FABP4 knockdown suppresses inflammation, apoptosis and extracellular matrix degradation in IL-1β-induced chondrocytes by activating PPARγ to regulate the NF-κB signaling pathway

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Received July 26, 2021; Accepted September 15, 2021

DOI: 10.3892/mmr.2021.12495

Abstract. Osteoarthritis (OA) is a common degenerative disease that can lead to severe joint pain and loss of function, seriously threatening the health and normal life of patients. At present, the pathogenesis of OA remains to be clarified. Recent studies have shown that fatty acid-binding protein 4 (FABP4) is increased in the plasma and synovial fluid of patients with OA. However, the effect of FABP4 on OA is unclear. The present study established IL-1β-induced ATDC5 cells with FABP4 knockdown. Next, cell viability was detected with Cell Counting Kit-8 assay. The content of inflammatory factors, prostaglandin E2 and glycosaminoglycan (GAG) was detected via ELISA. The levels of reactive oxygen species (ROS) and superoxide dismutase (SOD) in cells were detected by using ROS and SOD kits, respectively. TUNEL staining was used to detect the apoptosis level. Western blotting was used to detect the expression levels of proteins. The results revealed that FABP4 was upregulated in IL-1β-induced ATDC5 cells. Knockdown of FABP4 increased cell viability, reduced inflammatory damage, oxidative stress and apoptosis in IL-1β-induced ATDC5 cells. Following FABP4 knockdown, the expression of matrix metalloproteinases (MMP3, MMP9 and MMP13) of IL-1β-induced ATDC5 cells was reduced, and the expression of GAG was promoted. FABP4 knockdown also inhibited the expression of NF-κB p65 and enhanced peroxisome proliferator-activated receptor (PPAR)γ expression. However, the presence of PPARγ inhibitor blocked the aforementioned effects of FABP4 on IL-1β-induced ATDC5 cells. In conclusion, FABP4 knockdown suppressed the inflammation, oxidative stress, apoptosis and extracellular matrix degradation of IL-1β-induced chondrocytes by activating PPARγ to inhibit the NF-κB signaling pathway.

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Key words: osteoarthritis, fatty acid-binding protein 4, peroxisome proliferator- activated receptor γ, NF-κB signaling pathway, inflammation

Introduction

Osteoarthritis (OA) is the most common bone and joint disease in the elderly population. Its main characteristics include synovitis, degeneration and destruction of cartilage, and sclerosis of subchondral bone (1). Epidemiological statistics show that the incidence rate of OA among patients >55 years of age is 44-70% (1). Patients with OA have a poorer quality of life, present with numerous clinical symptoms and the efficacy of treatment is low, which poses a heavy burden for patients' relatives and society. There is currently no effective treatment to prevent the occurrence and delay the progression of OA (2,3). During OA, biochemical pathways of chondrocytes are altered, leading to an increase in inflammatory factors and the degradation of the extracellular matrix (ECM) (4). Abnormal gene expression in chondrocytes has been reported to be associated with cartilage erosion (5). Therefore, it is necessary to explore the regulatory mechanisms of genes affecting chondrocyte inflammation and ECM degradation.

Fatty acid binding protein (FABP) is a fat-binding protein present inside cells and is a member of the intracellular lipid-binding protein family (6), which consists of intracellular proteins with low molecular weight, 126-134 amino acid sequences and a strong affinity for long-chain fatty acids (7). FABP4 is a member of the FABP family, and is mainly expressed in mature adipocytes and macrophages, and is secreted by adipocytes in response to cAMP, thus playing a role in cellular lipid fluxes, metabolism and signaling (8). In addition to regulating lipid metabolism, FABP4 has been found to be involved in multiple biological processes, including inflammation (9). FABP4 has been found to contribute to the pathogenesis of inflammatory-mediated diseases, and downregulation of it can suppress inflammation, apoptosis and oxidative stress in these diseases (10-13). As an inflammatory arthritis, OA has been reported to be associated with the abnormal expression of FABP4. A previous study suggested that FABP4 expression is increased in plasma and synovial fluid in patients with OA (14). Another study validated that FABP4 could serve as

a biomarker of OA via bioinformatics analysis (15). Recently, FABP4 levels were found to be negatively associated with cartilage thickness in end-stage knee OA (16). The aforementioned reports provide evidence that FABP4 is involved in the occurrence and progression OA. However, the specific effect of FABP4 in the regulation of OA has not been investigated thus far, to the best of our knowledge. Therefore, the present study aimed to clarify the effect of FABP4 on IL-1β-induced chondrocyte inflammation and the potential mechanisms *in vitro*.

Materials and methods

Cell culture. ATDC5 cells were purchased from BeNa Culture Collection (Beijing Beina Chunglian Institute of Biotechnology). The cells were cultured in DMEM/Ham's F12 (Thermo Fisher Scientific, Inc.) supplemented with 5% FBS (Thermo Fisher Scientific, Inc.) and 100 U/ml penicillin/streptomycin in a humidified incubator at 37˚C with 5% CO_2. Cells were then treated with 10 ng/ml IL-1β (MilliporeSigma), with or without 10 μM GW9662 [peroxisome proliferator-activated receptor (PPAR)γ inhibitor; Sigma-Aldrich; Merck KGaA] co-treatment at 37˚C for 24 h.

Cell transfection. Small interfering RNAs (siRNAs) against FABP4 (si-FABP4-1 and si-FABP4-2) and siRNA negative control (NC; si-NC) were purchased from Shanghai GenePharma Co., Ltd. ATDC5 cells ($1x10^6$ per well) were seeded into 6-well plates and cultured with complete medium without antibiotics for ≥24 h prior to transfection. Next, cells were transiently transfected with 2 μg/ml siRNAs using Lipofectamine® 3000 (Invitrogen; Thermo Fisher Scientific, Inc.) and incubated at 37˚C for 6 h. The cells were harvested 48 h for analysis post-transfection. The sequences were as follows: si-FABP4-1, 5'-GACGUUGACCUGGACUGAAd TdT-3' and 3'-UUCAGUCCAGGUCAACGUCdTdT-5'; si-FABP4-2, 5'-GUGGGAUAUAUUGUUCAAAdTdT-3' and 3'-UUUGAACAAUAU-AUCCCACdTdT-5'; and si-NC, 5'-UUAUGCCGAUCGCGUCACATT-3' and 3'-TTAAUA CGGCUAGCGCAG-UGU-5'.

Bioinformatics analysis. To verify whether PPARγ was a target of FABP4, the database Search Tool for the Retrieval of Interacting Genes/Proteins (STRING; https://string-db.org/) was searched.

Cell Counting Kit-8 (CCK-8) assay. ATDC5 cells were plated into four 96-well plates ($2x10^4$ cells per well). Upon culture overnight for adherence, cells were treated with IL-1β or GW9662 co-treatment for 24 h, then CCK-8 solution (Dojindo Laboratories, Inc.) was diluted with culture medium and added to the 96-well plates, followed by incubation for 1 h at 37˚C. Finally, the absorbance at 450 nm was detected with a spectrophotometer (Thermo Fisher Scientific, Inc.).

TUNEL assay. TUNEL staining was performed by using an In Situ Apoptosis Detection Kit (MilliporeSigma) according to the manufacturer's protocol. Briefly, ATDC5 cells ($2x10^4$) cultured in 96-well plates were washed with PBS and fixed with 4% paraformaldehyde for 30 min at room temperature. Cells were then incubated for 90 min at 37˚C with terminal deoxynucleotidyl transferase (TdT) incubation buffer. NC cells were incubated without TdT enzyme. The reaction was terminated by washing the cells with PBS. The nuclei were stained with 5 μg/ml DAPI at room temperature for 5 min, followed by observation under a fluorescence microscope in three fields of view (Nikon Eclipse 80i; Nikon Corporation; magnification, x200).

ELISA. The levels of TNF-α (cat. no. PT512) and IL-6 (cat. no. PI326) in ATDC5 cell culture supernatant were measured with commercially available standard sandwich ELISA kits (Beyotime Institute of Biotechnology) in accordance with the manufacturer's instructions. Each sample was measured in triplicate. The level of prostaglandin E2 (PGE_2) was determined using a Prostaglandin E2 ELISA kit (cat. no. ab133021; Abcam). Reactive oxygen species (ROS) content in cells was detected by using a ROS/Superoxide Detection Assay kit (Cell-based) (cat. no. ab139476; Abcam). The expression of superoxide dismutase (SOD) was detected using a SOD kit (cat. no. ab277415; Abcam). The expression of glycosaminoglycan (GAG) was detected by spectrophotometry using 1,9-dimethylmethylene blue (DMMB; Sigma-Aldrich; Merck KGaA), which can be used to monitor the level of sulfated GAG, as previously reported (17).

Reverse transcription-quantitative PCR (RT-qPCR). Total RNA from ATDC5 cells was extracted with TRIzol® (Thermo Fisher Scientific, Inc.) according to the supplier's protocol. Next, RT kits (Takara Bio, Inc.) were used to reverse transcribe RNA into cDNA according to the manufacturer's protocol. Subsequently, 50 ng cDNA was used for qPCR using TB Green® Fast qPCR Mix (Takara Bio, Inc.) and an Applied Biosystems 7500 Real-Time PCR System (Thermo Fisher Scientific, Inc.). The following thermocycling conditions were used for qPCR: 95˚C for 2 min; followed by 40 cycles of 95˚C for 20 sec and 65˚C for 40 sec. The results were analyzed using the $2^{-\Delta\Delta Cq}$ method (18) with Gapdh as the internal reference gene. The primers were as follows: FABP4 forward, 5'-TTCCT TCAAACTGGGCGTGG-3' and reverse, 5'-GCCTTTCATAA CACATTCCACC-3'; matrix metalloproteinase (Mmp)3 forward, 5'-TCCCACATCACCTACAGGATTG-3' and reverse, 5'-CAG GCCCATC AAAAGGGACA-3'; Mmp9 forward, 5'-CAGCCG ACTTTTGTGGTCTTC-3' and reverse, 5'- CGGTACAAGTAT GCCTCTGCCA-3'; Mmp13 forward, 5'-GGAGCCCTGAT GTTTCCCAT-3' and reverse, 5'-GTCTTCATCGCCTGGA CCATA-3'; ADAM metalloproteinase with thrombospondin type 1 motif 4 (Adamts-4) forward, 5'-CAAGCATCCGAAAC CCTGTC-3' and reverse, 5'-ACACAGGTCCTGCCGGG-3'; and Gapdh forward, 5'-GGGTCCCAGCTTAGGTTCATC-3' and reverse, 5'-CCAATACGGCCAAATCCGTTC-3'.

Western blot assay. Total protein was extracted from ATDC5 cells using a RIPA kit (Beyotime Institute of Biotechnology). Harvested cells were lysed on ice. Protein concentrations of the cell supernatants were determined using a BCA Protein Assay kit (Beyotime Institute of Biotechnology). The samples were mixed with loading buffer, and heated in boiling water for 5 min. Equal quantities of proteins (40 μg per lane) were separated via 10-12% SDS-PAGE and then transferred to a

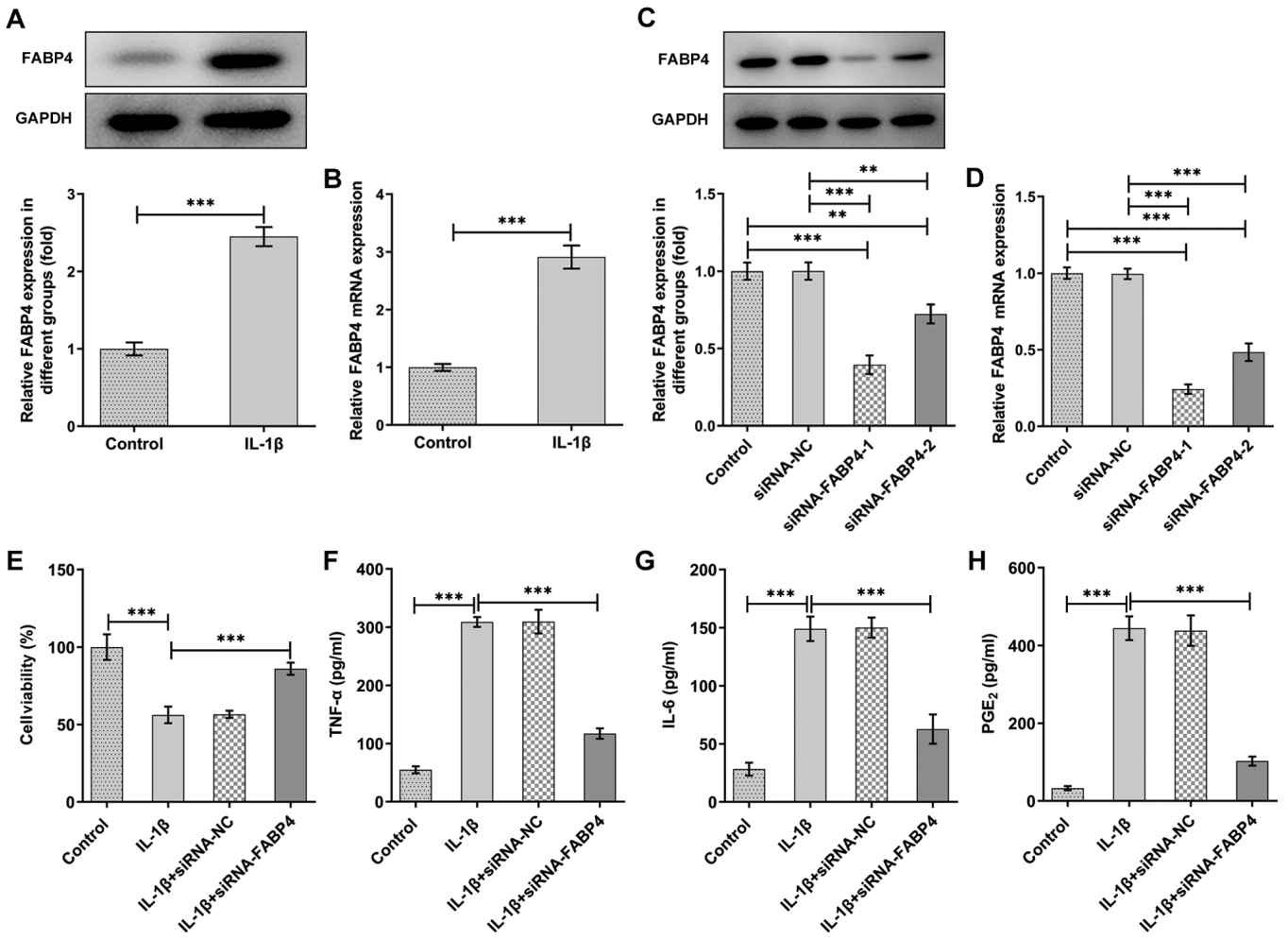

Figure 1. Knockdown of FABP4 suppresses the activity and inflammatory damage of IL-1β-induced ATDC5 cells. (A) Western blotting and (B) RT-qPCR revealed that FABP4 was significantly increased in IL-1β-induced ATDC5 cells compared with that in control ATDC5 cells (n=3). Transfection efficiency of siRNA-FABP4-1 and siRNA-FABP4-2 was verified by using (C) western blotting and (D) RT-qPCR (n=4). (E) Cell Counting Kit-8 assay was performed to detect changes in the viability of IL-1β-induced ATDC5 cells (n=3). The levels of (F) TNF-α, (G) IL-6 and the (H) cell growth and regulatory factor PGE_2 were determined by using ELISAs (n=5). $^{**}P<0.01$, $^{***}P<0.001$. RT-qPCR, reverse transcription-quantitative PCR; FABP4, fatty acid-binding protein 4; siRNA, small interfering RNA; PGE_2, prostaglandin E2; NC, negative control.

polyvinylidene fluoride membrane (MilliporeSigma). After blocking with 5% non-fat milk in TBS with Tween-20 (TBST) for 2 h at room temperature, the membranes were incubated at 4˚C overnight with specific primary antibodies. Following washing in TBST, the membranes were incubated with secondary goat anti-rabbit IgG antibody (Abcam; 1:10,000; cat. no. ab6721) at room temperature for 2 h and then washed again. Specific protein bands were visualized by using an ECL Plus kit (cat. no. WBKLS0500; MilliporeSigma) with a bio-imaging system (Quantity One, version 4.6.2; Bio-Rad Laboratories, Inc.). The densitometry analysis was performed using ImageJ software (version 1.8.0; National Institutes of Health). All experiments were performed three times independently. The rabbit primary antibodies (Abcam) used included: FABP4 (1:5,000; cat. no. ab92501), Bcl2 (1:1,000; cat. no. ab32124), Bax (1:5,000; cat. no. ab32503), cleaved caspase3 (1:500; cat. no. ab32042), caspase3 (1:2,000; cat. no. ab32351), MMP3 (1:10,000; cat. no. ab52915), MMP9 (1:10,000; cat. no. ab76003), MMP13 (1:1,000; cat. no. ab219620), phosphorylated (p)-NF-κB p65 (1:1,000; cat. no. ab76302), NF-κB p65 (1:5,000; cat. no. ab16502), PPARγ (1:1,000; cat. no. ab178860), β-actin (1:5,000; cat. no. ab8227) and GAPDH (1:10,000; cat. no. ab9485).

Statistical analysis. All experiments performed in the present study were repeated at least three times. The data were analyzed using GraphPad Prism 7.0 (GraphPad Software, Inc.), and are presented as the mean ± SD. Data were confirmed to be normally distributed using the Shapiro-Wilk test. The comparison of two groups was performed using an unpaired Student's t-test. Comparisons between multiple groups were analyzed using one-way ANOVA followed by Tukey's test. $P<0.05$ was considered to indicate a statistically significant difference.

Results

Knockdown of FABP4 suppresses the activity and inflammatory damage of IL-1β-induced ATDC5 cells. To detect the effect of FABP4 on IL-1β-induced ATDC5 cells, the expression of FABP4 was detected in untreated ATDC5 cells and IL-1β-induced ATDC5 cells by using RT-qPCR and western blotting. As shown in Fig. 1A and B, the expression of FABP4

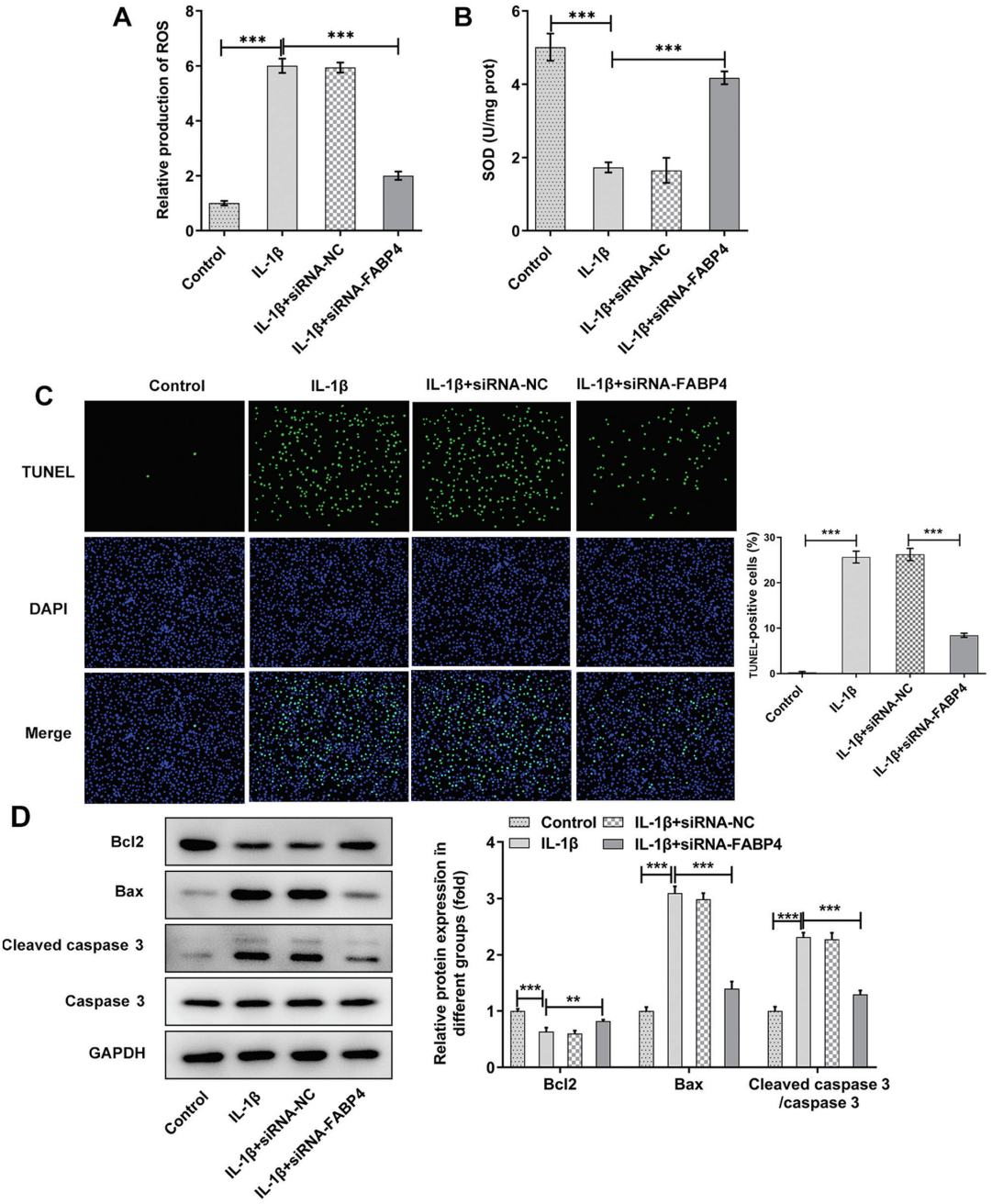

Figure 2. Knockdown of FABP4 suppresses the oxidative stress and apoptosis of IL-1β-induced ATDC5 cells. (A) ROS levels in cells were detected by using a ROS ELISA kit (n=6). (B) Expression of SOD was detected by using a SOD ELISA kit (n=6). (C) Apoptosis level was detected using TUNEL staining (n=4). (D) Western blotting was used to detect the expression of Bcl-2, Bax and cleaved caspase 3 (n=3). **P<0.01, ***P<0.001. FABP4, fatty acid-binding protein 4; siRNA, small interfering RNA; ROS, reactive oxygen species; SOD, superoxide dismutase; NC, negative control.

was upregulated in IL-1β-induced ATDC5 cells compared with that of control ATDC5 cells.

The expression of FABP4 was downregulated in the siRNA-FABP4 groups, and the inhibitory effect of siRNA-FABP4-1 was higher than that of siRNA-FABP4-2 (Fig. 1C and D). Thus, siRNA-FABP4-1 was selected for subsequent experiments. Next, a CCK-8 assay was performed to detect the changes in cell viability of IL-1β-induced ATDC5 cells. The results shown in Fig. 1E revealed that ATDC5 cell viability was reduced after induction with IL-1β, and the inhibitory effect was partly abolished with knockdown of FABP4.

Overproduction of pro-inflammatory cytokines plays a key role in the pathophysiology of OA (4). In the present study, it was observed that IL-1β could significantly elevate the levels of the pro-inflammatory cytokines, TNF-α and IL-6 (Fig. 1F and G), as well as those of the cell growth and regulatory factor, PGE_2 (Fig. 1H). However, knockdown of FABP4 suppressed the production of pro-inflammatory cytokines (Fig. 1F and G) and PGE_2 in IL-1β-induced ATDC5 (Fig. 1H).

Knockdown of FABP4 suppresses oxidative stress and apoptosis in IL-1β-induced ATDC5 cells. The levels of ROS and SOD were detected in untreated ATDC5 cells or IL-1β-induced ATDC5 cells. As shown in Fig. 2A, the expression of ROS was significantly increased (P<0.001) in IL-1β-induced ATDC5 cells and downregulated after the knockdown of FABP4. The

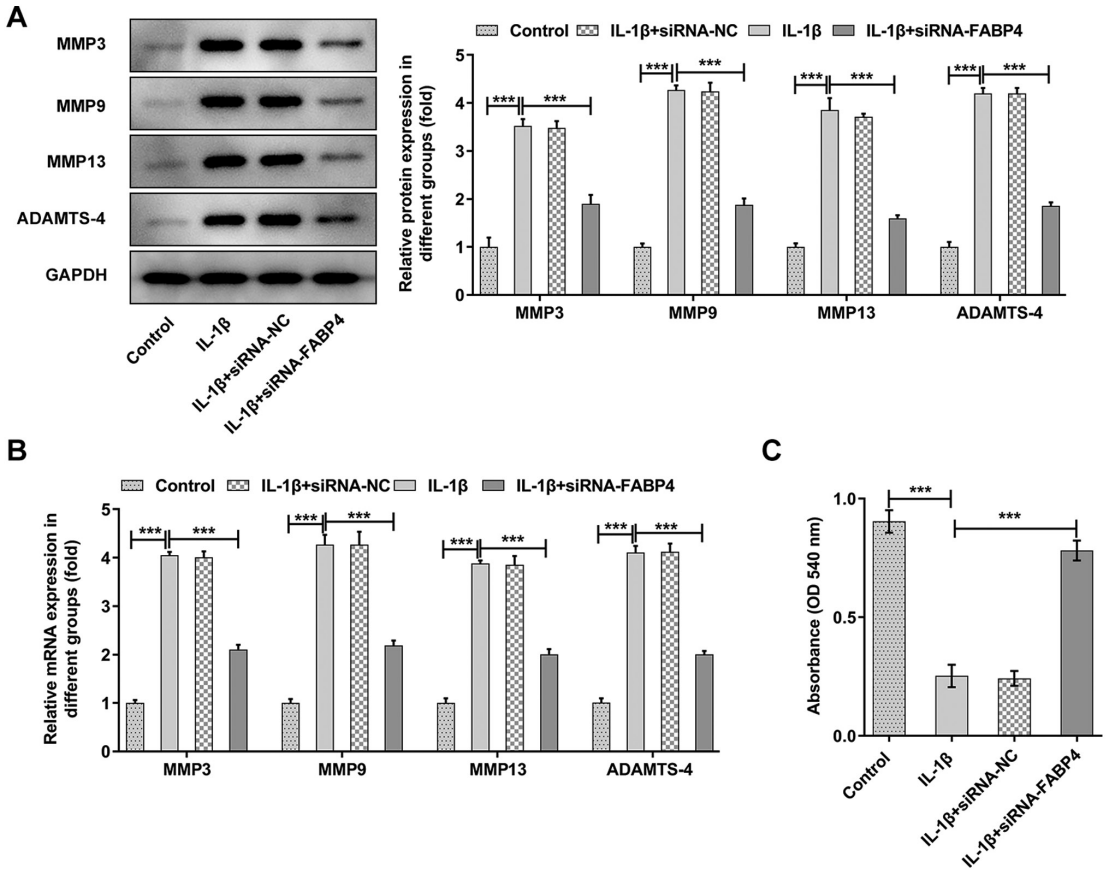

Figure 3. Knockdown of FABP4 suppresses matrix-degrading proteases and promotes the expression of GAG. (A) Western blotting and (B) reverse transcription-quantitative PCR assay were used to detect the expression of MMP3, MMP9, MMP13 and ADAMTS-4 (n=4). (C) Expression of GAG was detected by using a GAG ELISA kit (n=3). ***P<0.001. FABP4, fatty acid-binding protein 4; siRNA, small interfering RNA; GAG, glycosaminoglycan; ADAMTS-4, ADAM metalloproteinase with thrombospondin type 1 motif 4; NC, negative control.

expression trend of SOD showed the opposite trend to that of ROS (Fig. 2B).

TUNEL staining revealed that IL-1β increased apoptosis, and knockdown of FABP4 could weaken the pro-apoptotic effect of IL-1β (Fig. 2C). As shown in Fig. 2D, compared with IL-1β-induced ATDC5 cells, it was found that knockdown of FABP4 reduced cell apoptosis through downregulating Bax and cleaved caspase 3 expression, and upregulating Bcl-2 expression in IL-1β-induced ATDC5 cells.

Knockdown of FABP4 suppresses the expression of MMPs and promotes the expression of GAG. The expression of MMP3, MMP9, MMP13 and ADAMTS-4 was significantly increased in the IL-1β group (P<0.001), and knockdown of FABP4 could partly reverse the expression of MMP3, MMP9, MMP13 and ADAMTS-4 (Fig. 3A and B). Next, the expression of GAG was detected with a GAG ELISA kit. As shown in Fig. 3C, it was observed that IL-1β could significantly (P<0.001) reduce the levels of GAG, and knockdown of FABP4 could partly reverse this trend.

Knockdown of FABP4 suppresses the cell viability and levels of inflammatory factors in IL-1β-induced ATDC5 cells by activating PPARγ. Data from the STRING database showed that FABP4 had the potential to regulate the expression of PPARγ (Fig. 4A). The results of the present analysis also showed that the expression of p-NF-κB p65 was increased and that of PPARγ was decreased in IL-1β-induced ATDC5 cells. Knockdown of FABP4 significantly downregulated p-NF-κB p65 expression and upregulated the expression of PPARγ compared with that of IL-1β-induced ATDC5 cells (Fig. 4B).

To explore the potential mechanisms of FABP4 in ATDC5 cells, 10 μM GW9662 (a PPARγ inhibitor) was added to the cells, which were transfected with siRNA-FABP4 and treated with IL-1β. As shown in Fig. 4C, it was found that knockdown of FABP4 could increase the viability of IL-1β-induced ATDC5 cells by activating PPARγ. The expression levels of TNF-α (Fig. 4D), IL-6 (Fig. 4E) and PGE_2 (Fig. 4F) were upregulated in the IL-1β + siRNA-FABP4 + PPARγ inhibitor group compared with that in the IL-1β + siRNA-FABP4 group in IL-1β-induced ATDC5 cells. These findings demonstrated that knockdown of FABP4 suppressed inflammatory factors in IL-1β-induced ATDC5 cells by activating PPARγ.

Knockdown of FABP4 suppresses oxidative stress and apoptosis in IL-1β-induced ATDC5 cells by activating PPARγ. The expression of ROS and SOD was detected in the IL-1β + siRNA-FABP4 groups with or without PPARγ inhibitor treatment. As shown in Fig. 5A, the levels of ROS were significantly decreased in the IL-1β + siRNA-FABP4 group compared with those in the IL-1β group, and were upregulated after PPARγ inhibitor treatment. The expression trend of SOD

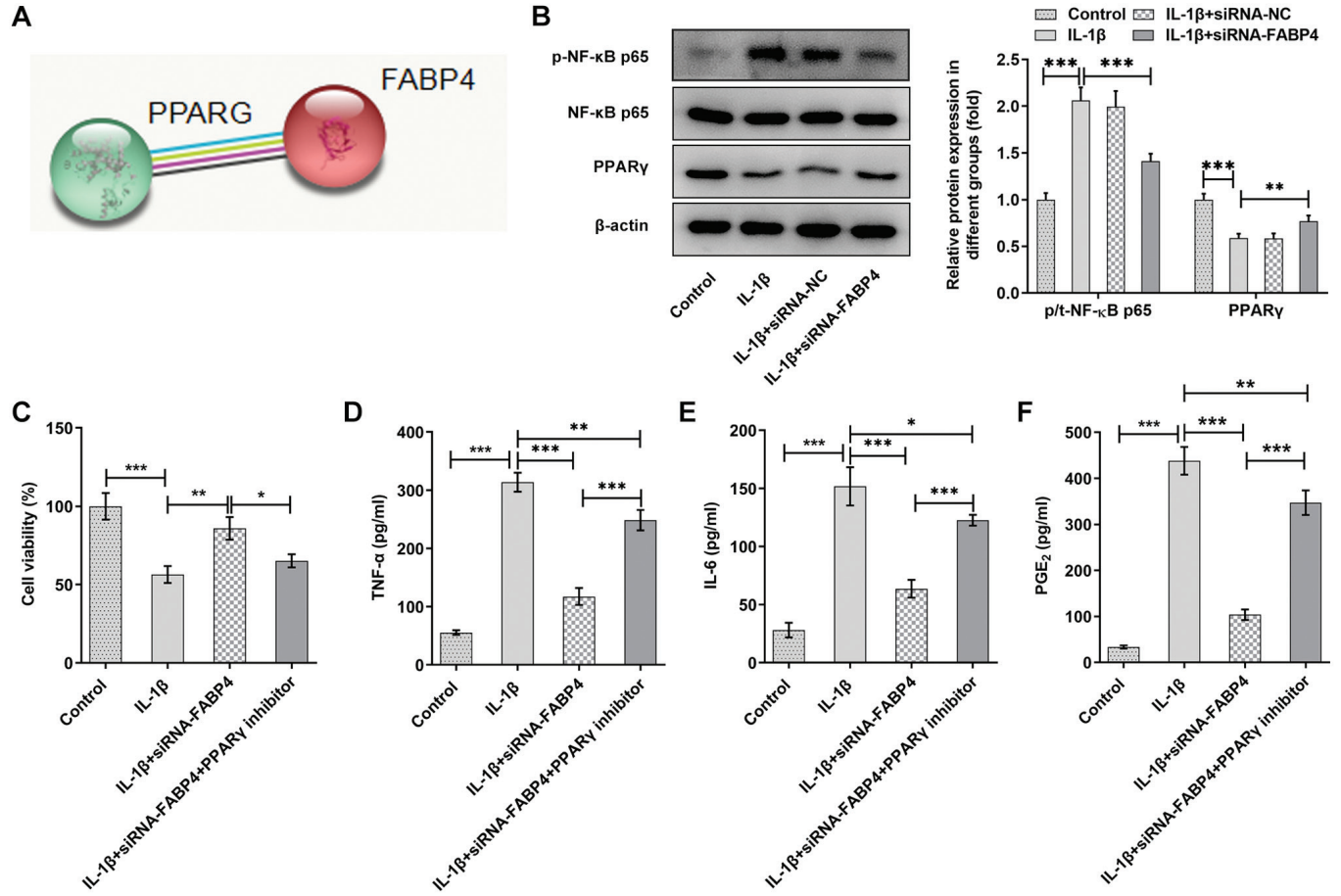

Figure 4. Knockdown of FABP4 suppresses the cell viability and inflammatory factors of IL-1β-induced ATDC5 cells by activating PPARγ. (A) Data from the Search Tool for the Retrieval of Interacting Genes/Proteins database showed that FABP4 had the potential to regulate the expression of PPARγ. (B) Expression levels of PPARγ and NF-κB p65 signaling proteins were detected using western blotting (n=3). (C) Cell Counting Kit-8 assay was performed to detect the viability of IL-1β-induced ATDC5 cells with or without PPARγ inhibitor treatment (n=6). The levels of (D) TNF-α, (E) IL-6 and (F) the cell growth and regulatory factor PGE_2 were measured by using ELISAs (n=4). *P<0.05, **P<0.01, ***P<0.001. FABP4, fatty acid-binding protein 4; PPARγ, peroxisome proliferator-activated receptor γ; PGE_2, prostaglandin E2; siRNA, small interfering RNA; NC, negative control; p-, phosphorylated; t-, total.

in the IL-1β + siRNA-FABP4 + PPARγ inhibitor group was opposite to that recorded for ROS (Fig. 5B). TUNEL staining (Fig. 5C) and western blotting (Fig. 5D) showed that the knockdown of FABP4 suppressed the apoptosis of IL-1β-induced ATDC5 cells by activating PPARγ.

Knockdown of FABP4 suppresses MMP expression and promotes the expression of GAG by activating PPARγ. The expression levels of MMP3, MMP9, MMP13 and ADAMTS-4 were detected using RT-qPCR and western blot analyses. The results revealed that the expression levels of MMP3, MMP9, MMP13 and ADAMTS-4 were downregulated in the IL-1β + siRNA-FABP4 group, which was reversed by the addition of the PPARγ inhibitor (Fig. 6A and B). Similarly, the expression of GAG was detected by using a GAG ELISA kit, and the PPARγ inhibitor also attenuated the FABP4 knockdown-induced upregulation of GAG compared with that of the IL-1β + siRNA-FABP4 group (Fig. 6C).

Discussion

OA is a complex disease regulated by multiple factors, including age, sex, genetic factors and physical trauma, but its pathogenesis remains unclear (2). It is generally accepted that the pathological features of OA are structural destruction and functional loss of articular cartilage caused by the imbalance of articular cartilage ECM synthesis and metabolism (4,5). Previous studies have suggested that FABP4 expression is increased in the plasma and synovial fluid of patients with OA (14,15). The present study found that FABP4 knockdown suppressed inflammation, apoptosis and ECM degradation of IL-1β-induced chondrocytes by activating PPARγ to regulate the NF-κB signaling pathway.

The release of inflammatory cytokines is one of the important inducing factors of OA (19). IL-1β serves an important role in altering the normal structure and function of chondrocytes, promoting the apoptosis of chondrocytes, degrading chondrocyte ECM, participating in synovial inflammatory lesions and affecting bone metabolism (20-22). Therefore, IL-1β was used in the present study to induce ATDC5 cells in order to establish an inflammatory environment.

Pro-inflammatory cytokines, such as TNF-α and IL-6, and ROS are known to be involved in the initiation and progression of OA (23). IL-6 is mainly secreted by osteoblasts and stromal cells, and plays a role in regulating osteoclast formation and bone resorption (24). Activated immature osteoclasts

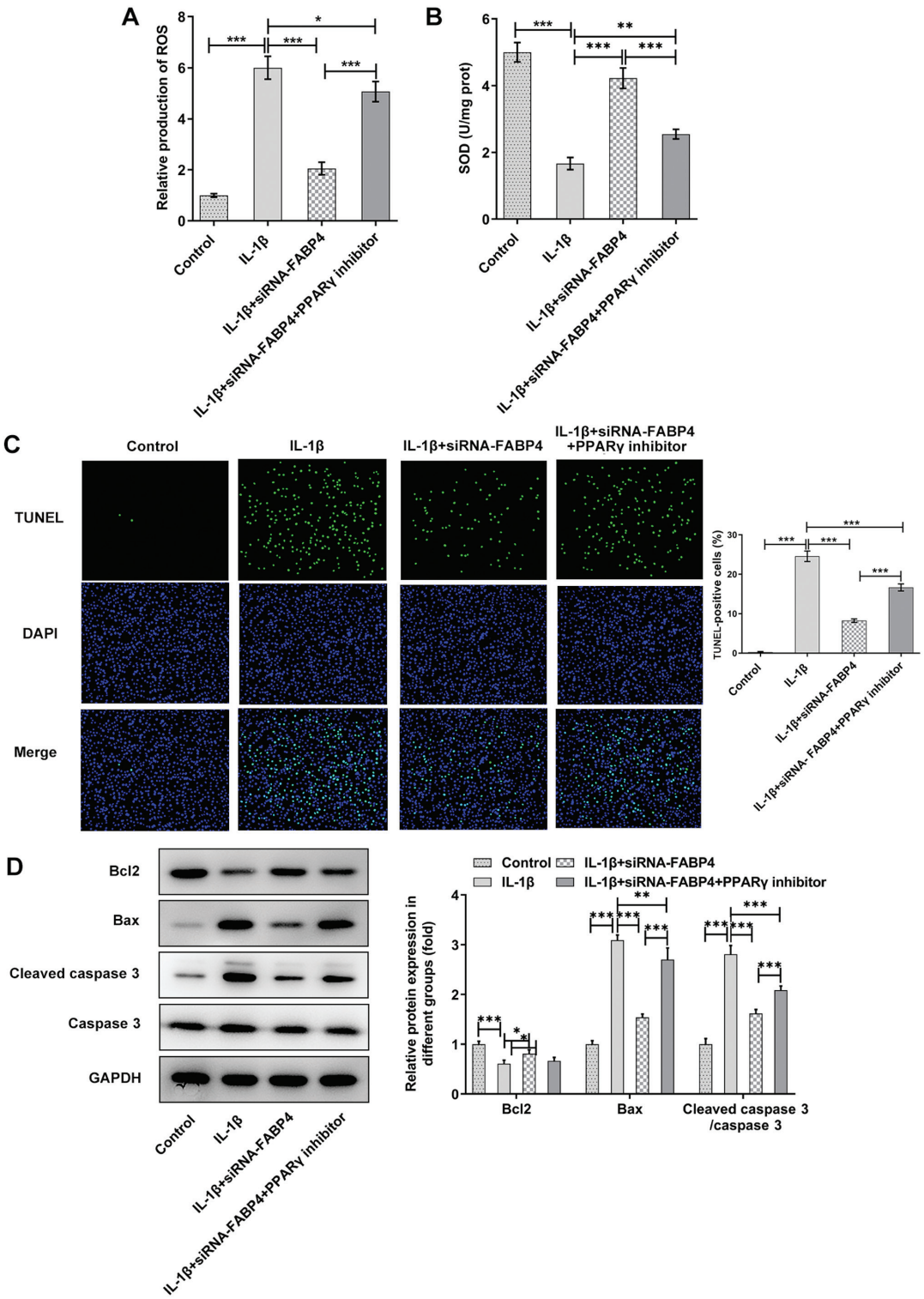

Figure 5. Knockdown of FABP4 suppresses the oxidative stress and apoptosis of IL-1β-induced ATDC5 cells by activating PPARγ. (A) ROS expression in cells was detected by using a ROS ELISA kit (n=4). (B) Expression of SOD was detected by using a SOD ELISA kit (n=4). (C) Apoptosis level was detected by using TUNEL staining (n=3). (D) Western blotting was used to detect the expression of Bcl-2, Bax and cleaved caspase 3 (n=4). *P<0.05, **P<0.01, ***P<0.001. FABP4, fatty acid-binding protein 4; PPARγ, peroxisome proliferator-activated receptor γ; ROS, reactive oxygen species; SOD, superoxide dismutase; siRNA, small interfering RNA.

participate directly in bone resorption, while MMPs degrade bone ECM and induce bone resorption (25). TNF-α induces the expression of MMPs and increases their activity, thereby inhibiting the synthesis of proteoglycan and collagen, and accelerating the decomposition of the cartilage ECM (25). IL-6 and TNF-α cause the destruction of the ECM, but also inhibit its repair (26). The current study found that knockdown of FABP4 suppressed pro-inflammatory cytokines and ROS production, but increased SOD concentration in IL-1β-induced ATDC5 cells. The degeneration of cartilage may be caused by

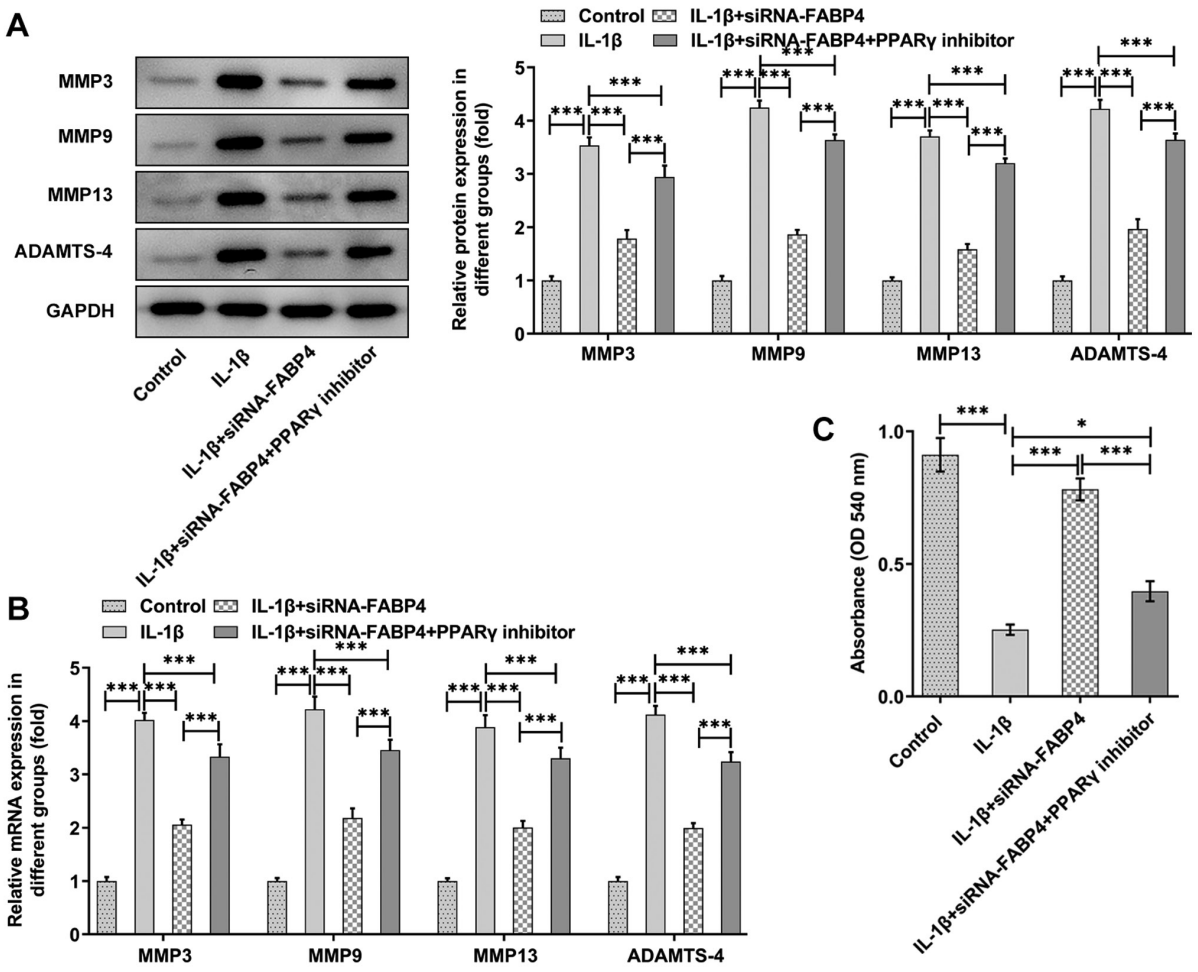

Figure 6. Knockdown of FABP4 suppresses matrix-degrading enzymes and promotes the expression of GAG by activating PPARγ. (A) Western blotting and (B) reverse transcription-quantitative PCR were used to detect the expression of MMP3, MMP9, MMP13 and ADAMTS-4 (n=4). (C) Expression of GAG was detected by using a GAG ELISA kit (n=5). *P<0.05, ***P<0.001. FABP4, fatty acid-binding protein 4; PPARγ, peroxisome proliferator-activated receptor γ; ADAMTS-4, ADAM metalloproteinase with thrombospondin type 1 motif 4; GAG, glycosaminoglycan; siRNA, small interfering RNA.

the reduced number of chondrocytes in the articular cartilage, which fails to regenerate and remodel the cartilage appropriately. Increased apoptosis of chondrocyte induced by ROS or pro-inflammatory cytokines has been documented to reduce the number of chondrocytes, resulting in OA initiation and progression (27). In the current study, IL-1β stimulation markedly increased ATDC5 cell apoptosis, but FABP4 knockdown effectively reduced the ratio of apoptotic cells.

OA is regulated by a variety of factors whose common pathway is the inability of chondrocytes to maintain a balance between ECM synthesis and degradation (28). The two main structural components of chondrocyte ECM are proteoglycan and type II collagen (29). OA is characterized by early loss of proteoglycans, followed by irreversible degradation of collagen, and the depletion of proteoglycans is mainly due to aggregation of proteoglycans (30,31). ADAMTS4 belongs to the ADAMTS family, and is a marker of cartilage degradation in OA (30). In addition, MMPs play an important role in cartilage degradation in OA, and the loss of GAG is a fundamental factor in the development of OA (32,33). Since knockdown of FABP4 suppressed pro-inflammatory cytokines production, the present study next detected the main components of chondrocyte ECM and the expression of MMPs. It was found that knockdown of FABP4 suppressed MMPs and ADAMTS-4 expression, and promoted the expression of GAG.

FABP4 was predicted to regulate PPARγ expression using the STRING database. Moreover, a previous study reported that FABP4 downregulated PPARγ expression to regulate adipogenesis (34). In addition, activation of PPARγ can inhibit the expression of NF-κB signaling, thereby suppressing the injury of chondrocytes caused by LPS, and ultimately alleviating OA (35,36). Upon stimulation, the activated p-NF-κB p65 can trigger the expression of an array of genes that induce destruction of the articular joint, leading to OA onset and progression (37). Therefore, to further explore the molecular mechanism of FABP4 involved in the regulation of OA, the present study analyzed the effect of FABP4 on PPARγ and NF-κB p65 expression levels. It was found that IL-1β significantly reduced PPARγ expression and increased p-p65 expression levels, but FABP4 knockdown recovered PPARγ and p-p65 expression levels. These results were in accordance with previous studies, which revealed that activation of PPARγ effectively suppressed IL-1β-induced inflammation in OA (38,39).

To further validate whether the effect of FABP4 knockdown on ATDC5 cell was dependent on activating PPARγ,

PPARγ inhibitor GW9662 was added. Following the addition of GW9662 to the cells, it was found that the presence of GW9662 blocked the effects of FABP4 knockdown on IL-1β-induced ATDC5 cell. However, these results were based on *in vitro* experiment, lacking the validation of *in vivo* studies. Besides, whether FABP4 exerted its effect on OA via other pathways needs to be elucidated. Moreover, the chondrogenic potential of ATDC5, such as the expression of collagen type 2 and aggrecan needs to be investigated in subsequent experiments.

Overall, the present findings clarified the effect of FABP4 on IL-1β-induced chondrocytes. The results indicated that FABP4 knockdown suppressed inflammation, apoptosis, oxidative stress and ECM degradation of IL-1β-induced chondrocytes by activating PPARγ to regulate the NF-κB signaling pathway. The findings of the present study could also provide a novel option for the clinical treatment of OA.

Acknowledgements

Not applicable.

Funding

The present study was supported by the Natural Science Foundation of Zhejiang Province (grant no. Y19H060053).

Availability of data and materials

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

Authors' contributions

HM and WW conceived and designed the study. HM, BH, HL and YT performed the experiments to acquire the data. HM and BH analysed and interpreted the data. HM and WW drafted the manuscript and revised it for critically important intellectual content. All authors read and approved the final manuscript. HM and WW confirm the authenticity of all the raw data.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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