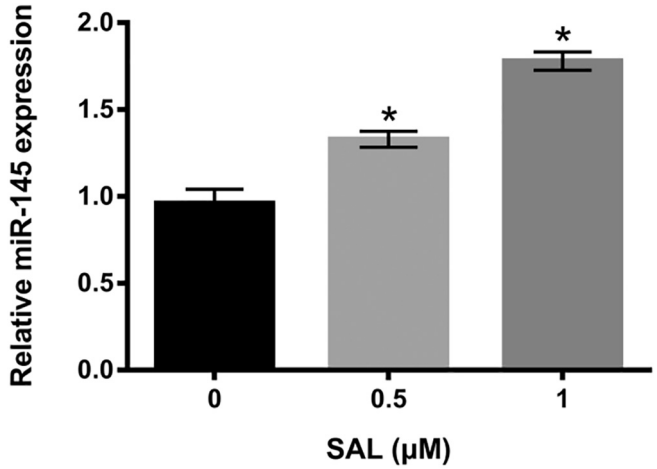

Fig. 3. MiR-145 was up-regulated by SAL in ATDC5 cells.
The different doses of SAL (0, 0.5 and 1 μM) were selected and used to stimulate ATDC5 cells, and then the expression level of miR-145 was examined by qRT-PCR.
MiR-145: microRNA-145; SAL: salidroside; qRT-PCR: quantitative reverse transcription PCR; *$P < 0.05$.

reversed the promoting effect of SAL on cell viability ($P < 0.001$). Meanwhile, the inhibitory effects of SAL on cell apoptosis and cleaved-caspase-3/-9 activation were also reversed by miR-145 suppression ($P < 0.001$, Fig. 4C and D). Further, the mRNA and protein levels of IL-6, MCP-1 and TNF-α were significantly up-regulated by miR-145 suppression in LPS and SAL treated cells ($P < 0.001$, Fig. 4E and F). The concentrations of IL-6, MCP-1 and TNF-α were also increased by miR-145 suppression in LPS and SAL treated cells ($P < 0.01$ or $P < 0.001$, Fig. 4G). In a word, these results fully indicated that SAL affected LPS-induced injury by regulation of miR-145 in ATDC5 cells.

3.5. SAL inactivated NF-κB and p38MAPK signaling pathways by regulation of miR-145

Finally, the effect of SAL and miR-145 on NF-κB and p38MAPK signaling pathways was investigated. As shown in Fig. 5A and B, the phosphorylated levels of p65 and IκBα were significantly increased by LPS stimulation ($P < 0.001$). However, the promoting effect was significantly declined by SAL ($P < 0.001$). MiR-145 suppression obviously reversed the inhibitory effect of SAL on NF-κB signaling pathway ($P < 0.01$ or $P < 0.001$). Similarly, the results in Fig. 5C and D revealed that the phosphorylated level of p38MAPK was also induced by LPS ($P < 0.05$). The LPS-induced increase was notably declined by SAL ($P < 0.05$). Furthermore, the inhibitory effect of SAL on p38MAPK signaling pathway was reversed by miR-145 suppression ($P < 0.05$).

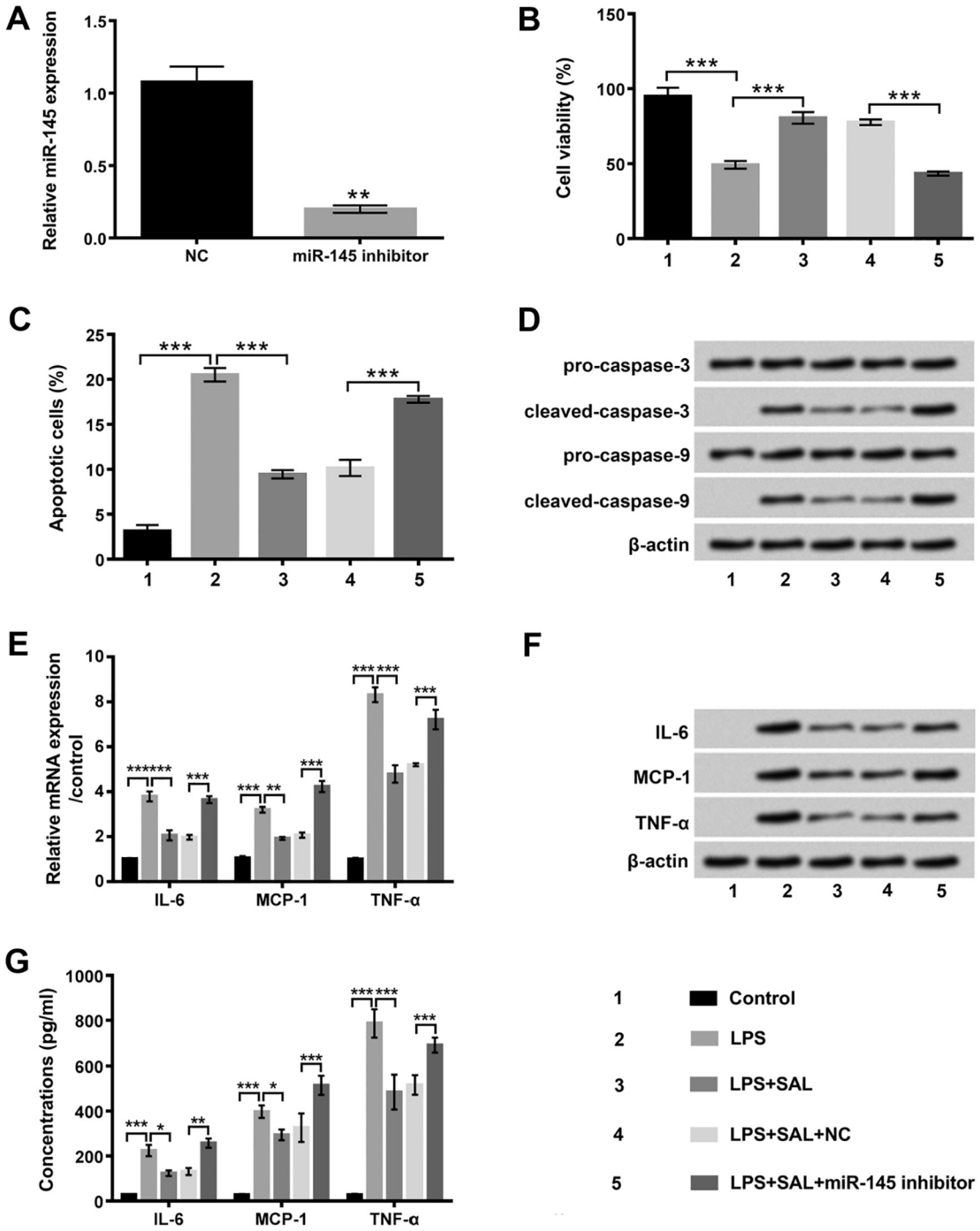

Fig. 4. SAL alleviated LPS-induced inflammatory injury by regulation of miR-145.

ATDC5 cells were transfected with miR-145 inhibitor and its corresponding control. After treatment of LPS and SAL, (A) the relative expression of miR-145 was examined by qRT-PCR; (B) cell viability (B) apoptosis and (C) apoptosis-associated factors were analyzed by CCK-8, flow cytometry and western blot; (D) The mRNA expression levels, (E) the protein levels and (F) the concentrations of inflammatory cytokines (IL-6, MCP-1 and TNF-α) were detected by qRT-PCR, western blot and ELISA.

SAL: salidroside; LPS: lipopolysaccharide; miR-145: microRNA-145; CCK-8: Cell Counting Kit-8; IL-6: interleukin-6; MCP-1: monocyte chemoattractant protein-1; TNF-α: tumor necrosis factor-α; qRT-PCR: quantitative reverse transcription PCR; ELISA: enzyme-linked immunosorbent assay; $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$.

Collectively, these data indicated that SAL inactivated NF-κB and p38MAPK signaling pathways by regulation of miR-145.

4. Discussion

OA is the most common joint disease, which seriously affects the health and quality life of elderly people [31]. It has been proved that the degeneration of articular cartilage is the most direct cause of OA, as well as prevention and control of articular cartilage degeneration is the keynote for the treatment of OA [32,33]. The conventional analgesic medications such as acetaminophen and NSAIDs are commonly used for the treatment of OA [34]. NSAIDs exerted the anti-inflammatory and analgesic effects mainly due to inhibit the ability of cyclooxygenase. Interestingly, Boer et al. performed the *in vivo* and *in vitro* experiments

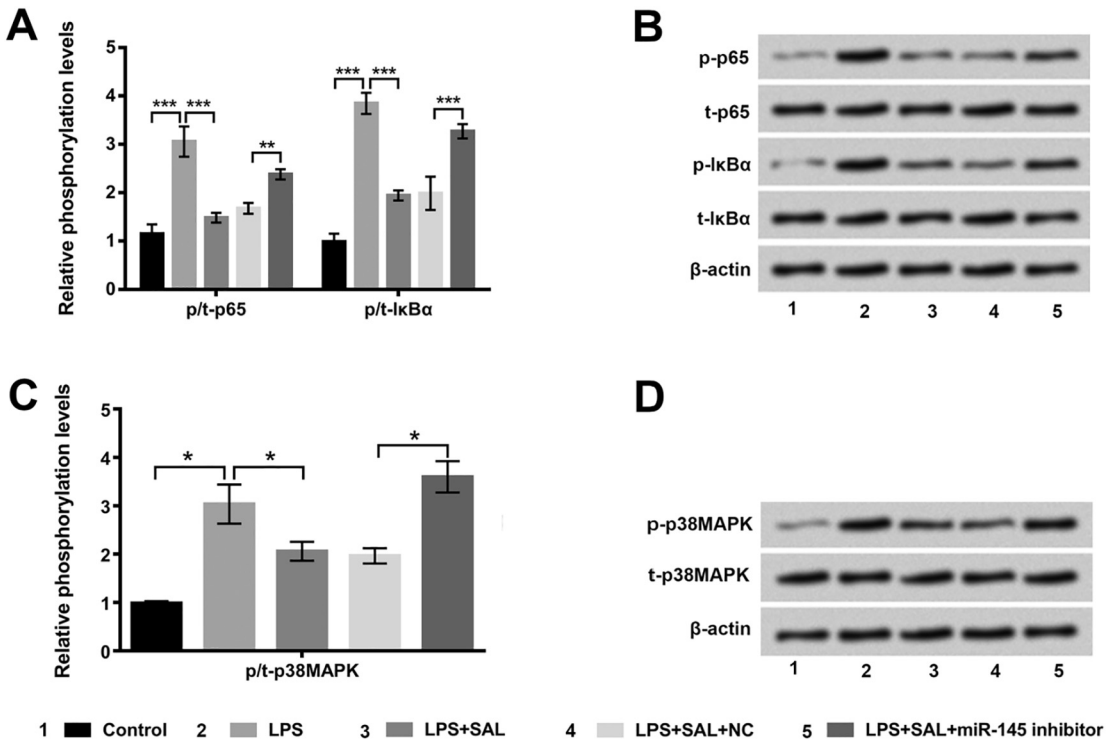

Fig. 5. SAL inactivated NF-κB and p38MAPK signaling pathways by regulation of miR-145.
ATDC5 cells were transfected with miR-145 inhibitor and its corresponding control. After treatment of LPS and SAL, (A and B) the relative mRNA and protein levels of p-p65, p65, p-IκBα, IκBα, and (C and D) the relative mRNA and protein level of p-p38MAPK, p38MAPK were examined by qRT-PCR and western blot.
SAL: salidroside; LPS: lipopolysaccharide; miR-145: microRNA-145; NF-κB: nuclear factor kappa B; p38MAPK: p38 mitogen-activated protein kinase; qRT-PCR: quantitative reverse transcription PCR; $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$.

to explore the effect of celecoxib (a selective COX-2 inhibitor) on OA. The study demonstrated the chondrobeneficial effect of celecoxib in patients with OA [35]. Cheleschi et al. carried out an *in vitro* experiment to analyze the anti-inflammatory effect of VA692 in comparison with celecoxib on OA, and the results indicated that VA692 had more beneficial effect compared with celecoxib [36]. Further, several studies reported that traditional Chinese medicine (TCM) could improve cartilage composition and metabolism, inhibit synovial inflammation, regulate abnormal cytokine levels, and reduce chondrocyte apoptosis in OA [37,38]. Evidence from Wang et al. revealed the analgesic and anti-inflammatory effect traditional Chinese herbal patches (TCHPs) on OA [39]. As mentioned above, plaster or compound Chinese medicine has been widely used for the treatment of OA. There is scarcely any report on the treatment of OA with a single TCM. Therefore, we explored whether SAL can be used to treat OA. In our study, we constructed an inflammatory injury model in ATDC5 cells to mimic OA. The functions of SAL in this *in vitro* model were explored. The results displayed that SAL significantly alleviated LPS-induced inflammatory injury by promoting cell viability, reducing apoptosis, and decreasing inflammatory cytokines expressions and secretions in ATDC5 cells. In addition, SAL enhanced the expression level of miR-145, and suppression of miR-145 reversed the protective effect of SAL on ATDC5 cells. Furthermore, SAL blocked NF-κB and p38MAPK signaling pathways by regulating miR-145.

Mounting evidences exhibited that LPS could induce cells inflammatory responses in various diseases, including OA [40,41]. ATDC5 is an excellent *in vitro* model cell line for skeletal development and it is widely used for investigating OA [42]. Therefore, in our study, the different concentrations of LPS were used to stimulate ATDC5 cells to mimic an inflammatory injury model *in vitro*. The results revealed that LPS significantly inhibited cell viability in a dose-dependent manner, and 8 μg/ml LPS was selected as the appropriate concentration. The subsequent experiments found that cell apoptosis and cleaved-caspase-3/-9 were promoted by LPS. IL-6, MCP-1 and TNF-α are vital pro-inflammatory cytokines, which play important role in inflammatory responses [43]. Our study also studied the expressions and secretions of IL-6, MCP-1 and TNF-α in LPS-stimulated ATDC5 cells. We found that LPS significantly up-regulated IL-6, MCP-1 and TNF-α expressions and secretions. These data fully suggested that LPS-induced ATDC5 cells injury model was successfully constructed.

SAL is one of main active components of *Rhodiola rosea L.*, which has been widely investigated in various diseases [44,45]. Modern pharmacological studies have proven that SAL has multiple biological activities, such as anti-aging, anti-inflammatory, anti-cancer, anti-oxidative stress and improve myocardial function [46,47]. A recent study revealed that SAL could alleviate TNF-α, IL-1β and IL-6 productions in LPS-stimulated RAW 264.7 macrophages and improve survival in murine endotoxemia [48]. In the mouse model, Guan et al. reported that SAL could protect LPS-induced acute lung injury (ALI) in mice [49]. However, the effect of SAL on LPS-induced ATDC5 cells injury has not been investigated. In our study, we found that SAL promoted cell viability, inhibited apoptosis and declined IL-6, MCP-1 and TNF-α expression and production in LPS-treated ATDC5 cells. These data revealed that SAL could protect ATDC5 cells against LPS-induced inflammatory injury. The finding indicated that SAL might be a potential therapeutic drug for OA.

MicroRNAs are small non-coding RNA that plays a crucial role in many disease processes, including malignancy and inflammatory processes [50]. Abnormal expression miRNAs, such as miR-9, miR-140, miR-146a and miR-558 have been found in OA [51]. MiR-145 is an important molecular marker, which has been proven to mediate chondrocyte homeostasis [52]. In terms of OA, Wang et al. displayed that miR-145 suppressed chondrocyte proliferation and fibrosis through targeting tumor necrosis factor receptor superfamily, member 11b (TNFRSF11B) [53]. Likewise, Yang et al. demonstrated that the expression of miR-145 was up-regulated in OA cartilage and

chondrocytes, and contributed to IL-1β-induced chondrocytes extracellular matrix (ECM) degradation [54]. These studies fully demonstrated that miR-145 was implicated in cartilage dysfunction in OA. However, whether miR-145 was involved in regulating LPS-induced ATDC5 cells injury remain unknown. In our study, we found that the expression of miR-145 was up-regulated by SAL, and suppression of miR-145 significantly alleviated the protective effect of SAL on LPS-injured ATDC5 cells.

It is generally known that NF-κB and p38MAPK signaling pathways are important pacificator in the process of inflammatory response [55]. Abnormal activation of NF-κB and p38MAPK signaling pathways are associated with OA chondrocytes degeneration [56]. Study from Wang et al. demonstrated that thymoquinone could alleviate IL-1β-induced inflammation injury in OA chondrocytes through inhibiting NF-κB and MAPKs signaling pathways [57]. Based on these evidences, we investigated the effect of SAL on NF-κB and p38MAPK signaling pathways. The results displayed that SAL significantly inactivated NF-κB and p38MAPK signaling pathways in LPS-stimulated ATDC5 cells. However, suppression of miR-145 alleviated the inhibitory effect of SAL on these two pathways. These data indicated SAL blocked NF-κB and p38MAPK signaling pathways might through regulation of miR-145.

5. Conclusion

Taken together, these data demonstrated that SAL alleviated LPS-induced inflammatory injury in ATDC5 cells through up-regulation of miR-145. Mechanistically, our results revealed that SAL functioned in LPS-induced injury by blocking NF-κB and p38MAPK signaling pathways. However, miR-145 suppression reversed these effects of SAL on ATDC5 cells. The findings suggested that SAL could protect ATDC5 cells against LPS-induced inflammatory injury, which might an effective therapeutic agent for OA. More studies are still needed for the clinical application in the future.

Statements

Declarations of interest

None.

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Authorship

Conceives and designed the experiments: Meihan Liu and Wenjun Wang. Performed the experiments: Meihan Liu and Jingzhe Zhang. Analyzed the data: Wanguo Liu. Wrote the paper: Wenjun Wang.

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