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FHL2 deteriorates IL-1β induced inflammation, apoptosis, and extracellular matrix degradation in chondrocyte-like ATDC5 cells by mTOR and NF-κB pathways

Yicheng Li[1†], Fei Wang[1†], Baochao Ji[1], Abdusami Amati[1] and Li Cao[1*]

Abstract

Background The role of nuclear translocation in osteoarthritis (OA) pathogenesis has garnered increasing attention in recent years. Extensive research has demonstrated that FHL2 acts as a nuclear transmitter through interactions with other nuclear transcription factors. We aimed to investigate the role of FHL2 in an osteoarthritis cell model.

Methods OA cartilage model was established by chondrocyte-like ATDC5 cells induced by 1% insulin-transferrin-selenium and then treated with interleukin-1β (IL-1β, 10 ng/mL). Lentivirus transfection was employed to suppress the expression of FHL2. Immunofluorescence and flow cytometry were used to examine nuclear transcription and apoptosis, respectively. Western blotting was performed to analyze the expression of metabolism-related proteins, autophagy-related proteins, apoptosis-related proteins, as well as proteins associated with the NF-κB and mTOR pathways.

Results The elevated expression of FHL2 occurred in both the cytoplasm and the nucleus. Knockdown of FHL2 could inhibit IL-1β-induced phosphorylation of NF-κB p65 and stabilize the extracellular matrix (ECM) by decreasing MMP-3 and MMP-13 expression, to suppress COL II degradation in chondrocyte-like ATDC5 cells. Meanwhile, the knockdown of FHL2-activated autophagy in IL-1β-treated chondrocytes through mTOR signaling, characterized by an increased LC3-II/LC3-I ratio and Beclin-1. FHL2 downregulation inhibited IL-1β-induced apoptosis by suppressing BAX and Caspase-3 expression, while enhancing BCL-2 protein levels. This mechanism may involve AKT phosphorylation and decreased expression of p-NF-κB p65.

Conclusions FHL2 knockdown activated autophagy while suppressing inflammation, apoptosis, and ECM degradation. The mechanism underlying these processes may involve the inhibition of the mTOR and NF-κB signaling pathways.

Keywords FHL2, IL-1β, mTOR, NF-κB, Signaling pathway, Osteoarthritis

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Background

Osteoarthritis (OA) is a leading cause of joint pain, disability, and increased health care costs [1], affecting more than 7% of the global population and over 500 million people worldwide in 2019 [2]. Several risk factors, such as obesity, aging, joint injuries, and genetic predisposition, contribute to the onset and progression of OA [3]. Recently, OA has been redefined as a whole-joint disease rather than solely a condition of cartilage degeneration. It involves complex pathological processes across multiple joint tissues, including the articular cartilage, meniscus, infrapatellar fat pad, subchondral bone, and synovial membranes [3, 4]. In OA pathophysiology, low-grade inflammation can damage the entire joint and contribute to disease progression [5]. Furthermore, inflammatory signaling driven by biomechanical stress can help foster the inflammatory environment, creating a vicious cycle [6]. Further exploration of the mechanisms underlying inflammatory pathophysiology is expected to yield novel treatments that may modify disease progression in OA.

Chondrocytes are the principal cell type in both articular cartilage and the meniscus. In OA, chondrocytes are profoundly influenced by both the inflammatory environment and biomechanical stress [7, 8]. These cells are highly specialized and metabolically active, playing a crucial role in the development, maintenance, and repair of the extracellular matrix (ECM). Chondrocytes have limited regenerative capacity, making the roles of apoptosis and autophagy crucial for ECM degradation and OA progression [9]. Conversely, the progression of OA has also been regulated by matrix metalloproteinases (MMPs), including MMP-3 and MMP-13, which may be stimulated by inflammatory mediators [10]. MMP-3 and MMP-13 are aggrecanase and collagenase enzymes, respectively, which degrade aggrecan, type II collagen, and other components of the ECM [11]. To understand the progression of OA, it is also important to understand the related pathways that are involved, a number of which are associated with OA, including NF-κB and mTOR signaling pathways [12, 13]. For example, the activation of NF-κB signaling pathway suppresses chondrocyte anabolism and triggers the expression of MMPs, leading to articular cartilage erosion [14].

The four-and-a-half LIM domain protein 2 (FHL2) is a member of the four-and-a-half LIM-only protein subfamily belonging to the LIM-only protein family [15]. As a multifunctional LIM domain-only protein, FHL2 binds cellular proteins and regulates various cellular processes via its LIM domains [16]. FHL2 played an important role in mediating many signaling pathways. Dahan et al. indicated that FHL2 positively activates NF-κB signaling in liver regeneration [17], and FHL2 inhibition has been shown to reduce apoptosis of early outgrowth cells by increasing AKT phosphorylation [18]. Additionally, FHL2 is also strongly associated with pro-inflammatory effects [19]. In post-injury inflammation, FHL2 induces upregulation of pro-inflammatory factors by activating NF-κB signaling [20]. However, the role of FHL2 has not been well established in the chondrocyte of osteoarthritis.

This study aims to investigate the expression pattern of FHL2 in response to IL-1β stimulation in chondrocyte-like ATDC5 cells. Additionally, we examined the effects of FHL2 knockdown on apoptosis-related, autophagy-related, and catabolic proteins, collagen expression, and the associated signaling pathways in an OA cell model.

Methods

Materials

Dulbeccco's Modified Eagle Medium Nutrient Mixture F-12 (C11330500BT), Penicillin Streptomycin (15140-122), and Insulin-Transferrin-selenium (ITS, 41400-045) were purchased from Gibco (Rockville, MD). The FHL2 antibody (21619-1-AP, 1:1,000 for WB, 1:100 for immunofluorescence) was purchased from Proteintech (Wuhan, Hubei, China). Antibodies against COX-2 (ab179800, 1:1,000 for WB), BAX (ab32503, 1:1,500 for WB), MMP-3 (ab52915, 1:1,000 for WB), MMP-13 (ab39012, 1:1,000 for WB), type II collagen (COL II) (ab34712, 1:1,000 for WB) were purchased from Abcam (Cambridge, UK). Antibodies against JNK (9252, 1:1,000 for WB), p-JNK (4668, 1:1,000 for WB), NF-κB p65 (8242, 1:1,000 for WB), p-NF-κB p65 (3033, 1:1,000 for WB), p38 (8690, 1:1,000 for WB), p-p38 (4511, 1:1,000 for WB), ERK1/2 (9102, 1:1,000 for WB), p- ERK1/2 (9101, 1:1,000 for WB), GAPDH (5174, 1:2,000 for WB), β-actin (4970, 1:2,000 for WB), Beclin-1 (3495, 1:1,000 for WB), LC3I/II (12741, 1:1,000 for WB) were purchased from Cell Signaling Technology (Danvers, MA). The recombinant murine IL-1β (211-11B) was purchased from Peprotech (Cranbury, NJ). The protease inhibitor cocktail (B14001) and phosphatase inhibitor cocktail (B15001) were purchased from Bimake (Shanghai, China). The RIPA buffer, WB transfer buffer, tris-glycine running buffer, and TBST buffer were purchased from Solarbio Life Sciences (Beijing, China). The electrochemiluminescence (ECL) reagent was purchased from Beijing Labgic Technology Co., Ltd. (Beijing, China).

Cell culture and treatment

The mouse cell line ATDC5 was purchased from Guangzhou Jennio Biotech Co., Ltd. (Guangzhou, China) and maintained in a complete medium including DMEM/F12 with 5% FBS and 1% penicillin/streptomycin in a humidified incubator with 5% CO_2 and a temperature of 37 °C. To induce differentiation into chondrocyte-like cells, 1% ITS was added to the medium after the cell confluence reached approximately 70%. The medium was then

changed every 2 days. Following 2 weeks of differentiation, the cells were used in subsequent experiments [21]. Unless otherwise noted, OA cell models were constructed by 10 ng/mL IL-1β stimulation for 24 h based on previous studies [22].

Transfection of lentivirus vector

The FHL2-knockdown lentiviral (FHL2-KD group) or empty vectors (control group) were packaged by Gene Chem Co. Ltd. (Shanghai, China). For the transfection of these vectors, chondrocyte-like ATDC5 cells were plated in 6-well plates at a density of 2×10^5 cells per well. The lentiviral vectors were transfected at a multiplicity of infection of 10. The prepared vectors were added to each well and incubated for 12 h. ATDC5 cells were treated with 12 μg/mL puromycin (Biosharp, Hefei, China) for 48 h. Afterward, cells were transferred into 25 cm^2 culture flasks for expansion. The earlier 10 generations were used for the following experiments. The details of lentiviral vectors in design and oligo information are shown in supplementary Tables S1 and S2.

Extraction of RNA for qRT-PCR

Total RNA was isolated from ATDC5 cells or chondrocyte-like ATDC5 cells by TRIzol reagent after washing twice with precooling phosphate-buffered saline (PBS). According to the manufacturer's protocol, 1,000 ng of RNA was reversely transcribed into cDNA using the first-strand cDNA synthesis kit with gDNA Eraser (RR047A, Takara, Japan). The primer pairs are shown in Table 1. Quantitative real-time polymerase chain reaction (qRT-PCR) was performed using a 7500 real-time PCR Instrument (Life Technologies Holdings Pte Ltd).

Extraction of protein and analysis via WB

After washing the collected cells three times with cold PBS, they were resuspended in RIPA buffer supplemented with the protease inhibitor cocktail and phosphatase inhibitor cocktail at a 1:100 (v/v) dilution ratio, and then incubated on ice for 30 min. The protein concentration was determined using the bicinchoninic acid assay kits (Thermo Fisher Scientific, Waltham, MA). Equal amounts of protein (20 μg) were subjected to 10% SDS-PAGE (Solarbio Life Sciences, Beijing, China) and subsequently transferred to PVDF membranes (Roche).

Table 1 List of primers for PCR

Gene	Forward	Reverse
COL II	5′- GCTCCCAGAACATCACCTACCA-3′	5′- ATGACGGTCTTGCCCCCACTTAC-3′
COL X	5′- GGATGCCGCTTGTCAGTGCTA-3′	5′- GGTCGTAATGCTGCTGCCTATTG-3′
β-actin	5′- AGGTCATCACTATTGGCAACGAG-3′	5′-CCATACCCAAGAAGGAAGGCT-3′

The phosphorylated protein blots were blocked with 5% BSA at room temperature for 1 h, while the blots of other proteins were blocked with 5% nonfat dry milk for the same duration. The membranes were then incubated overnight at 4 °C with primary antibodies. After washing three times with 1 × TBST for 5 min, the membranes were incubated with HRP-labeled secondary antibodies (1:2,000, bs-40295G-HRP, Beijing, China) for 1 h. Finally, the immunoreactive bands were detected by ECL using a Gel Doc 2000 Imager (Bio-Rad). Quantification of western blotting results was performed using the ImageJ software, version 1.8.0(NIH, Bethesda, MD, USA) [23].

Immunofluorescence

For immunofluorescence analysis, 3×10^4 chondrocyte-like ATDC5 cells were seeded onto a glass-bottomed dish (801001, NEST, Wuxi, Jiangsu, China), allowed to adhere, and subsequently stimulated with IL-1β. After removing the culture medium and washing the cells twice with PBS, chondrocytes were fixed in 4% paraformaldehyde for 15 min and permeabilized with 0.4% Triton X-100 for 10 min. We blocked chondrocytes with 5% goat serum for 30 min, washed them three times in PBS, and incubated the chondrocytes with antibodies against FHL2 overnight at 4°C. After washing three times with PBS, Alexa Fluor647-conjugated secondary antibodies were incubated for 1.5 h at room temperature before nuclear staining with 4′,6-diamidino-2-phenylindole (DAPI) for 3 min. To visualize the samples, a fluorescent microscope (Olympus Inc., Tokyo, Japan) was used. Digital images were analyzed using ImageJ software (NIH, Bethesda, MD, USA). Fluorescence intensity above background in the nucleus was compared to that in the cytoplasm to calculate the nuclear-to-cytoplasmic fluorescence ratio.

Flow cytometric analysis of cell apoptosis

We used a flow cytometric kit to detect the apoptosis ratios in chondrocyte-like ATDC5 cells between the FHL2-KD and control groups. After collecting the cells, they were resuspended in 1 × binding buffer and stained for 15 min at room temperature with 5 μl 7-aminoactinomycin D (7-ADD) and 5 μl phycoerythrin (PE)-labeled Annexin V in the dark. Following staining for 1 h,samples were immediately analyzed on FACSAria™ II flow cytometer using BD FACSDiva software (BD Biosciences).

Statistical analysis

The experiments were repeated at least three times each. Statistical analysis and graphing were performed using GraphPad Prism (Graphpad Software, San Diego, CA, USA). Statistical significance for two-group comparisons was assessed using an unpaired Student's t-test. For multiple group comparisons, a one-way analysis of variance (ANOVA) followed by Dunnett's multiple comparisons

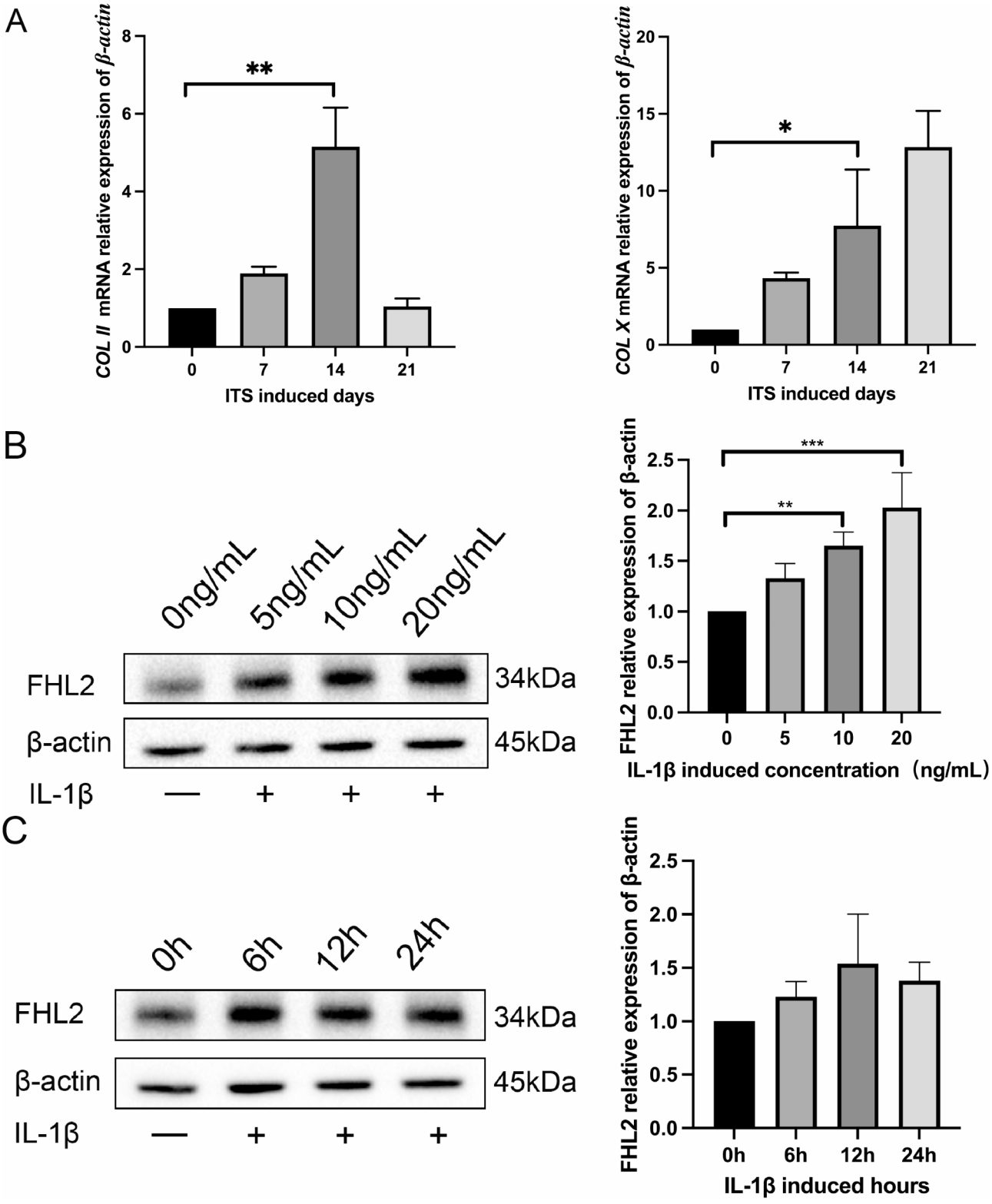

Fig. 1 (**A**) The mRNA relative expression of *COL II* and *COL X* with ITS stimulation was analyzed by qRT-PCR. (**B**–**C**) The protein relative expression of FHL2 by IL-1β stimulation was detected by WB. The results are presented as the mean ± standard deviation (SD) of three independent experiments. Significant differences between groups were determined by one-way ANOVA; *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$; $n = 3$

test was applied. $P < 0.05$ was considered to indicate statistical significance.

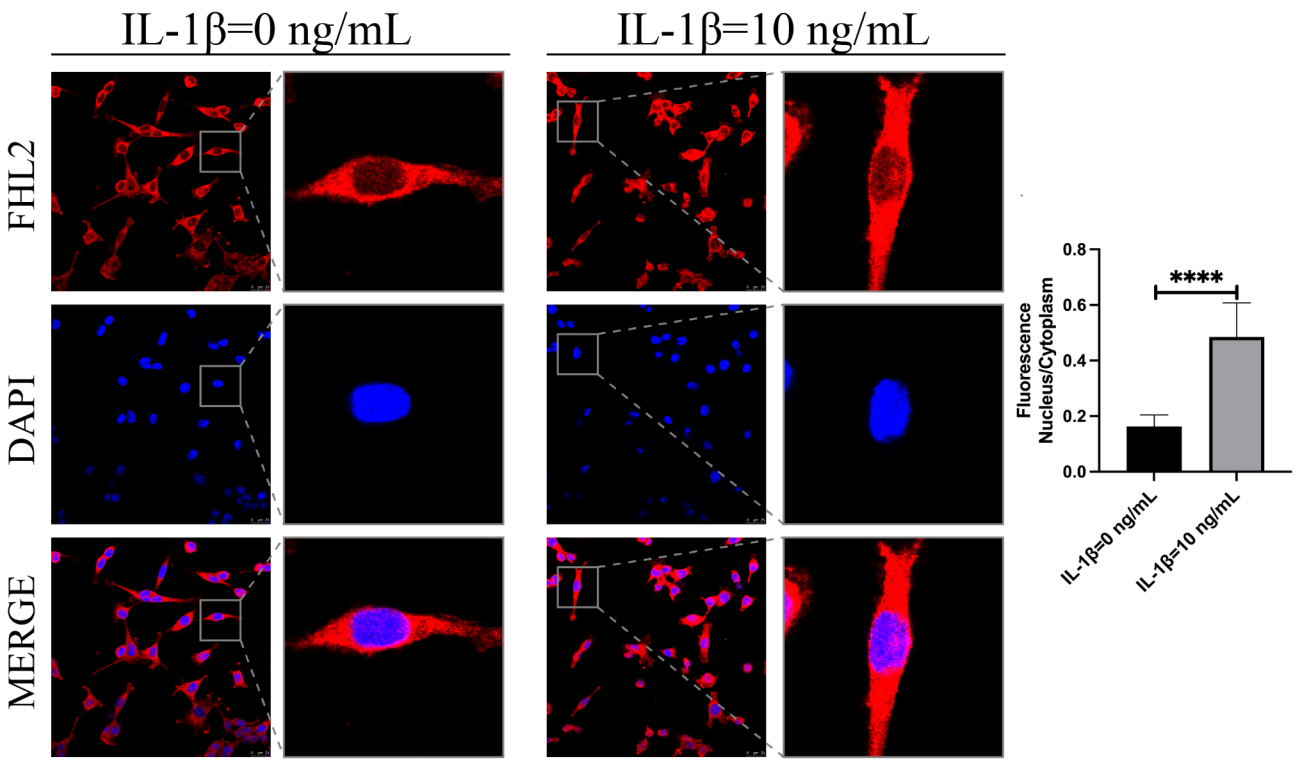

Fig. 2 IL-1β stimulation induced the expression and nuclear translocation of FHL2 in chondrocyte-like ATDC5 cells. FHL2 nuclear translocation was analyzed by the ratio of FHL2 in the nucleus to that in the cytoplasm. ****$P < 0.0001$; $n = 3$

Results

FHL2 expression IL-1β induced chondrocyte-like ATDC5 cells in a dose-dependent manner

As shown in Fig. 1A, *COL II* mRNA expression increased after ITS treatment and peaked at 2 weeks, whereas *COL X* mRNA expression increased between 1 and 3 weeks. In ATDC5 cells expressing *COL II* and *COL X*, differentiation from proliferative to hypertrophic chondrocytes was observed. Therefore, the following experiments were performed on ATDC5 cells that had been induced by ITS for 2 weeks.

Using Western blotting (WB), we detected the FHL2 expression in chondrocyte-like ATDC5 cells after IL-1β-stimulated treatment. As shown in Fig. 1B and C, the expression of FHL2 was upregulated in IL-1β-induced ATDC5 cells in a dose-dependent manner, but not a time-dependent manner.

An immunofluorescence assay was then performed to investigate the localization of FHL2 during IL-1β stimulation in chondrocyte-like ATDC5 cells. Immunofluorescence staining for FHL2 (red) exhibits increased expression in the nucleus and cytoplasm after IL-1β treatment, as well as FHL2 nuclear translocation (Fig. 2).

Knockdown of FHL2 expression in ATDC5 Cells and establishment of an OA model in vitro

As shown in Fig. 3A, the results of WB analyses showed that the FHL2-interfering sequence knocked down the expression of FHL2 in chondrocyte-like ATDC5 cells by >70%. After 10 ng/mL IL-1β treatment for 24 h, the expression of COX-2 and MMP-13 was significantly upregulated compared to that in the control group, while the expression of COL II was decreased (Fig. 3B). These results imply that the OA model in vitro was successfully established by treatment with IL-1β.

Knockdown of FHL2 promotes anabolism and suppresses catabolism in IL-1β-induced chondrocyte-like ATDC5 cells

Overproduction of pro-inflammatory cytokines and MMPs, and degradation of the ECM promotes the progression of OA. In this study, it was observed that the downregulation of FHL2 could significantly decrease the levels of COX-2, MMP-3, and MMP-13 with IL-1β stimulation, while elevating the expression of COL II (Fig. 4). However, in the absence of IL-1β stimulation, the levels of other proteins, except MMP-13, were not affected by the knockdown of FHL2.

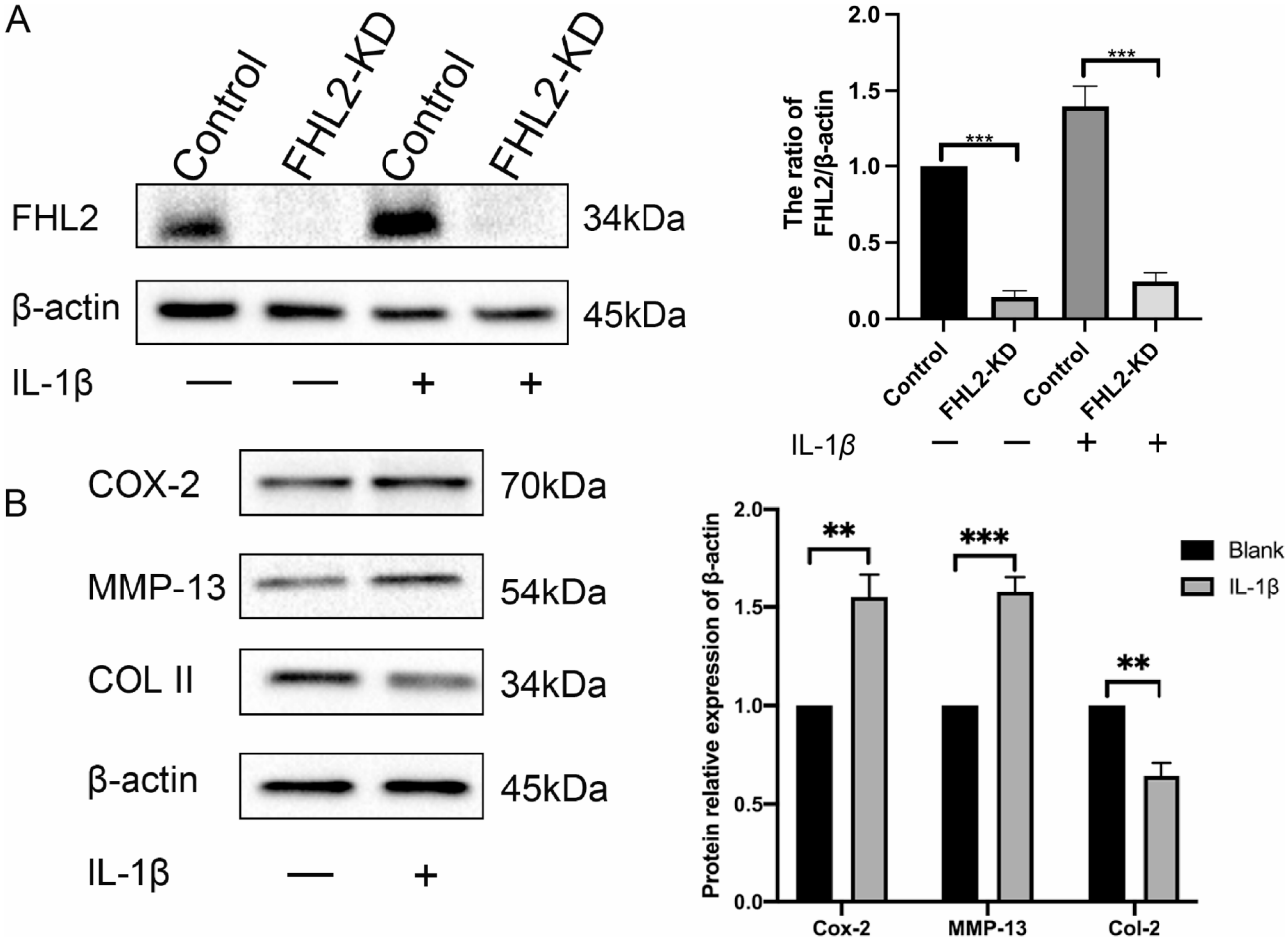

Fig. 3 (**A**) Identification for knockdown of FHL2 in chondrocyte-like ATDC5 cells with and without IL-1β stimulation. ***$P < 0.001$; $n = 3$. (**B**) The expression of COX-2 and MMP-13 was increased, while the COL II level was decreased by IL-1β stimulation in chondrocyte-like ATDC5 cells. The data are presented as the mean ± SD; Significant differences between groups were determined by Student's t-test; **$P < 0.01$, ***$P < 0.001$; $n = 3$

Knockdown of FHL2 suppressed the apoptosis and activated autophagy of IL-1β-induced chondrocyte-like ATDC5 cells

To investigate the role of FHL2 on chondrocyte apoptosis induced by IL-1β, WB and flow cytometric analyses were performed. As shown in Fig. 5A, FHL2 knockdown largely reduced the expression of the proapoptotic protein BAX and Caspase-3 in the IL-1β-induced chondrocytes and increased the expression of the anti-apoptotic protein BCL-2, whereas the expression of Caspase-7 was not affected. Consistent with the results of WB, the flow cytometry results revealed that the apoptosis rate in the control group was significantly higher compared with that of FHL2 knockdown following treatment with IL-1β (Fig. 5B). Although the expression of BAX and Caspase-3 were decreased in FHL2 knockdown chondrocytes without IL-1β stimulation, the decreased trend results in apoptosis of flow cytometry were not significant ($P > 0.05$).

WB was performed to ascertain whether FHL2 regulated autophagy in chondrocyte-like ATDC5 cells under IL-1β stimulation. As shown in Fig. 5C, the knockdown of FHL2 efficiently increased the levels of Beclin1 and the ratio of LC3-II/LC3-I. By contrast, in chondrocyte-like ATDC5 cells without IL-1β treatment, the downregulation of FHL2 decreased the expression of Beclin1 and the ratio of LC3-II/LC3-I.

Knockdown of FHL2 suppressed mTOR and NF-κB signaling while activating AKT signaling in IL-1β-induced chondrocyte-like ATDC5 cells

To further explore the molecular mechanism of the role of FHL2 in anabolism, catabolism, apoptosis, and autophagy in IL-1β-induced chondrocyte-like ATDC5 cells, key components of multiple MAPK signaling pathways, the NF-κB pathway, and the PI3K/AKT/mTOR pathway were analyzed by WB. As shown in Fig. 6A, the protein expression of phosphorylated NF-κB p65 was significantly decreased by FHL2-knockdown in chondrocytes

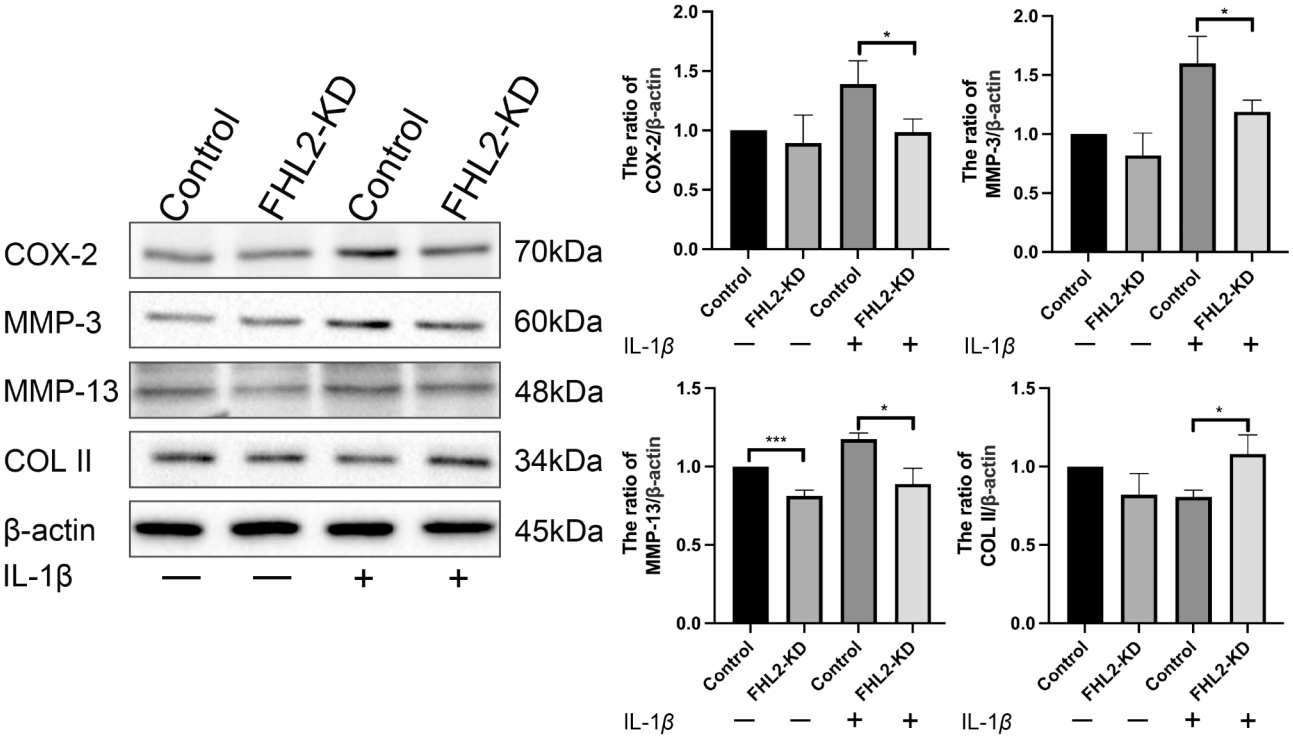

Fig. 4 Knockdown of FHL2 alleviated IL-1β-induced ECM degradation and inflammation in chondrocyte-like ATDC5 cells. The data are presented as the mean ± SD. Significant differences between groups were determined by Student's t-test; * $P < 0.05$, *** $P < 0.001$; $n = 3$

with IL-1β administration compared with the control group. However, phosphorylated NF-κB p65 expression was upregulated by FHL2-knockdown in chondrocytes without IL-1β treatment, and phosphorylated p38 protein expression was downregulated. Additionally, p-AKT protein expression increased in the FHL2-knockdown group both with and without IL-1β stimulation. The ratio of phosphorylated mTOR/mTOR in IL-1β-induced chondrocytes from the FHL2-knockdown group was lower than that in the control group. Conversely, the ratio of p-mTOR/mTOR in chondrocytes without IL-1β stimulation from the FHL2-knockdown group was significantly higher than that in the control group (Fig. 6B).

Discussion

We identified a role for FHL2 in the progression of IL-1β-induced chondrocyte-like ATDC5 cells. The protein expression of FHL2 was increased by IL-1β stimulation in the dose-dependent manner, while the IL-1β-induced osteoarthritis was associated with nuclear translocation of FHL2. These findings reveal that FHL2 is an important mediator of osteoarthritis.

OA is a heterogeneous disease featuring articular cartilage degeneration, which consists of the degradation with ECM, the apoptosis of chondrocytes, and the inhibition of autophagy in chondrocytes [24, 25]. There are several ECM proteins that play critical roles in the maintenance of homeostasis for chondrocytes, including collagens, proteoglycans, and other extracellular matrix macromolecules. The degradation of the ECM is performed by MMPs, which include MMP-3 and MMP-13. The MMPs further degrade COL II, which is the main component of the ECM, and IL-1β is able to inhibit the production of COL II [26]. In addition, COX-2 is a key mediator in inflammation and pain [27]. We demonstrated that the changes in FHL2 expression did not affect COX-2, MMP-3, or COL II expression in chondrocyte-like ATDC5 cells without IL-1β treatment. However, downregulation of FHL2 increased COL II expression, while inhibited the expression of COX-2, MMP-3, and MMP-13 in OA. Additionally, we found that FHL2 knockdown inhibited p-NF-κB p65 expression in OA, which may be linked to the suppression of ECM degradation. Similar to previous studies, inhibiting the NF-κB signaling pathway can reduce ECM degradation in IL-1β-stimulated chondrocytes, thereby contributing to OA treatment [28, 29].

Autophagy plays a critical role in the regulation of metabolism as a highly conserved degradation system, which usually protect cells under abnormal physiological conditions, especially in OA [30, 31] Kao WC, Chen JC, Liu PC, et al. The Role of Autophagy in Osteoarthritic Cartilage. Biomolecules. 2022;12(10):1357. Published 2022 Sep 23. doi:10.3390/biom12101357.

Lv X, Zhao T, Dai Y, et al. New insights into the interplay between autophagy and cartilage degeneration in osteoarthritis. Front Cell Dev Biol. 2022;10:1089668.

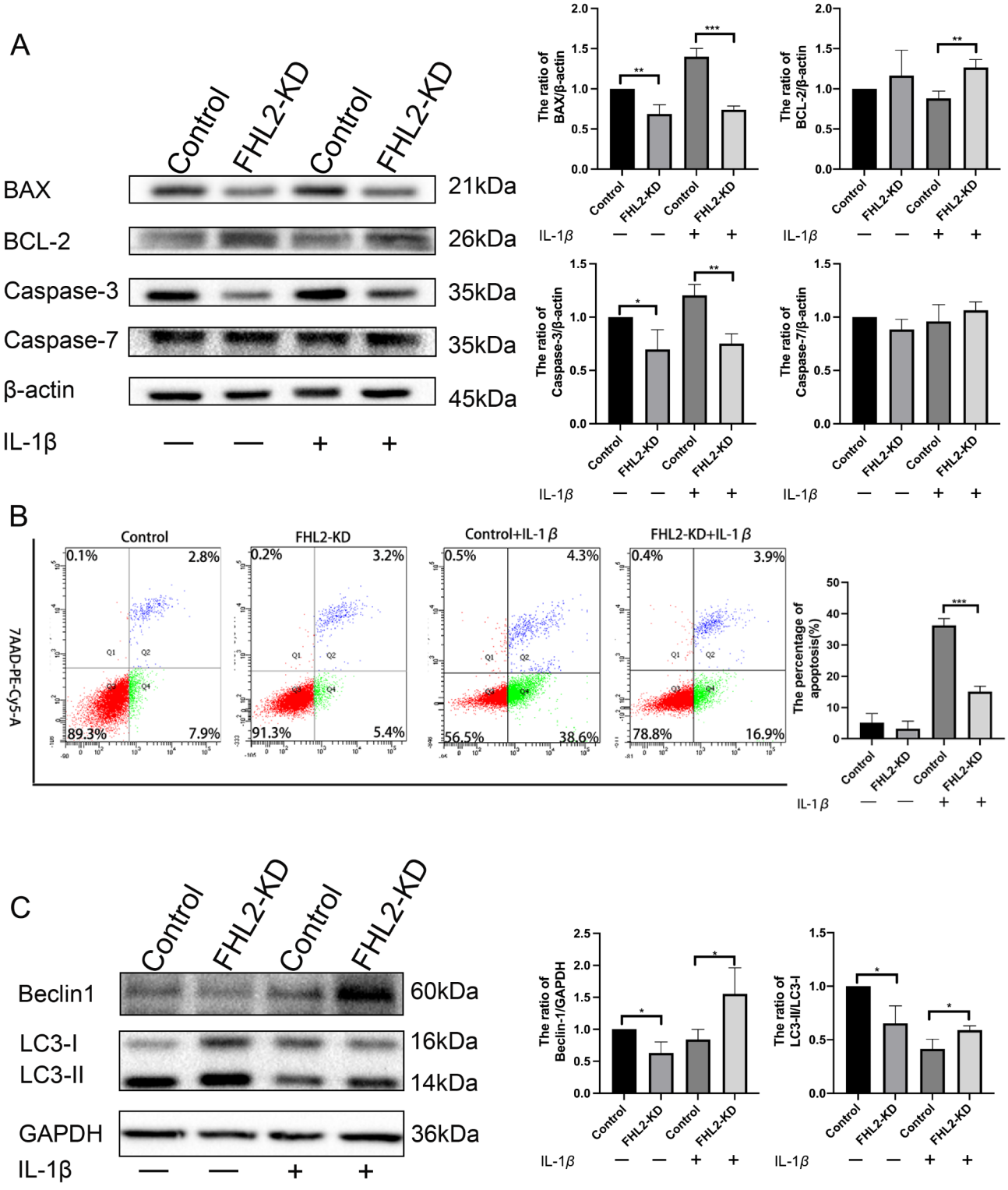

Fig. 5 FHL2 knockdown suppressed apoptosis and promoted autophagy in IL-1β induced chondrocytes, and downregulation of FHL2 inhibited both apoptosis and autophagy in chondrocytes without IL-1β stimulation. (**A**) The protein expressions of BAX, BCL-2, Caspase-3, and Caspase-7 were detected by WB. (**B**) Apoptotic rates of different groups were determined by flow cytometric analyses. (**C**) The levels of Beclin1 in protein expression and the ratio of LC3-II/LC3-I were detected by WB. The data are presented as the mean ± SD. Significant differences between groups were determined by Student's t-test; * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$; $n = 3$

Published 2022 Dec 5. doi:10.3389/fcell.2022.1089668. In recent studies, the level of autophagy in OA cartilage

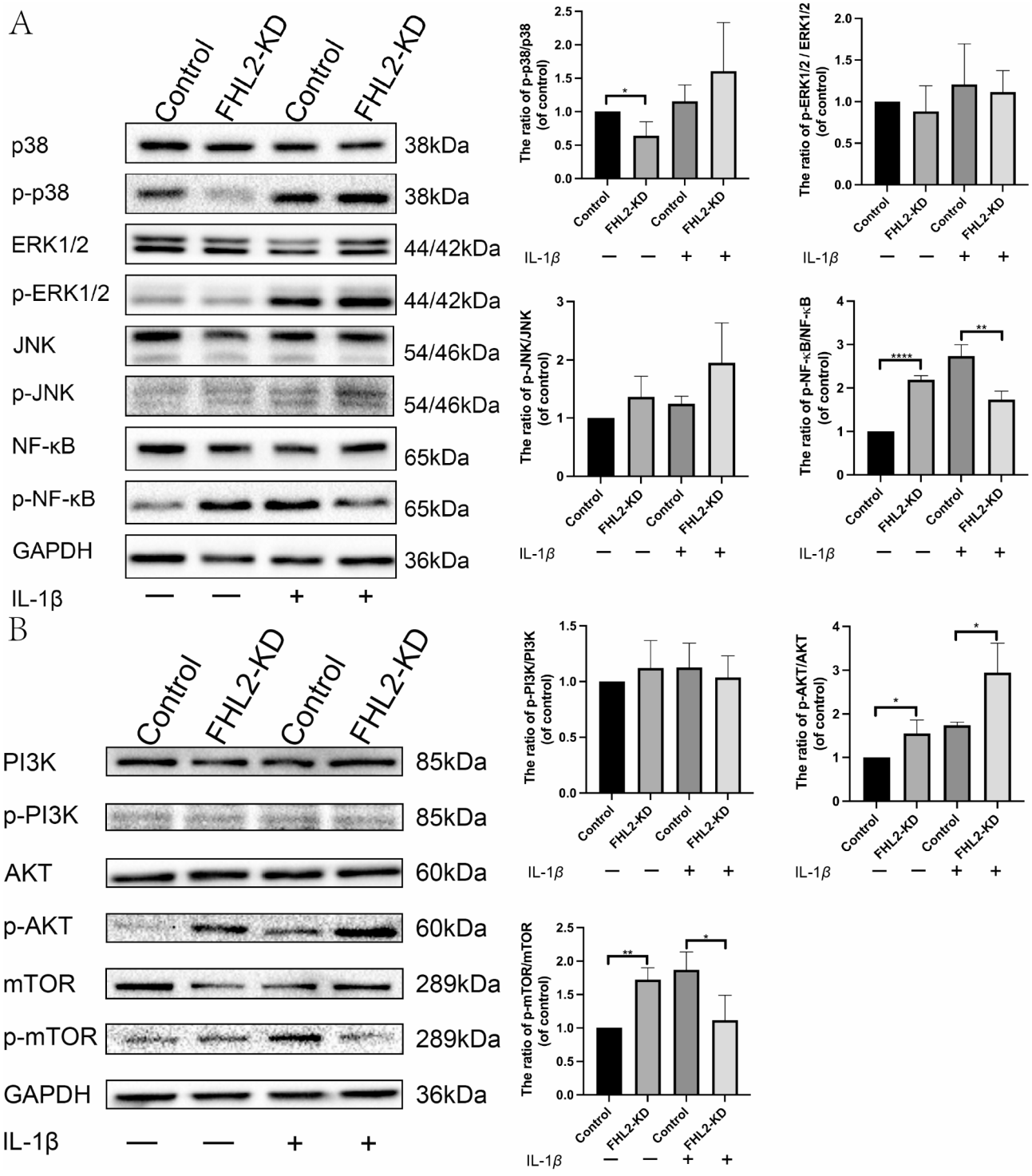

Fig. 6 Knockdown of FHL2 exerts a multitude of beneficial effects through different mechanisms in IL-1β-induced chondrocyte-like ATDC5 cells. (**A**) Western blot analysis of p38, ERK1/2, JNK, and NF-κB p65 in the chondrocyte-like ATDC5 cells treated with and without IL-1β. (**B**) Western blot analysis of PI3K, AKT, and mTOR in the chondrocyte-like ATDC5 cells treated with and without IL-1β. The data are presented as the mean ± SD. Significant differences between groups were determined by Student's t-test; * $P < 0.05$, ** $P < 0.01$, **** $P < 0.0001$; $n = 3$

was shown to be decreased, and the activation of autophagy played a protective role in chondrocytes [32]. Classic autophagy consisted of several proteins which are dynamic and interactive. Beclin1 acts as an autophagy regulator which could affect engulfment and endosome maturation. LC3-I is a pivotal biomarker of

autophagosome formation, which could generate another autophagy marker, LC3-II, when conjugated with phosphatidylethanolamine [33]. We demonstrated here that the downregulation of FHL2 activated autophagy by upregulation of Beclin1 and the ratio of LC3-II/LC3-1. In the signaling pathway, mTOR is upregulated and mediates the inhibition of autophagy signal transduction in the articular cartilage, which further promotes cartilage degeneration in OA, which is accordant to our results [34]. Additionally, AKT is one of the upstream regulators of the mTOR signaling pathway, which can directly mediate autophagy in chondrocytes by the AKT-mTOR signaling pathway [35]. However, our study demonstrated that the downregulation of FHL2 induced the high expression of phosphorylated AKT and the low expression of phosphorylated mTOR, which indicated that knockdown of FHL2 expression-induced autophagy might be related of the low expression of the phosphorylated mTOR pathway but not the PI3K/AKT pathway.

Apoptosis also plays a critical role in the initiation and progression of OA [36]. The BCL-2 family members control mitochondrial apoptotic signaling by modulating mitochondrial membrane permeability, and are classified into anti-apoptotic and pro-survival members [37]. The BCL-2 family proteins can be heterodimerized by protein–protein interactions between pro- and anti-apoptotic BCL-2 family members, which determines whether cell survival or the apoptosis signal proceed [38]. Furthermore, the intact collagen fibril is necessary to sustain chondrocyte survival. We demonstrated that the expression of COL II, MMP-3, and the BCL-2 family proteins were in accordance with this; chondrocyte death and matrix loss may form a vicious cycle, with the progression of one aggravating the other. Furthermore, the pro-apoptotic protein, BAX, could translocate from the cytosol to the mitochondria following exposure of cells to apoptotic stresses[39]. We further observed that upregulation of BCL-2 and the expression of BAXand Caspase-3decreased, while FHL2 was down regulated, which indicated that the expression of FHL2 might promote apoptosis. Additionally, the activation of AKT could inhibit expression of BAX to suppress apoptosis [40]. The role of NF-κB in chondrocytes varies depending on the stimulus. In normal chondrocytes, activation of the NF-κB pathway is generally linked to chondrocyte proliferation, maturation, and inhibition of apoptosis [41]. This is consistent with the decreased expression of BAX and Caspase-3 observed in our study.However, in OA, the NF-κB signaling pathway plays the opposite role due to abnormal activation [42]. This suggests that FHL2 knockdown could maintain cell viability in normal chondrocytes by promoting NF-κB activation, whereas in OA chondrocytes, it could reduce apoptosis and ECM degradation by inhibiting aberrant NF-κB activation.

There are several limitations in this study. First, while the in vitro nature of the study provides valuable mechanistic insights under controlled conditions, it does not fully replicate the complexity of in vivo environments. Further studies should focus on validating these findings in animal models or clinical trials to establish the relevance of the role of FHL2 in human OA pathology. Second, the ATDC5 cell line was used in this study because it is more stable than human chondrocytes, especially for signaling pathway research. To enhance clinical relevance, future studies will include human chondrocytes. Finally, using IL-1β as the only inflammatory factor in this study may not fully capture the complexity of OA. Although IL-1β stimulation does not completely replicate OA progression, it remains one of the most widely used and accepted in vitro models for OA research.

Conclusions

In IL-1β-induced chondrocyte-like ATDC5 cells,the downregulation of FHL2 inhibited ECM degradation and apoptosiswhile autophagy was activated. The protected role of FHL2 downregulation in osteoarthritis might be partially associated with the nuclear translocation of FHL2, as well as the phosphorylation of NF-κB, mTOR, and AKT. This indicates that FHL2 might function as a molecular transmitter, linking various signaling pathways to transcriptional regulation in osteoarthritis.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12891-025-08536-9.

Supplementary Material 1

Supplementary Material 2

Supplementary Material 3

Acknowledgements
Not applicable.

Author contributions
YCL and FW accountable for the integrity and analysis of the data, and the writing of the manuscript. ABDSM accountable for the collected and analyzed data. BCJ analyzed data and gave experimental support. LC planned the study. All authors read and approved the final manuscript.

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Data availability
All relevant data analyzed during the current study are within the paper.

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

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