Original Research

FOXM1 activates JAK1/STAT3 pathway in human osteoarthritis cartilage cell inflammatory reaction

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Impact statement

This study evaluated the signal reaction of JAK1/STAT3 with FOXM1 in the inflammatory process of lipopolysaccharides (LPS)-exposed chondrocytes. The results showed chondrocyte proliferation and survival were reduced after LPS induction, and LPS could induce activation of the JAK1/STAT3 signal pathway, suggesting that the JAK1/STAT3 signal pathway participated in LPS-induced inflammation in chondrocytes. In addition, FOXM1 was found to interact with STAT3 and was positively associated with activation of the JAK1/STAT3 pathway, indicating that the JAK1/STAT3 signaling pathway participated in the LPS-treated inflammation reaction and cell apoptosis pathway in cartilage cells via mediating FOXM1 expression. And the fundamental mechanism of the JAK1/STAT3 pathway inactivation on chondrocyte protection might be realized by inhibiting the expression of FOXM1. Therefore, this study offers new clues in the effects of JAK1/STAT3 in the cellular morbidity course of osteoarthritis.

Abstract

Osteoarthritis (OA), the most prevalent form of arthritis disease, is characterized by destruction of articular cartilage, osteophyte development, and sclerosis of subchondral bone. Transcription factors Janus kinase 1/signal transducer and activator of transcription 3 (JAK1/STAT3) and Forkhead box M1 (FOXM1) are key mediators of this inflammatory reaction. In this study, we investigated the interaction between JAK1/STAT3 and FOXM1 in OA. Inflammation is related to the cartilage damage, and lipopolysaccharides (LPS) are a major pro-inflammatory inducer, so LPS was utilized to stimulate chondrocytes and establish a cell-based OA model. We found LPS treatment caused a generation of inflammatory cell factors (IL-1β, IL-6, and TNF-α), and upregulation of inducible nitric oxide synthases (iNOS), cyclooxygenase-2 (COX-2), nitric oxide (NO), prostaglandin E2 (PGE2) and other inflammatory mediators. Cell viability of chondrocytes was impaired with LPS stimulation, along with an upregulation of JAK1 expression, and phosphorylation and nuclear accumulation of STAT3. The administration of STAT3 inhibitor WP1066, which abated activation and nuclear location of STAT3, depleted the effect of LPS on inflammation and cell death. Co-immunoprecipitation showed that STAT3 was able to bind to FOXM1, and deactivation of STAT3 resulted in the downregulation of FOXM1. Moreover, FOXM1 silencing inhibited the generation of inflammatory cytokines induced by LPS, and the attenuation of cell survival. These findings indicated that the interaction between JAK1/STAT3 and FOXM1 may play a key role in OA pathogenic studies, and suggest the JAK1/STAT3 pathway may be a potential target for OA therapy.

Keywords: Osteoarthritis, chondrocytes, lipopolysaccharides, inflammation, JAK1, STAT3, FOXM1

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Introduction

Osteoarthritis (OA), the most prevalent disease of joint regressive, causes patients to suffer pain and articular dyskinesia.[1] OA is characterized by constantly decomposing articular cartilage, causing an imbalance of composition and decomposition in extracellular matrix in articular cartilage cells.[2] The main cellular OA characteristics are decreased composition of aggrecan and type II collagen,[3,4] and an increase in matrix degrading enzyme matrix metalloproteinases (MMPs).[5] OA pathogenesis is reported to be strongly associated with variable degrees of inflammation;[6,7] however, the underlying regulatory mechanism that causes aberrant gene expression in inflammatory OA cartilage has not yet been clarified.

The Janus kinase (JAK)/signal transducer and activator of transcription (STAT) pathway is a signaling pathway which was activated during inflammation. It is triggered after a ligate binds a receptor.[8,9] The JAK/STAT pathway then participates in the transmission of the signal in cell factor receptors into nucleus. JAKs are stimulated

following the activation of the cell factor receptors, which leads to the phosphorylation and activation of the STAT family in transcription factors. Activation of JAK/STAT is considered important in the drive of OA chronic inflammation.[10] Forkhead box M1 (FOXM1) is a transcription factor in the Forkhead box family, and a key mediator of inflammatory reaction.[11,12] Previous studies have suggested that FOXM1 is a key factor in OA chondrocyte inflammation.[13] However, the influence of JAK/STAT-FOXM1 cross-communication in the inflammatory reaction and apoptosis of human OA chondrocytes is still not clear.

The pro-inflammatory LPS is a glycolipid composed of a polysaccharide O-antigen, a core oligosaccharide and a highly conserved lipid A moiety which contributed to the toxicity of gram-negative bacteria and biological activities of LPS. LPS activates cells of the innate immune system, such as macrophages and neutrophils, which synthesize proinflammatory factors, such as IL-1β and TNF, MMPs and free radicals that lead to dramatic secondary inflammation in tissues.[14] Recurrent exposure to subclinical LPS increases mortality and induces cardiac fibrosis in mice.[15] Because of its pro-inflammatory properties, exogenous LPS has long been utilized in conjunction with collagen to induce arthritis in experimental animal models and cell models.[14,16,17] The gram-negative flora of the terminal ileum and large intestine constitutes a large reservoir of LPS, and intestinal absorption of LPS contributes to low-grade inflammation.[18] Low levels of LPS can be detected in the systemic circulation with chronic diseases, including rheumatoid arthritis (RA),[19] and OA.[20]

In the present study, chondrocytes were exposed to lipopolysaccharides (LPS) to establish an OA cell model, as previously reported.[21] The cell proliferation, viability, inflammation, and apoptosis of LPS-exposed chondrocytes were examined. Furthermore, the role of signal reaction of JAK1/STAT3 with FOXM1 on the inflammatory process of LPS-exposed chondrocytes was also determined.

Materials and methods

Original cartilage cell culture in human OA

Original chondrocytes are cartilage specimens from human OA joints.[8] The cartilage samples ($n = 3$) derived from patient underwent joint replacement surgery and had not taken non-steroidal anti-inflammatory drugs or steroids for at least two weeks prior to surgery or had not any intra-articular injection for at least one month prior to surgery. K/L Image Criterion score is II. The cells were precipitated via centrifugation, washed with DMEM/F12, and cultured in six-well tissue culture plates after the filtration of dissected cartilage specimens was digested with pronase and collagenase P. Cells were obtained after continuous passage. The first generation of cells were cultured in DMEM/F12 with 10% of fetal bovine serum (Invitrogen, Carlsbad, CA, USA) at 37°C, after electroplating in tissue culture plate with 12 wells. 0.5 or 1 μg/mL LPS was employed to treat the chondrocytes of the LPS stimulation group for 48 h, and 500 ng/mL (1.5 μmol/L) WP1066 (a novel inhibitor of STAT3 with IC50 of 2.43 μmol/L[22]) was employed to treat the chondrocytes of the WP1066 treatment group for 48 h.

ELISA

Based on the manufacturer instructions, IL-1β, IL-6, TNF-α, and prostaglandin E2 (PGE2) levels in the culture supernatant were evaluated by commercial ELISA kits (R&D Systems, Minneapolis, MN, USA).

Western blotting

The cultured cells were treated with protease inhibitor cocktail (Roche Applied Science, Basel, Switzerland) and RIPA buffer (pH 8.0) to prepare whole cell lysate. Protein content was evaluated with a bicinchoninic acid kit. Proteins were transferred electrically to the PVDF membrane (Millipore, MA, USA) using SDS-PAGE electrophoresis. The proteins were washed with TBST to block unoccupied sites on the membrane, and then incubated with primary antibodies overnight at 4°C. The information of primary antibodies were displaying as follows: anti-GAPDH antibody (1:5000, ab8245, Abcam), anti-STAT3 antibody (1:1000, ab5073, Abcam), anti-pSTAT3 antibody (1:200, ab30647, Abcam), anti-JAK1 antibody (1:1000, ab125051, Abcam), anti-FOXM1 antibody (1:1000, ab180710, Abcam), anti-FLAG M2 antibody (1:5000, B3111, Sigma), and anti-Myc antibody (1:2500, ab9106, Abcam). The membrane was then washed with secondary antibodies at room temperature for 1 h incubation. Following multiple washes with TBST, Super Signal West Femto Maximum Sensitivity Substrate Kit (Thermo, MA, USA) was employed to develop the bands on the membrane for the WB. VersaDoc 4000 MP imaging system (Bio-Rad Laboratory, Hercules, California, USA) with Quantity One software was utilized to analyze the band density.

Quantitative real-time PCR

TRIzol reagent (Invitrogen) was used to separate total RNA from the chondrocytes, and then Nanodrop2000 (OD260) was used to evaluate the concentration. MMLV First-Strand Kit (Invitrogen) and Oligo (dT) 20 primer were employed to perform the reverse transcription needed for cDNA preparation. SYBR Select Master Mix (Invitrogen) was then used to prepare the cDNA for a qPCR. Following the manufacturer's instructions, the related kits were used to detect qPCR in miR-1225 and U6. The reaction system used a 10 min denaturation at 95°C, a 15 s denaturation at 95°C for 40 cycles, and a 40 s denaturation cycle at 60°C. The expression levels of U6 or GAPDH mRNA were used as internal reference controls. The $2^{-\Delta\Delta CT}$ method was used to quantify target mRNAs expression levels, and all tests were performed in triplicate.

Nitric oxide detection

Following the manufacturer's instructions, the Griess method and Nitric Oxide Assay Kit (Beyotime, Shanghai, China) were used to evaluate NO levels in the cell culture supernatant. A microplate reader (Bio-Tek) was used to

detect absorbance at 540 nm after 15 min in culture, using 50 μL of cultured medium and 100 μL of Griess reagent.

Cell proliferation

Following the manufacturer's instructions, the CCK-8 assay was used to evaluate cell division rates. The 96-well plates were seeded with cells and CCK-8 (10 μL) was added to each well to culture for 2 h at 37°C. An auto-microplate reader (infinite M200, Tecan, Männedorf, Switzerland) was used to detect the optical density at 450 nm.

Cell survival

The cell survival rate was assessed via MTT assay. Formazan dye was dissolved in 10 min by rotating and adding DMSO (150 μL) to 20 μL MTT-treated cells (0.5 mg/mL) without supernatant in each well. Then, absorbance at 540 nm was detected using the Infinite M200 microplate reader (Tecan).

Flow cytometry (FCM)

Cells were collected, washed, and resuspended. Following the addition of 5 μL Annexin V (BD Pharmingen; BD Biosciences, Franklin Lakes, New Jersey, USA) and 5 μL PI (BD Pharmingen; BD Biosciences), cells were incubated at room temperature for 20 min in the dark, washed with PBS and re-suspended with 300 mL of PBS. Cell apoptosis rate was calculated using Flow Jo software (v10.1.1 FlowJo LLC).

Immunofluorescence assays

Cells were grown in 24-well plates with covers, and then fixed in 4% paraformaldehyde. Cells were then treated with a 15-min permeation with PBST at 25°C, blocked for 1 h

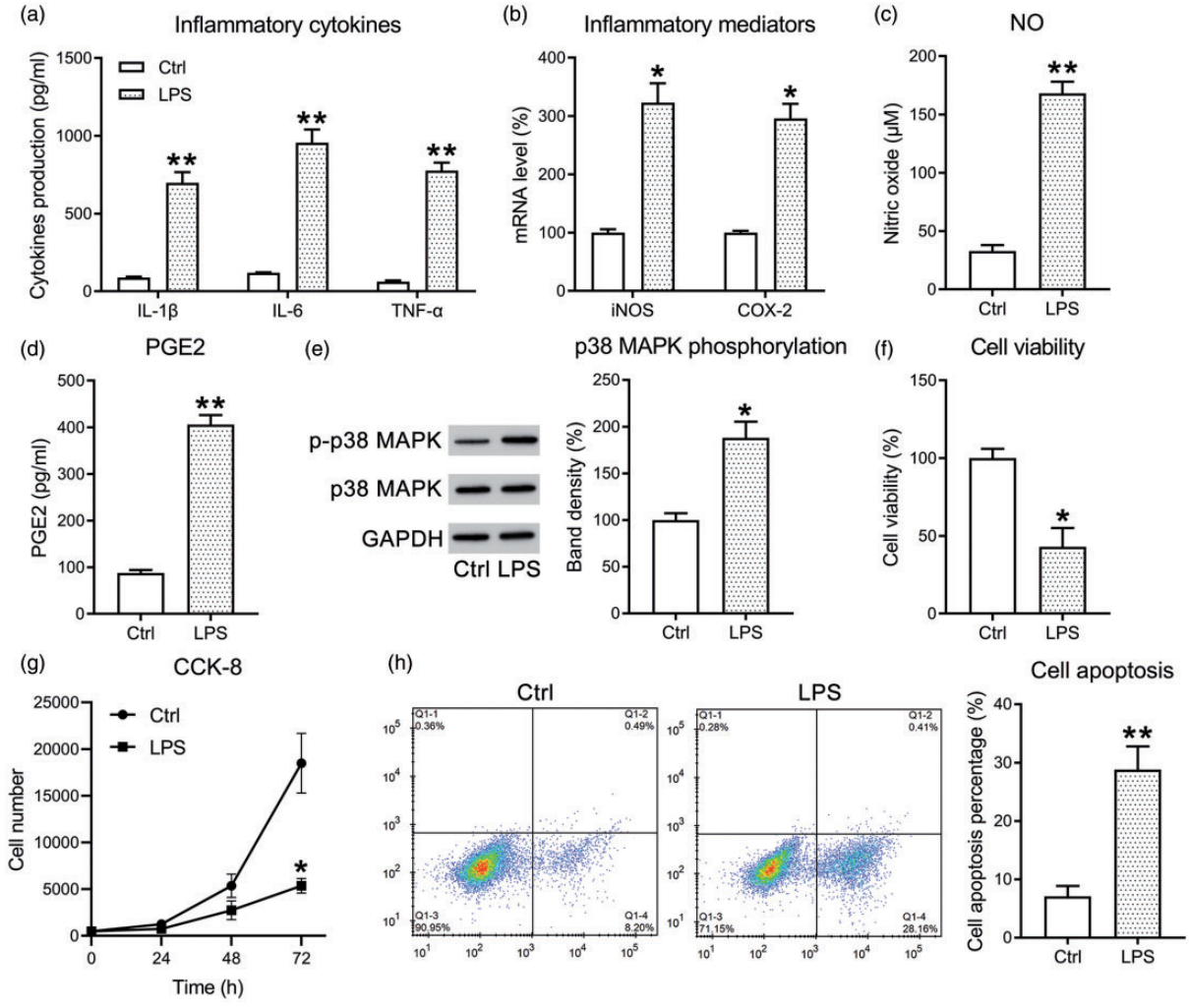

Figure 1. LPS treatment induced inflammation and cell death of primary chondrocytes. Chondrocytes was treated with 1 μg/mL LPS. (a) ELISA was carried out to detect expression levels of IL-1β, IL-6, and TNF-α in chondrocytes with (or without) LPS treatment. (b) qPCR analysis was performed to detect iNOS and COX-2 levels in each group. (c) NO levels were detected via Griess method. (d) ELISA was used to detect the expression levels of PGE2. (e) Expression and phosphorylation of p38 MAPK was assessed by WB. (f) MTT assay was used to detect cell survival rates of chondrocytes after LPS treatment. (g) CCK-8 assay was utilized to examine the cell proliferation rates of chondrocytes after LPS treatment. (h) FCM was used to detect cell apoptosis of chondrocytes after LPS induction. $^{*}P < 0.05$, $^{**}P < 0.01$ vs. indicated group. (A color version of this figure is available in the online journal.)

with 0.4% BSA in PBST at 37°C, and then incubated for 1 h with STAT3 antibodies, diluted with 0.2% BSA in PBST at 37°C. Nuclear staining with DAPI was performed after a 1 h wash with PBST, followed by a 1 h cell incubation with 0.2% BSA-diluted goat anti-rabbit antibody labeled by TRITC in PBST at 37°C, and finally a 1 h wash with PBST. STAT3 staining in cells was analyzed by confocal laser scanning fluorescence microscopy (Olympus LSCMFV500, Tokyo, Japan). At least 100 cells from each group were scored as predominantly nuclear STAT3 cells, and the percentage of total cells is calculated.

SiRNA silencing experiment

Following the manufacturer's instructions, Lipofectamine 2000 (Invitrogen) was used to transfect synthesized siRNAs that targeted FOXM1 (siRNA-FOXM1) in the chondrocytes, or scramble siRNAs (NC siRNA; Invitrogen) as a control. The cells were collected for analyses 48 h after transfection.

Statistical analysis

All data are expressed with means ± SD. A one-way variance analysis (ANOVA) was employed to compare the differences among multiple groups, and the unpaired *T*-test was used to compare the differences between two groups. The differences were marked as significant if $P < 0.05$.

Results

JAK1/STAT3 pathway was activated in LPS-induced chondrocytes

LPS was used to induce inflammation and cell death in primary chondrocytes. As showed in Figure 1(a), three important inflammatory markers (IL-1β, IL-6, and TNF-α) were up-regulated in the supernatant in the cell culture medium checked by ELISA. The qPCR results showed two inflammation mediators, inducible nitric oxide synthases (iNOS) and cyclooxygenase-2 (COX-2), were also elevated due to LPS stimulation (Figure 1(b)). We found that LPS induction resulted in the robust manufacturing of NO and PGE-2 in the cell culture supernatant (Figure 1 (c) and (d)). Because p38 MAPK signaling pathway attributed to IL-1β upregulation in chondrocytes,[23] we examined the activation of p38 MAPK after LPS stimulation. The results showed that MAPK pathway is activated after LPS

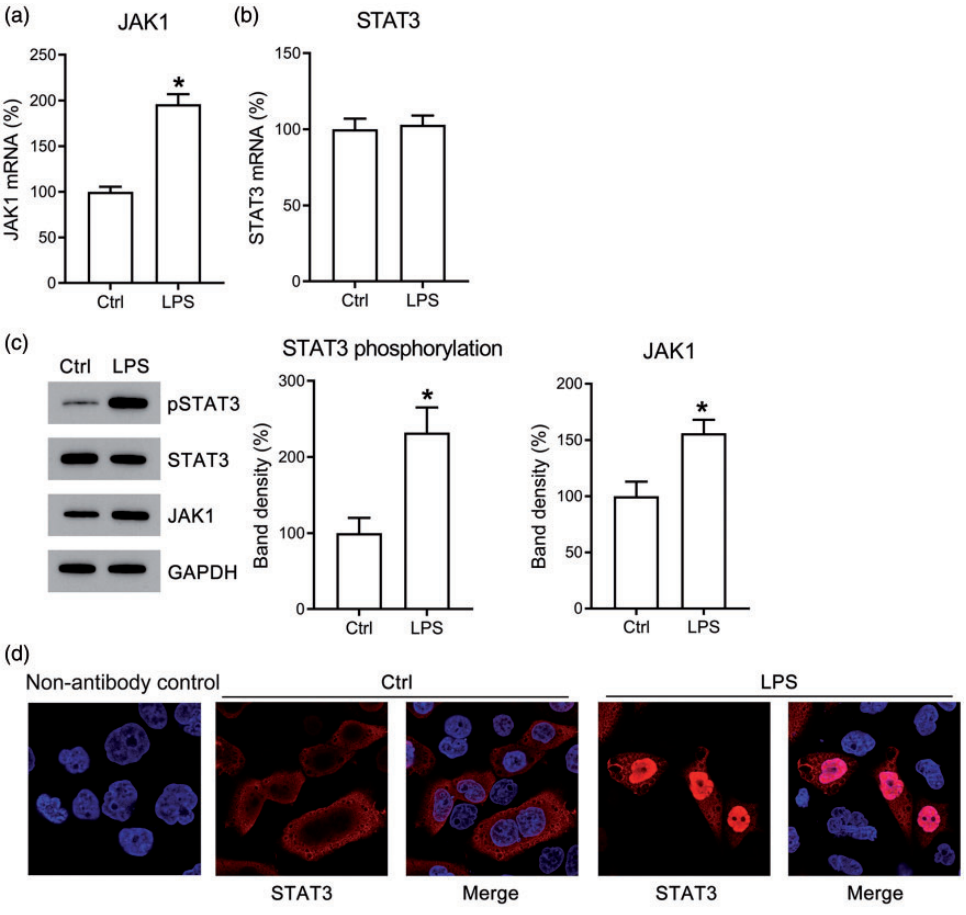

Figure 2. JAK1 and STAT3 pathway in LPS-treated chondrocytes. Chondrocytes were treated with 1 μg/mL LPS. (a and b) qPCR was carried out to measure the expression levels of JAK1 and STAT3 in chondrocytes. (c) Expression levels of JAK1 and STAT3, and phosphorylated STAT3, were examined via WB. (d) Subcellular localization of STAT3 in chondrocytes was detected via IFA. STAT3 (red) and nuclear DNA (blue). Data are expressed with mean ± SD. *$P < 0.05$ vs. indicated group. (A color version of this figure is available in the online journal.)

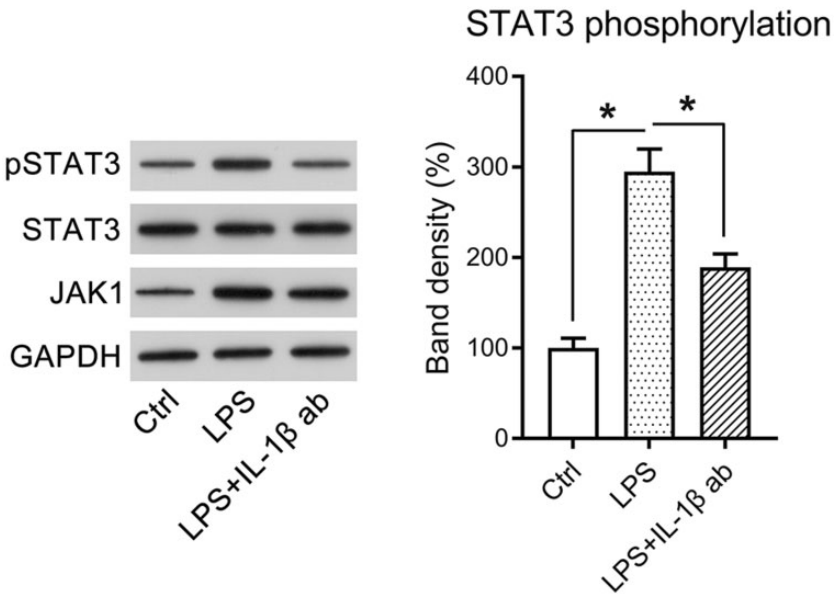

Figure 3. Effect of IL-1β antibody incubation on STAT3 phosphorylation in LPS-treated chondrocytes. Chondrocytes were treated with 1 μg/mL LPS and IL-1β antibody (1:2500) for 12 h. Expression levels of JAK1 and STAT3, and phosphorylated STAT3, were examined via WB. Data are expressed with mean ± SD. $^{*}P < 0.05$ vs. indicated group.

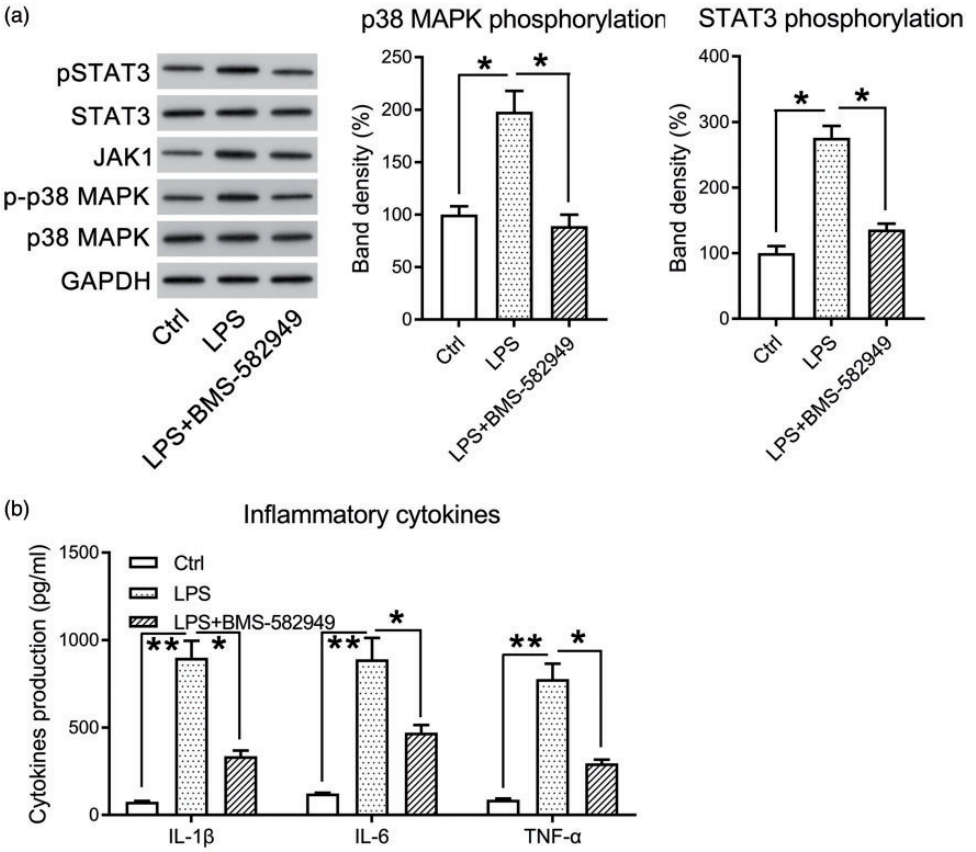

Figure 4. BMS-582949 treatment reduced inflammation and deactivated STAT3 sensor. Chondrocytes were treated with 1 μg/mL LPS with (or without) 5 μM BMS-582949. (a) Expressed p38 MAPK, JAK1 and STAT3, and phosphorylated p38 and STAT3 were examined by WB analysis. (b) ELISA was carried out to detect expression levels of IL-1β, IL-6, and TNF-α in chondrocytes with LPS treatment. Data are expressed with mean ± SD. $^{*}P < 0.05$, $^{**}P < 0.01$.

treatment, as evidenced by p38 MAPK phosphorylation (Figure 1(e)). We then assessed the role of LPS treatment and cell viability. Both the CCK-8 and MTT assays showed chondrocyte proliferation and survival was reduced after LPS induction (Figure 1(f) and (g)). FCM data also indicated that LPS induction triggered cell apoptosis in chondrocyte (Figure 1(h)).

To assess the effects of the JAK1/STAT3 pathway during LPS-induced inflammation in chondrocytes, we first examined JAK1 and STAT3 expression at both mRNA and protein levels. qPCR and WB testing showed JAK1 expression was elevated due to LPS stimulation at the level in both mRNA and protein, while STAT3 expression levels were not significantly changed after LPS administration (Figure 2(a) to (c)); however, the WB detected that STAT3 phosphorylation was significantly increased (Figure 2(c)). Immunofluorescence assay (IFA) showed that LPS treatment also led to a nuclear accumulation of STAT3 in the chondrocytes (Figure 2(d)). These results indicate LPS could induce activation of the JAK1/STAT3 signal pathway.

Deactivation of JAK1/STAT3 signal pathway abated the effect of LPS on chondrocytes

We next attempt to assess whether monoclonal antibodies that neutralizing IL-1β can block the induction of JAK1 and the phosphorylation of STAT3 in the presence of LPS, since IL-1β, IL-6, and TNF-α can activate JAK-STAT signaling. WB analysis showed that expression of JAK1 and phosphorylation of STAT3 was partially blocked by antibody incubation, suggesting that IL-1β partially contributed to LPS-induced activation of JAK1/STAT3 pathway (Figure 3).

Moreover, to evaluate whether p38 MAPK activation is responsible for inflammatory cytokines generation and STAT3 phosphorylation, ELISA and WB were performed after the cells were treated with BMS-582949 (5 μM), a p38 MAPK inhibitor,[24] for 6 h. The data showed that production of inflammatory cytokines and phosphorylation of STAT3 were repressed by BMS-582949 treatment (Figure 4(a) and (b)), suggesting that MAPK pathway acts as upstream modulator for LPS-induced inflammation in chondrocytes.

We utilized WP1066, a STAT3 inhibitor,[25] to block JAK1/STAT3 and evaluate the impact of JAK1/STAT3 on inflammation and chondrocyte cell survival. A WB analysis showed that WP1066 treatment did not result in the any changes in expression of both JAK1 and STAT3; however, STAT3 phosphorylation was observed as reduced by WP1066 (Figure 5(a)). Meanwhile, IFA showed that WP1066 treatment blocked the nuclear import of STAT3 in chondrocytes (Figure 5(b)). These findings suggest that the JAK1/STAT3 pathway was blocked due to WP1066.

Furthermore, we found that WP1066 treatment resulted in decreased expression levels of inflammatory cell factors (IL-1β, IL-6, and TNF-α; Figure 6(a)), and two inflammatory

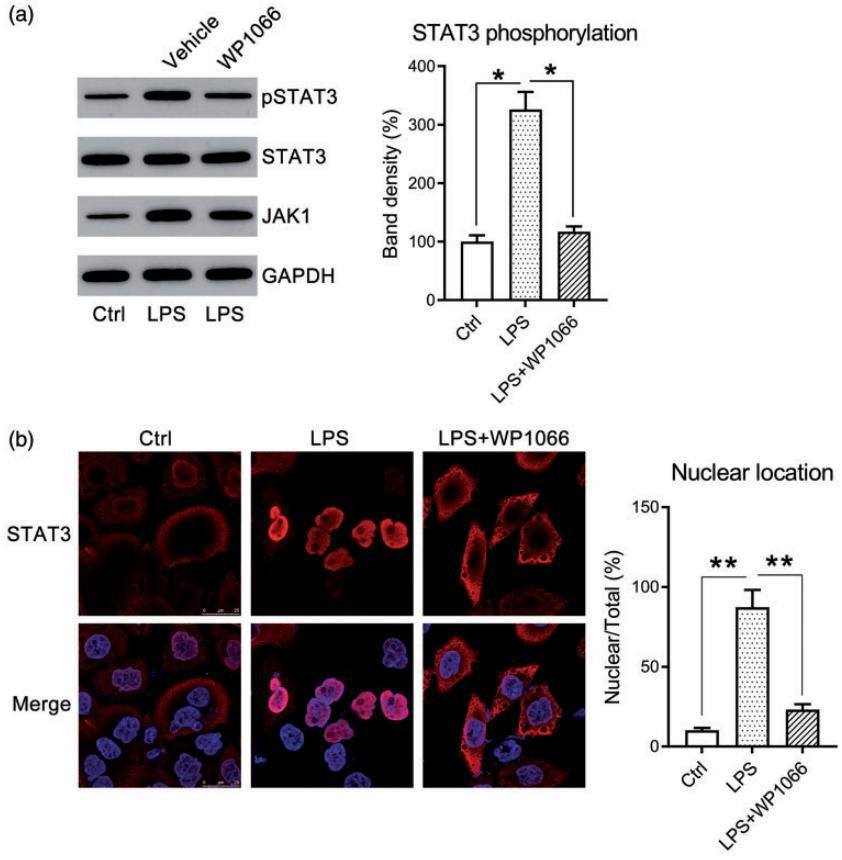

Figure 5. WP1066 treatment deactivated STAT3 sensor. Chondrocytes were treated with 1 μg/mL LPS with (or without) 500 ng/mL WP1066. (a) Expressed JAK1 and STAT3, and phosphorylated STAT3 were examined by WB analysis. (b) Subcellular localization of STAT3 in chondrocytes was detected via IFA. STAT3 (red) and nuclear DNA (blue). Data are expressed with mean ± SD. $^{*}P < 0.05$, $^{**}P < 0.01$. (A color version of this figure is available in the online journal.)

mediators (iNOS and COX-2; Figure 6(b)). The robust generation of NO and PGE-2 was ameliorated after WP1066 administration (Figure 6(c) and (d)). On the other hand, the CCK-8 and MTT assay indicated that WP1066 treatment was able to restore cell survival and proliferation (Figure 6(e) and (f)). Furthermore, FCM data displayed that WP1066 treatment repressed LPS-induced apoptosis to a normal level (Figure 6(g)) suggesting that the JAK1/STAT3 signal pathway participated in LPS-induced inflammation and cell death in chondrocytes.

FOXM1 interacts with STAT3 and positively associates with activation of the JAK1/STAT3 pathway

Previous studies have demonstrated FOXM1 is a key regulator of inflammation during OA pathogenesis.[13] We sought to further examine the relationship between FOXM1 and the JAK1/STAT3 pathway. Co-immunoprecipitation (Co-IP) assays showed that Myc-FOXM1 was capable of interacting with the Myc-STAT3 protein (Figure 7(a)), rather than JAK1 (data not shown). We then used both qPCR and WB analysis to evaluate if the

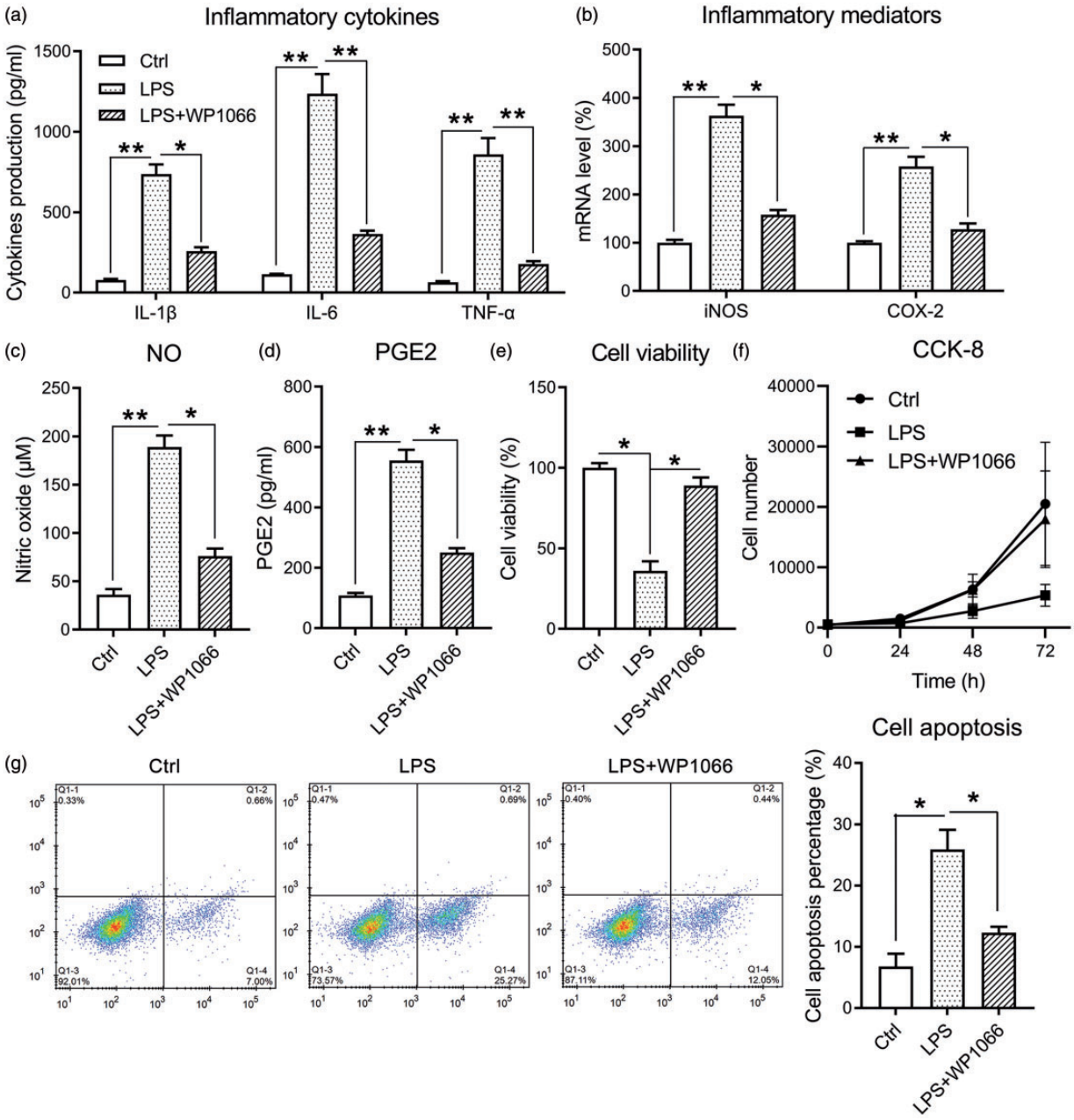

Figure 6. WP1066 treatment reduced inflammation and cell death of chondrocytes. Chondrocytes were treated with 1 μg/mL LPS with (or without) 500 ng/mL WP1066. (a) ELISA was carried out to detect expression levels of IL-1β, IL-6, and TNF-α in chondrocytes with (or without) LPS/WP1066 treatment. (b) qPCR analysis was performed to detect iNOS and COX-2 levels in each group. (c) NO levels were detected via Griess method. (d) ELISA was used to detect expression levels of PGE2. (e) MTT assay was used to detect cell survival rates of chondrocytes after LPS treatment. (f) CCK-8 assay was carried out to examine the cell proliferation rates of chondrocytes after LPS treatment. (g) FCM was performed to assess the cell apoptosis rates in chondrocytes after LPS stimulation. $^{*}P < 0.05$, $^{**}P < 0.01$ vs. indicated group. (A color version of this figure is available in the online journal.)

expressed and phosphorylated JAK1 and STAT3 were correlative to that of FOXM1 in LPS-stimulated chondrocytes. Cells were induced with 0.5 μg/mL LPS, 1 μg/mL LPS or none. JAK1 expression and STAT3 phosphorylation gradually increased in cells following with LPS treatment, while expression levels of FOXM1 also elevated (Figure 7(b) and (d)). In order to further identify the regulatory role of STAT3 activation to FOXM1 expression, WP1066 was administrated. We found that FOXM1 expression was clearly downregulated due to WP1066 treatment (Figure 7(c) and (e)). This data suggests FOXM1 is a downstream modulator of the JAK1/STAT3 pathway.

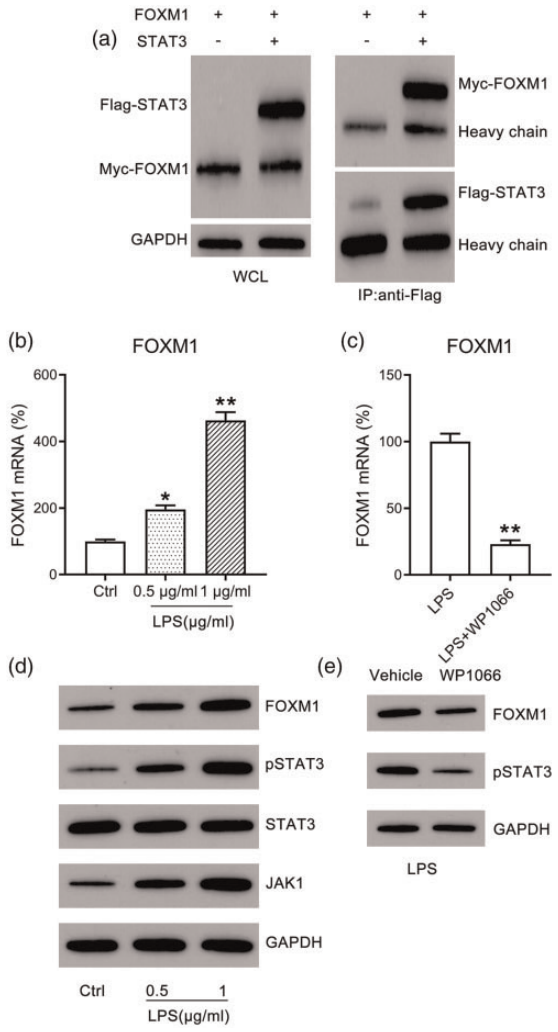

Figure 7. FOXM1 interacts with STAT3, and was regulated through activation of JAK1/STAT3 pathway. (a) Chondrocytes were transfected with pCDNA3-Flag-STAT3 and/or pCMV-Myc-FOXM1 plasmids. Co-IP was carried out to assess the interaction of STAT3 and FOXM1. WB was performed to examine Flag-STAT3 and Myc-FOXM1 expression. (b and d) Chondrocytes were co-induced with 0.5, 1 μg/mL LPS or none. qPCR and WB analysis were carried out to detect the expression levels of JAK1, STAT3, and FOXM1, as well as phosphorylated STAT3 levels. (c and e) Chondrocytes were treated with 1 μg/mL LPS with (or without) 500 ng/mL WP1066. Chondrocytes were co-induced with 0.5 and 1 μg/mL LPS, or none. Expression levels of FOXM1 and phosphorylated STAT3 were examined using qPCR and WB analysis. $^{*}P < 0.05$, $^{**}P < 0.01$ vs. indicated group.

FOXM1 was involved in the JAK1/STAT3 mediated inflammation of chondrocytes

To confirm the effect of FOXM1 in JAK1/STAT3-regulated inflammation and cell death in LPS-induced chondrocytes, siRNA was used to knockdown FOXM1 in LPS-treated cartilage cells. Both qPCR and WB demonstrated that FOXM1 expression was silenced after transfection with FOXM1 siRNA (Figure 8(a) and (b)). Meanwhile, ELISA and qPCR data showed that three inflammatory cell factors (IL-1β, IL-6, and TNF-α) and four inflammatory mediating factors (iNOS, COX-2, NO, and PGE2) were partially downregulated due to FOXM1 silencing (Figure 8(c) to (f)). MTT and CCK-8 assays showed that the proliferation and survival of LPS-induced chondrocytes was restored after FOXM1 silencing (Figure 8(g) and (h)). FCM results indicated that FOXM1 silencing caused a reduction of apoptotic cell proportion (Figure 8(i)). These results indicate FOXM1 is involved as a downstream regulator in the JAK1/STAT3-regulated inflammation and cell death of chondrocytes.

Discussion

OA is the most prevalent arthropathy, usually caused by aging and the destruction of the articular cartilage surface. Aging, mechanical loading abnormality, trauma, genetic inheritance, and other factors are associated with OA occurrence. An increasing number of studies have confirmed that chondrocyte survival is related to joint damage in OA patients. In this research, the effects of JAK1 and STAT3 in the regulation of OA were studied through the chondrocytic inflammation pathway induced by LPS. It has been reported that LPS is the regulatory factor for the inflammatory reaction seen in the disease progression of OA, LPS activates cells of the innate immune system, such as macrophages and neutrophils, which synthesize proinflammatory factors, such as IL-1β and TNF, MMPs and free radicals that lead to dramatic secondary inflammation in tissues.[14] Therefore, LPS was employed to form an OA cell model for *in vitro* study.[17] This study showed that JAK1 expression and STAT3 phosphorylation were significantly increased after LPS simulation of chondrocytes, suggesting that activation of the JAK1/STAT3 pathway may act as a regulatory pathway in LPS-induced chondrocyte inflammation. Our data also suggest the JAK1/STAT3 signal pathway promotes inflammation and cell death in LPS-treated chondrocytes.

The JAK/STAT signaling pathway could be negatively affected by the mediating effects of the suppressor of cytokine signal protein, an inhibitor in RA and OA.[26] Lim and Kim report that the JAK2/STAT1/2 signal participated in induction of MMP-13 in IL-1β-induced cartilage cells.[27] It was suggested that the IL-6 receptor was also an underlying therapeutic target in OA drug development, which could improve degradation of cartilage extracellular protein through the JAK/STAT signal pathways.[28] Previous studies have also reported that FOXM1 knockdown could weaken the inflammatory reaction of cartilage cells in human OA,[13] and FOXM1 expression was increased in cartilage cells treated by IL-1β. FOXM1 knockdown weakened IL-1β-induced cell viability decline, and inhibited the

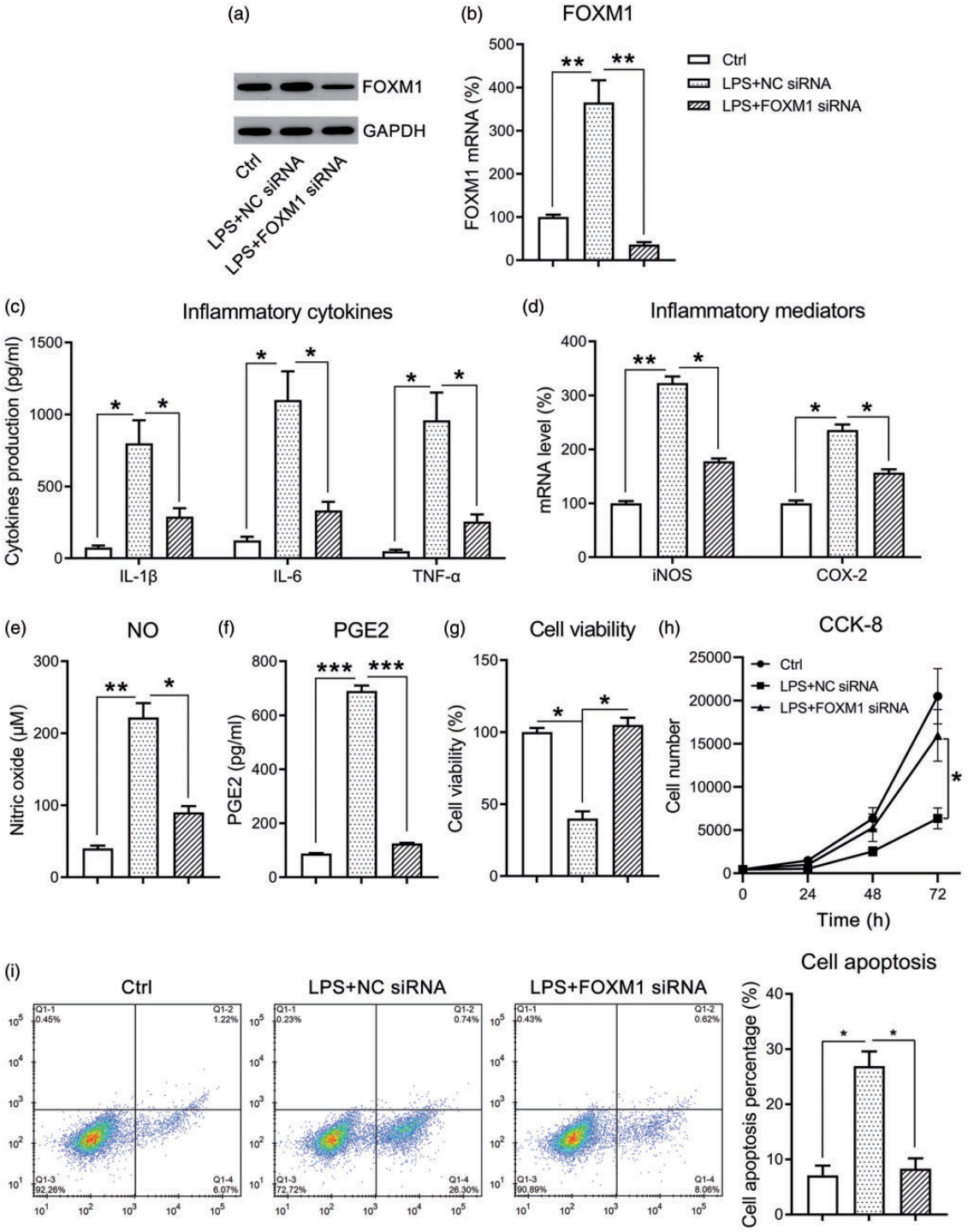

Figure 8. FOXM1 silencing reversed LPS-treated inflammation and apoptosis of chondrocytes. Primary chondrocytes experienced 12 h transfections with siRNA-FOXM1 or siRNA-NC, and were treated with LPS for 36 h. (a and b) Expression levels of FOXM1 were examined using WB and qPCR analysis. (c and d) ELISA was carried out to detect expression levels of IL-1β, IL-6, and TNF-α in chondrocytes undergoing different treatments. (e) qPCR analysis was used to detect iNOS and COX-2 levels in each group. (f) NO levels were detected via Griess method. (d) ELISA was used to detect the expressed levels of PGE2. (g) MTT assay was used to detect the survival rates of chondrocytes after LPS treatment. (h) CCK-8 assay was carried out to examine the cell proliferation rates in chondrocytes after LPS treatment. (i) FCM was performed to assess the cell apoptosis rates in chondrocytes after LPS stimulation. Data have been expressed with mean ± SD. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ vs. indicated group. (A color version of this figure is available in the online journal.)

generation of TNF-α, IL-6 and other inflammatory cell factors treated by IL-1β. It was also seen that silencing FOXM1 regressed the activation of IL-1β-treated NF-κB in cartilage cells.[13] Fatty acid binding protein 4 caused airway inflammation and epithelial barrier dysfunction through ROS-activated FOXM1, while the suppression of FOXM1 inhibits the influence of fatty acid binding protein 4 in an inflammatory reaction and improves epithelial barrier dysfunction.[11] Overexpression of lncRNA CRNDE increased LPS-treated cell death and inflammation by upregulating

the expression of FOXM1 in diploid fibroblasts derived from embryonic lung tissue.[12] In this study, the data showed that LPS stimulation could activate the JAK1/STAT3 and FOXM1 signal pathway in chondrocytes, and WP1066 could repress the role of JAK1/STAT3 signaling in the inflammation reaction, and cell apoptosis in cartilage cells. Moreover, data from Co-IP assays indicated that STAT3 could interact with FOXM1, and deactivation of STAT3 led to reduction of FOXM1 expression. These findings show that the JAK1/STAT3 signaling pathway participated in the LPS-treated inflammation reaction, and cell apoptosis pathway in cartilage cells via mediating FOXM1 expression.

In our study, the co-IP assay for the interaction between STAT3 and FOXM1 also showed both proteins are key modulators of inflammation. The fundamental mechanism of the JAK1/STAT3 pathway inactivation on chondrocyte protection might be realized by inhibiting the expression of FOXM1.

These results offer new clues in the effects of JAK1/STAT3 in the cellular morbidity course of OA.

AUTHORS' CONTRIBUTIONS

ZRM and LXH conceived the study and designed the experiments. LJ, RZJ, and FJZ contributed to the data collection; LZW and ZWT performed the data analysis and interpreted the results. ZRM wrote the manuscript; ZRM and LXH revised the article. All authors read and approved the final manuscript.

DECLARATION OF CONFLICTING INTERESTS

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