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Astragaloside IV protects ATDC5 cells from lipopolysaccharide-caused damage through regulating miR-203/MyD88

Dexin Li[a], Guangcheng Li[b], Yang Chen[c], Yifei Li[b], Junfeng Zhang[b], Dexuan Gao[b], Linglong Sun[a] and Bo Liu[b]

[a]Department of Sports Medicine, Jilin City Central Hospital, Jilin, China; [b]Department of Joint Surgery, Jilin City Central Hospital, Jilin, China; [c]Department of Dermatology, Jilin City Central Hospital, Jilin, China

ABSTRACT

Context: Osteoarthritis (OA) is a degenerative arthrosis sickness. Astragaloside IV (AS-IV) functions by relieving inflammatory damage.

Objective: We aimed to investigate the mechanism by which AS-IV protects ATD cells from lipopolysaccharide (LPS)-induced damage.

Materials and methods: ATDC5 cells were transfected with miR-203 inhibitor and NC inhibitor (150 nM) or pEX-MyD88 and sh-MyD88 (50 nM) for 48 h, pre-treated by 15 μg/mL AS-IV for 24 h, then treated by 5 μg/mL LPS for 12 h. Dual-luciferase activity testing was used to determine whether miR-203 could bind to MyD88. CCK-8 and flow cytometry were used to detect cell activity and apoptosis, respectively, and qRT-PCR, western blots, and ELISA were performed to detect expression levels of miR-203 and inflammatory cytokines.

Results: Based on the 50% inhibiting concentration (IC_{50}), there was no significant difference of AS-IV (0 to 15 μg/mL) on cell viability. Fifteen μg/mL was the optimal concentration of AS-IV in treating LPS-induced inflammatory damage in subsequent experiments since this was a semi-lethal concentration. AS-IV significantly reduces LPS-induced viability, apoptosis and the release of TNF-α, IL-6 and iNOS mainly through up-regulating miR-203. Further, MyD88 was a target gene of miR-203 and negatively regulated by miR-203. Knockdown of MyD88 inhibited LPS-induced inflammatory damage by inhibiting the NF-κB signal pathway.

Discussion and conclusions: AS-IV protects ATDC5 cells against LPS-induced damage mainly via regulating miR-203/MyD88. Our results support a theoretical basis for in-depth study of the function of AS-IV and the clinical cure of OA.

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KEYWORDS
AS-IV; inflammatory; osteoarthritis

Introduction

Osteoarthritis (OA) is a degenerative arthritis, which is a general term for a series of clinical manifestations caused by degeneration or damage of articular cartilage (Hunter et al. 2014). It commonly occurs in the elderly, including degradation of artivular cartilage and joint inflammation (Wu et al. 2017). Basic literature showed that apoptosis or chondrosis is an important part in the OA pathological process (Deng et al. 2019). The increased apoptosis in chondrocytes leads to a decrease in cell viability (Charlier et al. 2016). Researchers also found that inflammatory factors like tumour necrosis factor (TNF) and interleukins (ILs) could inhibit the proliferation of chondrocyte (Goldring and Otero 2011). Thus, searching for drugs to reduce cartilage inflammation has been thought to be a significant method for the clinical application of OA.

Astragali Radix, taken from the root of *Astragalus membranaceus* (Fisch) Bge (Leguminosae), has been used as a Chinese medicine for hundreds of years (Lau et al. 2012). Astragaloside IV (AS-IV) (3-*O*-β-D-xylopyranosyl-6-*O*-β-D-glucopyranosylcyl-cloastragenol) (Figure 1) is the primary active ingredient of *Astragali Radix*, whose content is the main criterion for evaluating the quality of *Astragali Radix* (Li et al. 2017). Clinical study indicated diverse pharmacological effects of AS-IV, such as anti-inflammation (Gui et al. 2013), antioxidation (Chen T et al. 2016), hypoglycaemia (Lv et al. 2010), protective myocardium (Lu et al. 2015), antiviral myocarditis (Chen et al. 2011), protection of brain tissue (Qu et al. 2009), and antihepatitis B virus (Wang et al. 2009). Therefore, the application of AS-IV may be an effective method for relieving inflammatory lesions in ATDC5 cells.

MicroRNAs (miRNA) are a kind of non-coding RNAs with 22 bases in length and can regulate target genes in post-transcriptional level in different biological processes, such as proliferation, differentiation and apoptosis (Krol et al. 2010). MiR-203 was found to be a tumour inhibitor because of controlling cells viability and metastasis (Xu et al. 2015; Zhao G et al. 2015), and its high expression could reduce the active anti-inflammation in preeclampsia (Wang et al. 2016). MiR-203 was lowly expressed in osteoarthritis cells and considered to be a critical regulator of lipopolysaccharide (LPS) (Zhao et al. 2017). However, the regulation mechanism between AS-IV and miR-203 has not been studied. In addition, myeloid differentiation factor 88 (MyD88) has been considered to be an essential mediator in the development of OA (Ellman et al. 2012; Hwang et al. 2015). It is a general adaptor protein in toll-like receptor 4 (TLR4) pathway,

CONTACT Bo Liu liu62bo@163.com Department of Joint Surgery, Jilin City Central Hospital, No.4 Nanjing Street, Jilin 132011, Jilin, China

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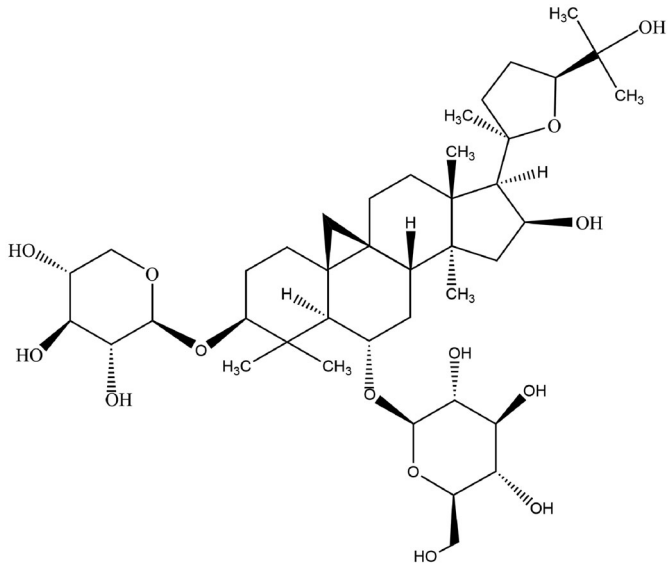

Figure 1. Chemical structure of AS-IV.

playing a crucial role in promoting the signal transduction of downstream inflammatory cytokine (Qiao et al. 2019). Therefore, this research was undertaken to research miR-203 and MyD88 to further disclose the possible defensive mechanism of AS-IV in protecting ATDC5 cells against LPS-induced inflammatory damage, which will provide effective treatment strategies for OA.

Materials and methods

Cell culture and treatment

ATDC5 cells were bought from the American Type Culture Collection (Manassas, VA, USA) and then kept at 37 °C in complete RPMI-1640 (Gibco, Grand Island, NY, USA) with 10% foetal bovine serum (FBS; HyClone, Logan, UT, USA) additive in a humidified 5% CO_2 incubator. Cells between the fifth and tenth passages were used in this study. Cells were cultured in growth medium in a 75 cm^2 flask. Fresh medium was changed every 3 days to achieve the confluence. Cells were treated by 5 μg/mL LPS (Sigma-Aldrich, St. Louis, MO, USA) for 12 h. Astragaloside IV ($C_{41}H_{68}O_{14}$, molecular weight = 784) was bought from Sigma-Aldrich (ref: 74777). It was dissolved in dimethyl sulfoxide (DMSO) at a dilution concentration of 1:1,000 and pre-treated cells for 24 h.

Cell Counting Kit-8 assay

Cell viability was tested through a CCK-8 (Dojindo Molecular Technologies, Gaithersburg, MD, USA). Inoculating cells in 96-well plate with 5000 cells/well and then adding CCK-8 solution to culture medium after stimulation. Cells were kept in humidified 95% air and 5% CO_2 at 37 °C for 1 h. Measure absorbance at 450 nm through a Microplate Reader (Bio-Rad, Hercules, CA, USA).

Apoptosis assay

Propidium iodide (PI) and fluorescein isothiocynate (FITC)-conjugate Annexin V staining (BD Pharmingen, San Diego, CA, USA) were performed to analysis cell apoptosis. Generally, cells were washed by using phosphate-buffered saline (PBS) for 3 times, and stained in PI/FITC-Annexin V with 50 μg/mL RNase A (Sigma-Aldrich). Treated cells were cultured in the dark at room temperature for 1 h. Apoptotic cells and necrotic cells were differentiated through flow cytometry analysis by using a FACS can (Beckman Coulter, Fullerton, CA, USA). Our data were resolved by FlowJo software (Tree Star Software, San Carlos, California, USA).

qRT-PCR

The extraction of total RNA from cells was performed by Trizol reagent (Life Technologies Corporation, Carlsbad, CA, USA) following directions. The Taqman MicroRNA Reverse Transcription Kit and Taqman Universal Master Mix II with the TaqMan MicroRNA Assay of miR-203 and U6 (Applied Biosystems, Foster City, CA, USA) were used to detect expression level of miR-203 in cells. In addition, for inflammatory factors (TNF-α and IL-6) and inducible NOS (iNOS), the SuperScript RT kit (Invitrogen, Carlsbad, CA, USA) was used to reverse transcription of RNA, and the 7500c real-time PCR detection system (Applied Biosystems, Carlsbad, CA, USA) with SYBR premix EX Taq (TaKaRa) was used to detect expression levels of mRNA, with β-actin as internal control.

Cell transfection

Full-length of MyD88 sequences and short-hairpin RNA were ligated into the pEX-2 and U6/Neo plasmids (GenePharma, Shanghai, China), referring to pEX-MyD88 and sh-MyD88, respectively. Cells were transfected with pEX-MyD88 and sh-MyD88 (50 nM) through the lipofectamine 3000 reagent (Life Technologies Corporation) following manufacturer's instructions. The negative control (NC) of sh-MyD88 was the plasmid carried a non-targeting sequence. The medium containing 0.5 mg/mL G418 (Sigma-Aldrich) was used to select stably transfected cells. G418-resistant cell clones were created after about 4 weeks. Synthetic (Life Technologies Corporation) miR-203 inhibitor and the relative NC (150 nM) were transfected into cells. 48 h post-transfection was thought to be the harvest time in following experiments.

Enzyme-linked immunosorbent assay (ELISA)

After cells were treated with AS-IV and/or LPS, and/or transfection with miR-203 or NC inhibitor, and/or transfection with pEX-MyD88 and sh-MyD88, the supernatant was collected from 24-well plates. Inflammatory factors (TNF-α and IL-6) concentration were determined through ELISA kits (# SMTA00B and # SM6000B, respectively, R&D Systems, Abingdon, UK) according to the manufacturer's instructions and standardized to cell protein concentrations.

Western blot

Protein was extracted through RIPA lysis buffer (Beyotime Biotechnology, Shanghai, China) complemented with protease inhibitors (Roche, Basel, Switzerland). Protein quantification was measured by using the BCA™ Protein Assay Kit (Pierce, Appleton, WI, USA). Then a western blot system was established through a Bio-Rad Bis-Tris Gel system according to manufacturer's directions. Then, the protein samples were transferred onto PVDF membrane. Primary antibodies specific against

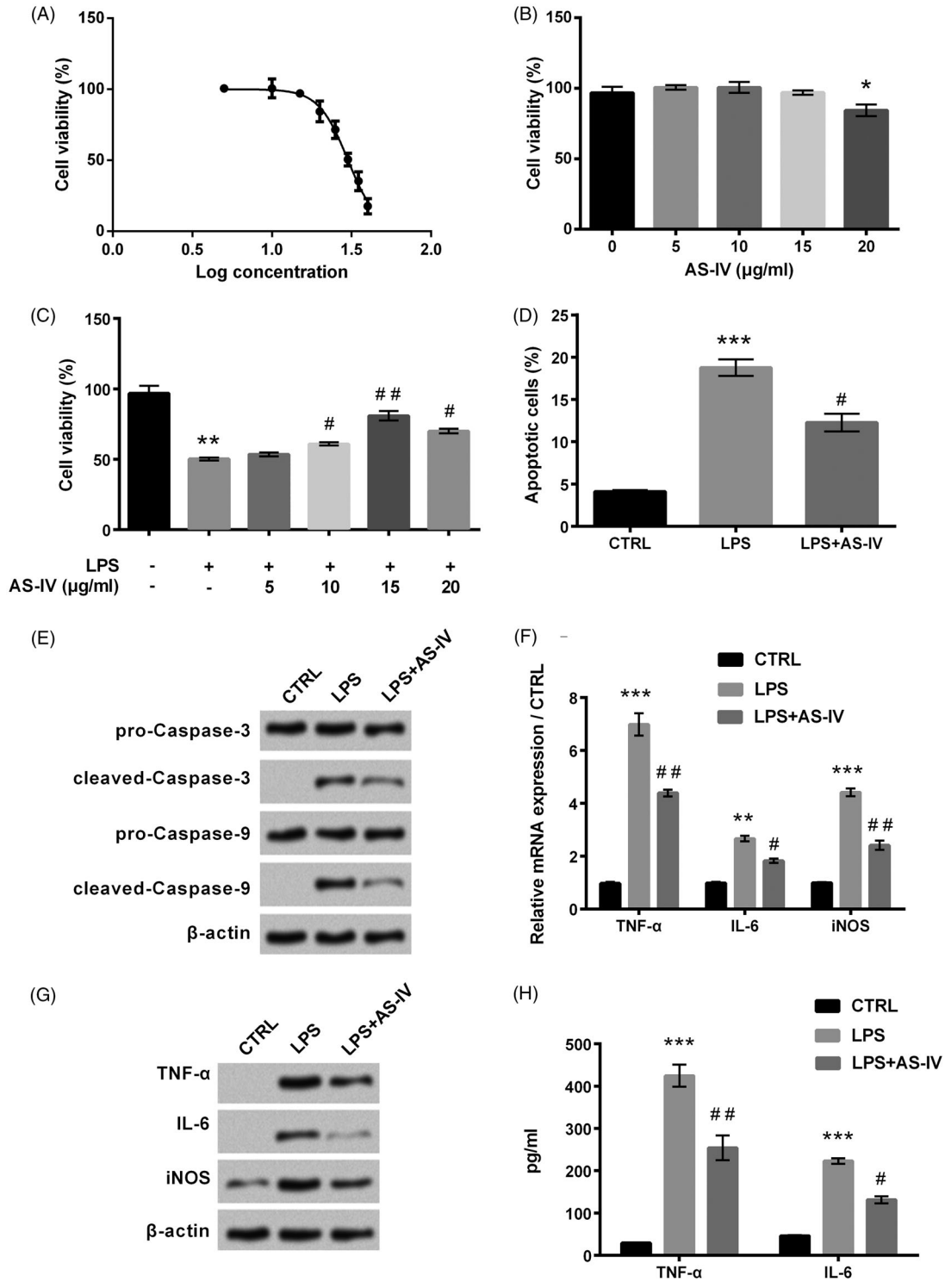

Figure 2. Defensive effects of AS-IV. (A) IC_{50} of AS-IV on the viability of ATDC5 cells was examined. (B) Various concentrations of AS-IV (0, 5, 10, 15 and 20 μg/mL) were used to detect their effects on cell viability. (C) Various concentrations of AS-IV (0, 5, 10, 15 and 20 μg/mL) were used to attenuate LPS-induced inhibition of cell viability. 15 μg/mL was selected in the later experiments. (D) Apoptosis was detected through flow cytometry. (E) The related protein expressions of apoptotic process were tested through western blot. (F-G) The levels of TNF-α, IL-6 and iNOS were detected through western blot analysis. (H) Concentration of TNF-α and IL-6 were tested using ELISA. $^{*}p < 0.05$, $^{**}p < 0.01$ and $^{***}p < 0.001$ compared to control group, $\#p < 0.05$ and $\#\#p < 0.01$ compared to LPS group.

pro-caspase-3 (ab32499, Abcam, MA, USA), cleaved-caspase-3 (ab2302), pro-caspase-9 (ab135544), cleaved caspase-9 (ab2324), TNF-α (ab1793), IL-6 (ab6672), iNOS (ab15323), β-actin (ab8226), MyD88 (ab2064), t-IκBα (ab7217), p-IκBα (ab7217), t-p65 (ab16502) and p-p65 (ab6503) were prepared in 5% blocking buffer. Primary antibody was incubated with the membrane overnight at 4 °C. Next washing the primary antibody and incubated with secondary antibody carrying with horseradish

peroxidase (HRP)-conjugates for 1 h at room temperature. Signals were observed and quantified through Image Lab™ Software (Bio-Rad).

Dual-luciferase activity assay

MyD88 3′UTR target sequences were cloned into the multiple cloning sites (MCS) of pMIR-Report Luciferase vector (Promega, Madison, WI, USA). Co-transfecting miR-203 mimic with expression vector MyD88-wild-type (MyD88-wt) or MyD88-mutated-type (MyD88-mut) into ATDC5 cells. Results were detected through the dual-luciferase assay system (Promega) according to the manufacturer's information.

Statistics analysis

Our experiments were performed with five biological replicates and three technical replicates. Data of multiplex experiments are showed as the mean ± SD. Statistical analyses were conducted through Graphpad Prism 6.0 software (GraphPad Software Inc., La Jolla, CA, USA). The p-values were determined through a one-way analysis of variance (ANOVA). p-value of <0.05 represented a statistically significant result.

Results

Astragaloside IV relieved LPS-induced inflammatory damage of ATDC5 cells

According to our experiment, the IC_{50} of AS-IV in inducing the cellular damage model was 30 μg/mL (Figure 2(A)). Then, the AS-IV cell toxicity assay was performed in Figure 2(B). There was no significant difference of AS-IV on cell viability at concentration of 0, 5, 10 and 15 μg/mL. At higher concentration (20 μg/mL), AS-IV significantly reduced the cell viability compared with control ($p < 0.05$, Figure 2(B)). In Figure 2(C), cell viability was notably reduced after LPS treatment ($p < 0.01$), while was apparently relieved by AS-IV under the concentration of 10, 15 and 20 μg/mL ($p < 0.05$, $p < 0.01$ and $p < 0.05$). Data revealed that the cell viability was optimal when the concentration of AS-IV was 15 μg/mL, which was also the cell viability semi-lethal concentration and the difference was extremely significant under this concentration. Thus, 15 μg/mL was used in later experiments. Besides, Figure 2(D) indicated that the apoptosis caused by LPS was significantly diminished after AS-IV treatment ($p < 0.05$). Western blot assay revealed that cleaved-caspase-3 and cleaved-caspase-9 were apparently down-regulated after AS-IV treatment (Figure 2(E)). Besides, western blot analysis indicated that LPS could significantly promote expression of TNF-α, IL-6 and iNOS ($p < 0.001$, $p < 0.01$ and $p < 0.001$, Figure 2(F,G)). However, AS-IV could relieve this promotion ($p < 0.01$, $p < 0.05$ and $p < 0.01$, Figure 2(F,G)). Inflammatory factors concentrations were apparently decreased in LPS + AS-IV group ($p < 0.01$ and $p < 0.05$) in contrast with LPS group (Figure 2(H)). These data indicated that AS-IV could alleviate LPS-induced inflammatory damage in ATDC5 cells.

As-IV up-regulated the expression of miR-203

The qRT-PCR testing revealed that miR-203 level was notably down-regulated in LPS-induced ATDC5 cells in comparison with control ($p < 0.01$). However, miR-203 expression was specifically up-regulated after AS-IV treatment ($p < 0.01$, Figure 3). This result suggested that AS-IV significantly up-regulated miR-203.

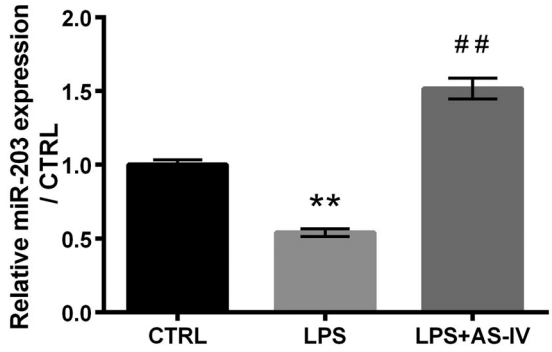

Figure 3. MiR-203 was up-regulated by AS-IV. Expression of miR-203 was tested through qRT-PCR. $^{**}p < 0.01$ compared to control group, ## $p < 0.01$ compared to LPS group.

As-IV attenuated cellular inflammatory damage mainly by means of up-regulating expression level of miR-203

MiR-203 expression was detected through qRT-PCR testing. Results revealed that miR-203 expression was obviously suppressed after transfection with miR-203 inhibitor ($p < 0.01$, Figure 4(A)). Inhibition of miR-203 could partly decrease cell activity ($p < 0.05$) and increase apoptotic process ($p < 0.05$, Figure 4(B,C)). Levels of cleaved-caspase-3 and cleaved-caspase-9 were apparently raised in group of LPS + AS-IV + miR-203 inhibitor (Figure 4(D)). Besides, we detected the role of miR-203 on TNF-α, IL-6 and iNOS. In Figure 4(E,F), levels of TNF-α, IL-6 and iNOS were partly raised when miR-203 was inhibited compared with NC ($p < 0.01$, $p < 0.05$ and $p < 0.01$). Moreover, the concentrations of two inflammatory cytokines were notably increased when miR-203 was inhibited in LPS + AS-IV + miR-203 inhibitor group in comparison with its NC group ($p < 0.01$ and $p < 0.05$, Figure 4(G)). The above results indicated that AS-IV could reduce inflammation mainly by means of up-regulating miR-203.

MyD88 acted as the target gene for miR-203

To further study the regulation of miR-203, we performed qRT-PCR, western blot and dual luciferase activity assay. qRT-PCR testing revealed that expression of MyD88 was increased when miR-203 was inhibited ($p < 0.01$, Figure 5(A)). Knockdown of miR-203 could up-regulate MyD88 expression (Figure 5(B)). Luciferase activity in cells co-transfected with MyD88-wt and miR-203 mimic was notably decreased ($p < 0.05$, Figure 5(C)). Our findings revealed that MyD88 was a target gene of miR-203.

Overexpression of MyD88 up-regulated LPS-induced inflammatory cytokines

The transfected effects of MyD88 were further detected in Figure 6(A,B). The mRNA and protein levels of MyD88 were obviously increased when cells were transfected with pEX-MyD88 ($p < 0.01$), whereas were diminished when cells were transfected with sh-MyD88 ($p < 0.05$). Besides, in Figure 6(C,D), expression levels of TNF-α, IL-6 and iNOS were raised in LPS + pEX-MyD88 group ($p < 0.01$, $p < 0.05$ and $p < 0.05$), whereas levels

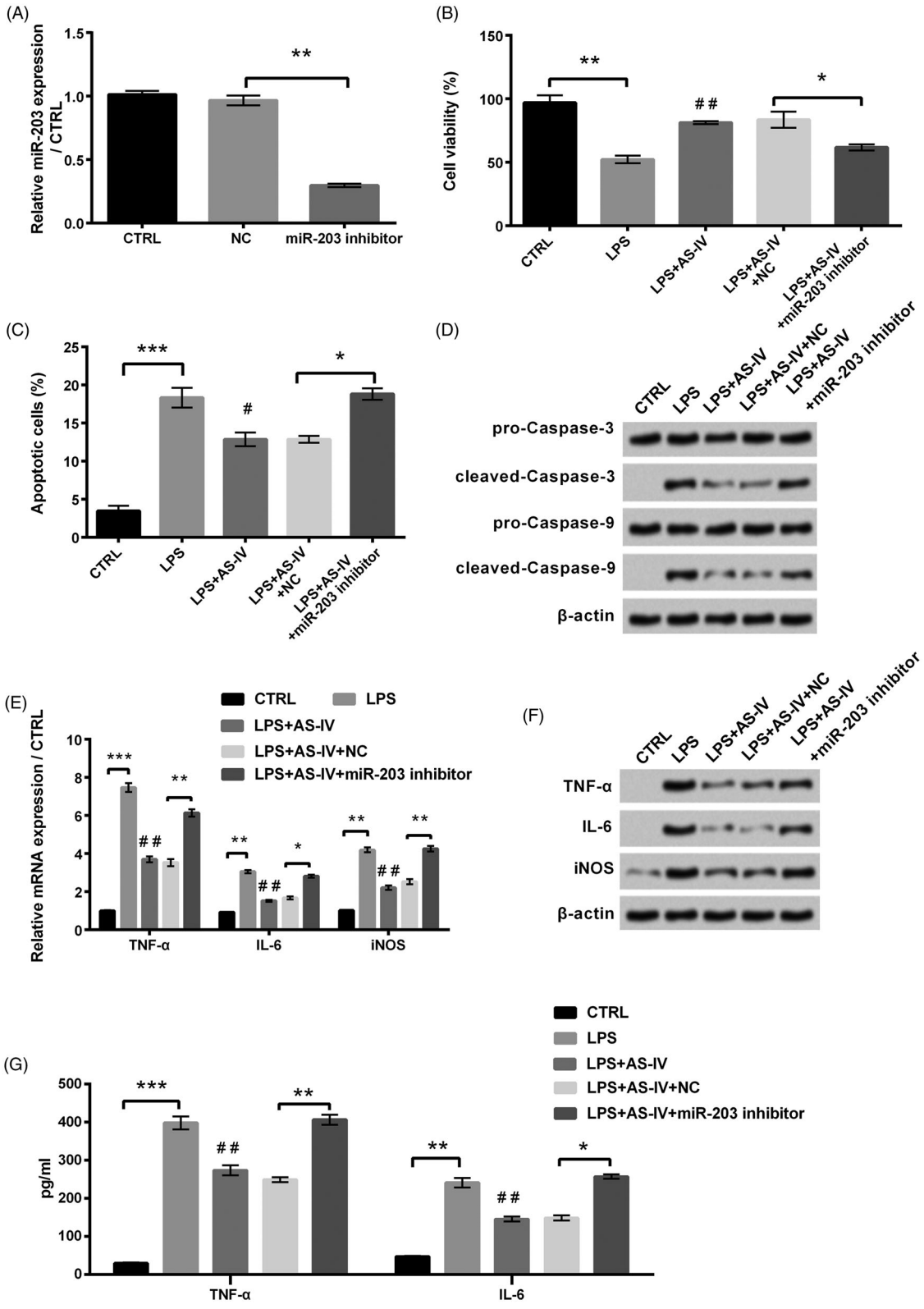

Figure 4. Mechanism of AS-IV was tested after transfected with miR-203 inhibitor in LPS-induced inflammatory damage. (A) The level of miR-203 was tested through qRT-PCR. (B) Cell viability was measured through CCK-8 assay. (C) Apoptosis was measured through PI and FITC-conjugate Annexin V staining. (D) The related protein expressions of apoptotic process were tested through western blot. (E-F) The levels of TNF-α, IL-6 and iNOS were detected through western blot analysis. (G) Concentration of TNF-α and IL-6 were tested using ELISA. *$p < 0.05$, **$p < 0.01$ and ***$p < 0.001$ compared to the indicated group, #$p < 0.05$ and ##$p < 0.01$ compared to LPS group.

were reduced in LPS + sh-MyD88 group ($p < 0.01$, $p < 0.05$ and $p < 0.05$). ELISA assay showed that concentration of TNF-α and IL-6 were apparently raised in LPS + pEX-MyD88 group ($p < 0.01$ and $p < 0.05$), whereas were lessened in LPS + sh-MyD88 group ($p < 0.01$ and $p < 0.05$, Figure 6(E)). Our findings revealed that up-regulation of MyD88 enhanced LPS-induced inflammatory damage, knockdown of MyD88 relieved LPS-induced inflammatory damage.

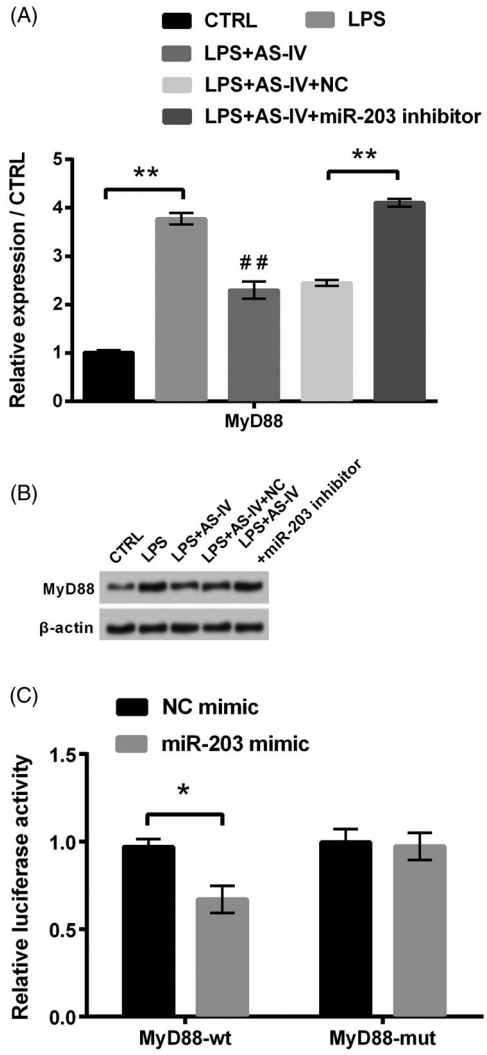

Figure 5. MyD88 was a target gene of miR-203. (A) Expression of MyD88 was tested through qRT-PCR. (B) Protein level of MyD88 was tested through western blot. (C) Luciferase activity testing was used to determine the targeting relationship between miR-203 and MyD88. *$p < 0.05$ and **$p < 0.01$ compared to control group, ##$p < 0.01$ compared to LPS group.

MyD88 regulated LPS-induced inflammatory damage through regulating nuclear factor (NF-κB) signal pathway

The western blot analysis results suggested that the phosphorylation standards of IκBα and p65 were apparently up-regulated when MyD88 was overexpressed (both $p < 0.05$), while levels were down-regulated after knockdown of MyD88 ($p < 0.01$ and $p < 0.05$, Figure 7(A,B)). These results indicated that overexpression of MyD88 could activate NF-κB signal pathway to enhance inflammatory damage, however knockdown of MyD88 could alleviate inflammatory damage by inhibiting the NF-κB signal pathway.

Discussion

OA is a complex inflammatory disease caused by many components (Rainbow et al. 2012), resulting in articular cartilage degeneration damage. Chondrocyte death is important in the process of OA. Therefore, protecting inflammatory damage can help to relieve inflammation. AS-IV, known as the effective ingredient of *Astragali Radix*, could offer protection against the formation of cerebral infarction by reducing infarct volume (Luo et al. 2004), stimulating cell proliferation (Zhang et al. 2011) and reducing inflammatory responses (Gui et al. 2013), which suggested that AS-IV has potential protective function of relieving disease damage. Therefore, our goal was to investigate the protective mechanism of AS-IV in LPS-induced inflammatory damage in ATDC5 cells. We found that AS-IV was beneficial in protecting ATDC5 cells from LPS-induced damage. Protective mechanism of AS-IV was achieved mainly through the regulation of miR-203/MyD88. These results strongly indicated that AS-IV performed a key role in LPS-induced ATDC5 cells inflammatory damage.

Previous studies showed that AS-IV has immunomodulatory effects on lymphocyte proliferation and production of pro-inflammatory factors (Liu et al. 2016). The increased pro-inflammatory factors in chondrocytes are associated with the process of OA joints (Philp et al. 2017). The latest study demonstrated that LPS could promote to generate inflammatory factors like TNF-α and IL-6, which were the elements of the formation of OA (Wang et al. 2018). AS-IV presented an immunomodulating effect on the generation of inflammatory factors (Liu et al. 2017). Therefore, we detected the effects of AS-IV in ATDC5 cells. Our findings showed that AS-IV could effectively increase cell activity, inhibit apoptotic process and suppress production of inflammatory factors (TNF-α, IL-6) and iNOS. These findings indicated that AS-IV was a saponin with potential anti-inflammatory activity (Gui et al. 2013) and indeed relieved inflammatory damage in ATDC5 cells.

MiRNAs play critical roles through regulating target genes in inflammatory response, tumour progress and other biological function, such as cell proliferation and apoptosis (Marques-Rocha et al. 2015). The regulation between AS-IV and miRNA is a research hotspot during recent years. For example, AS-IV could down-regulated miR-203a and miR-92a to protect rat cardiomyocytes from hypoxia-caused damage by down-regulation of (Gong et al. 2018; Yu et al. 2018). Besides, AS-IV could inhibit oxidised low-density lipoprotein-induced endothelial damage via up-regulating miR-140-3p (Qian et al. 2019). These findings indicate the regulatory mechanism between AS-IV and miRNAs. MiR-203 is an inflammation-associated miRNA (Mohan et al. 2016), maybe a key regulator against LPS infection (Wei et al. 2013), and was able to regulate key pro-inflammatory factor-like TNF-α (Primo et al. 2012). In addition, miR-203 increased the generation of matrix metalloproteinase 1 (MMP-1) and IL-6 to promote the activated phenotype of synovia fibroblasts in rheumatoid arthritis (RA) (Stanczyk et al. 2011). Our findings firstly demonstrated the potential regulation mechanism between AS-IV and miR-203 in LPS-induced ATDC5 cell damage that miR-203 was up-regulated by AS-IV to alleviate inflammatory damage. Furthermore, MyD88 is a key downstream adaptor for most Toll-like regulators and IL-1 receptors, it takes key roles in elevating the signal transduction of downstream inflammatory factors (Li et al. 2019; Qiao et al. 2019). Li et al. (2019) found that tetramethylpyrazine attenuated LPS-triggered ATDC5 cell injury through down-regulating MyD88. Consistently, we also found that up-regulation of MyD88 enhanced LPS-induced inflammatory damage, while MyD88 knockdown inhibited this damage. In addition, regulatory mechanism between miR-203 and MyD88 has been reported in many studies. For example, miR-203 could inhibit MyD88 generation to reduce neuronal inflammation through directly binding to its 3′UTR (Yang et al. 2015). Consistently, we also found that MyD88 was a target gene of miR-203 in dual luciferase activity testing. Further work is needed to explore whether

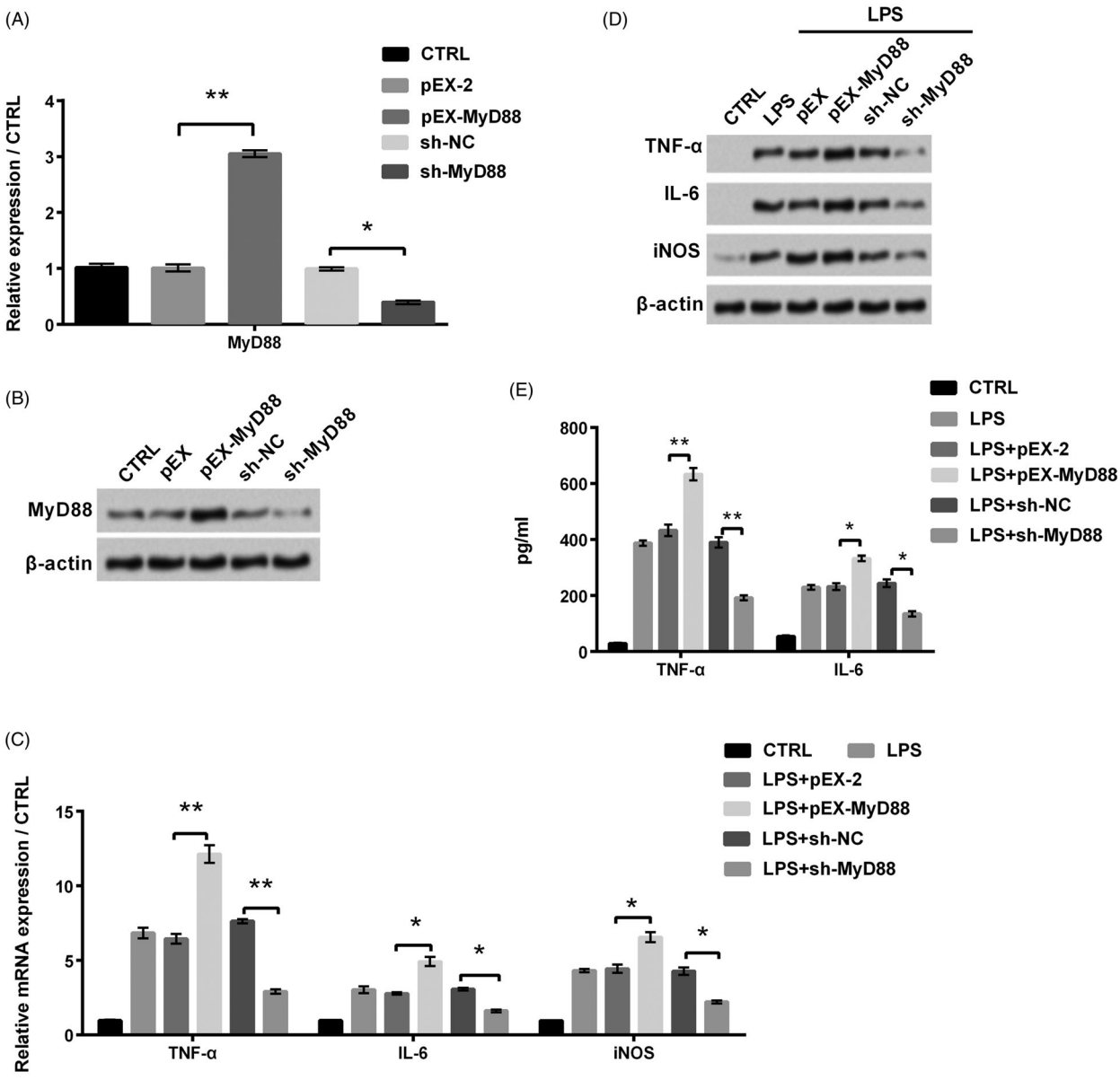

Figure 6. Role of MyD88 in regulating inflammatory cytokines in LPS-induced inflammatory damage in ATDC5 cells, which were transfected with pEX-MyD88 and sh-MyD88. (A) Expression of MyD88 was measured through qRT-PCR. (B) Protein level of MyD88 was detected through western blot. (C-D) The levels of TNF-α, IL-6 and iNOS were detected through western blot analysis. (E) The concentration of TNF-α and IL-6 were measured through ELISA. $^{*}p < 0.05$ and $^{**}p < 0.01$ compared to relative negative control group.

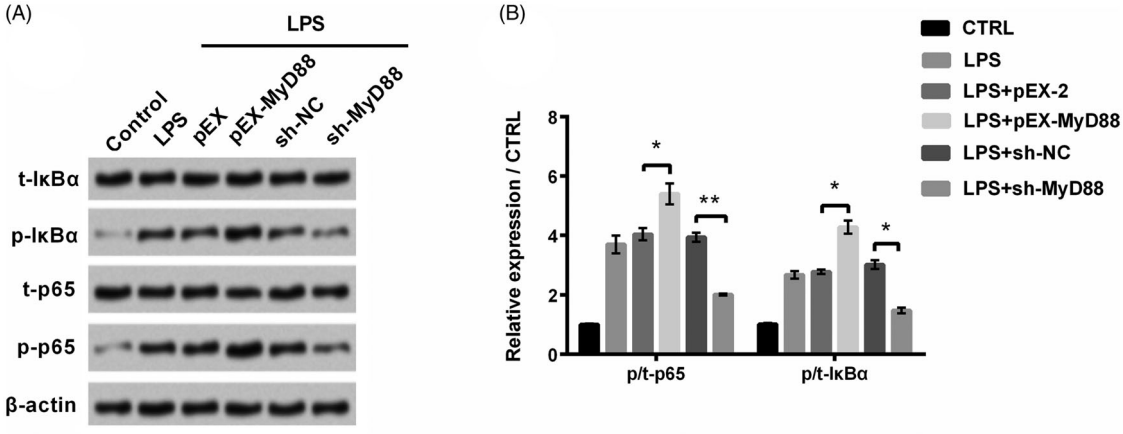

Figure 7. Regulatory mechanism between MyD88 and NF-κB signal pathway was tested in LPS-induced inflammatory damage in ATDC5 cells, which were transfected with pEX-MyD88 and sh-MyD88. (A-B) Levels of p/t-p65 and p/t-IκBα were detected through western blot analysis. $^{*}p < 0.05$ and $^{**}p < 0.01$ compared to relative negative control group.

this targeting regulatory relationship is involved in the protective mechanism of AS-IV against LPS-induced inflammatory damage in ATDC5 cells to enrich the theoretical basis of AS-IV's anti-inflammatory effect.

Several studies showed that the TLR4/NF-κB signal pathway could mediate inflammatory factors expression (Avlas et al. 2011). Five members make up the NF-κB dimers, including p65/RelA, p50, RelB, cRel and p52 (Oeckinghaus et al. 2011). IκB proteins naturally bind to NF-κB dimers. It is phosphorylated, ubiquitinated and degraded when cells are stimulated (Mulero et al. 2013). NF-κB signalling was participated in OA pathophysiology, activated in chondrocytes, and induced inflammation-related factors, such as iNOS, TNF-α and IL-1β (Saito and Tanaka 2017). A previous study has reported that MyD88 overexpression activated NF-κB pathway through improving the expression rates of p/t-IκBα and p/t-p65 in ATDC5 cells (Ren and Liang 2018). In addition, another study indicated that long non-coding RNA ATB (lncRNA-ATB) alleviated LPS-triggered inflammatory damage in ATDC5 cells through blocking MyD88/NF-κB pathway (Ying et al. 2019). As we expected, the knockdown of MyD88 could suppress NF-κB signal pathway in LPS-induced ATDC5 cell inflammatory damage.

Conclusions

We found that AS-IV might up-regulate miR-203 expression to attenuate inflammation in ATDC5 cells, accompanied by the down-regulation of MyD88 and the inhibition of NF-κB signal pathway. In sum, AS-IV might be an effective therapeutic strategy for OA.

Disclosure statement

The authors declare that there are no conflicts of interest.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

ORCID

Bo Liu http://orcid.org/0000-0002-0508-0703

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