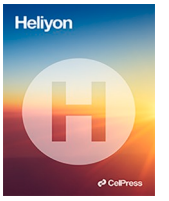
Heliyon 10 (2024) e25080

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon



Research article

RNA binding protein PUM2 promotes IL-1β-induced apoptosis of chondrocytes via regulating FOXO3 expression

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ARTICLE INFO

Keywords:
Apoptosis
FOXO3
Chondrocyte
PUM2
Osteoarthritis

ABSTRACT

Objective: RNA-binding proteins (RBPs) have been recently proven to be involved in the pathogenesis of several diseases. However, few studies elaborated RBPs in regulating osteoarthritis. This study aims to define the function and mechanism of RBPs-PUM2 in chondrocyte apoptosis during osteoarthritis.
Methods: Cartilage tissue samples and human juvenile chondrocyte cell line C28/I2 were collected for further study. PUM2 expression in the human tissues and cells was determined using qRT-PCR. Chondrocyte viability and apoptosis were determined by MTT and flow cytometry. ROS generation was determined by flow cytometry. The regulation of PUM2 on FOXO3 translation was evaluated by RNA immunoprecipitation, RNA pull-down, and Luciferase gene reporter analysis.
Results: PUM2 is upregulated in both cartilage tissue of osteoarthritis patients and IL-1β-stimulated chondrocytes. PUM2 overexpression reduces cell viability and promotes cell apoptosis and ROS generation of chondrocytes. PUM2 silencing increases cell viability and ameliorates cell apoptosis as well as ROS generation in chondrocytes induced by IL-1β. PUM2 inhibits FOXO3 expression via binding its mRNA 3′-UTR. PUM2 forms a signaling axis with FOXO3 in IL-1β induced chondrocyte damage.
Conclusion: PUM2 is upregulated in cartilage tissue of osteoarthritis and positively regulates chondrocytes apoptosis through controlling FOXO3 protein expression.

1. Introduction

Osteoarthritis is a chronic degenerative joint disease involving cartilage and its surrounding tissues, which can eventually lead to joint failure with pain and disability. Its pathological features are the focal absence of articular cartilage, osteophyte formation, subchondral bone changes, and synovial hyperplasia [1]. Its prevention and therapeutic intervention are always impeded owing to ill-defined pathogenesis. According to what is already known, osteoarthritis is a multifactorial disease and also involved multiple pathogeneses including biological and biomechanical alteration, physiological cellular behavior dysfunction, and immunologic dissonance [2]. It's worth noting that the dysfunction of the chondrocyte, the main active cells in the structure of cartilage tissue, is the

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https://doi.org/10.1016/j.heliyon.2024.e25080
Received 13 July 2023; Received in revised form 18 January 2024; Accepted 19 January 2024
Available online 27 January 2024

focus of current researchers. Especially, the unusual cell proliferation, differentiation, and apoptosis of chondrocytes under pathogenic factors are considered very important in osteoarthritis. Therefore, researchers are aroused to discern the underlying mechanism.

RNA-binding proteins (RBPs) are an interesting biomarkers due to their functions on gene expression during disease occurrence and progression via post-transcriptional regulation of mRNA [3,4]. Studies have shown that the excessive accumulation and dysfunction of RBPs are the cause of inflammation-related diseases and maybe serves as a potential therapeutic target [5]. However, the involvement of RBPs in osteoarthritis is less well documented. PUM2, a known member of RBPs, is recognized as a critical regulator in aging-related diseases by affecting the homeostasis of organelles mediated by impairing cellular proteostasis at the level of metabolite or translation efficacy of target mRNAs [6,7] suggesting the critical role of PUM2 in degenerative joint disease. This triggered our interest in the possibility that PUM2 might also play a role in the pathogenesis of osteoarthritis.

The Forkhead box O (FoxO) family has recently been highlighted as an important transcriptional regulator of crucial proteins associated with the many diverse functions of cells [8]. FoxO3a is been identified in humans, and plays an improtant role in disease progression by regulating biological processes involved in apoptosis, cell cycle arrest, and oxidative stress resistance [8,9]. In the development period of bones, FOXO3a in chondrocytes is critical for endochondral bone formation [10]. In osteoarthritic chondrocytes, FOXO3a affects chondrocyte function and survival by regulating gene expression [11,12]. In the pathologic process of disease, FOXO3 expression can be regulated by RBPs [13,14]. So far, there have been rare explorations about whether FOXO3 can be regulated by PUM2. The present study investigated the regulating role of PUM2 on FOXO3 expression and their roles in chondrocytes induced by inflammatory cytokine interleukin (IL)-1β, a kind of pro-inflammatory cytokine that is believed to be a key mediator of osteoarthritis via affecting chondrocytes survival and functionality [15,16]. The exploration in the present research may provide an especially insightful perspective on the function of PUM2 on chondrocyte apoptosis in osteoarthritis and reveal a potential therapeutic target for delaying the progression of osteoarthritis. The present study first reveal the interaction between PUM2 and FOXO3, and their role on chondrocytes. We also aimed to investigate the impact of biological pathways and their mediation effect on osteoarthritis.

2. Materials and methods

2.1. Patients and cartilage tissue tissues

A total of 35 patients with osteoarthritis (aged 28–75 years with an average of 51.4) who underwent a discectomy in our hospital were included in the study. Another 12 healthy objects (aged 29–68 years with an average of 47.9) were recruited as the controls. Cartilage tissue samples of knee joint from OA patients with knee replacement surgery and controls person who under the surgery of knee fracture were collected after obtaining informed consent and immediately frozen in the liquid nitrogen being used for qRT-PCR and Western blot assay. All protocols involved have been approved by the committee of the Wuhan Third Hospital. The study was approved by the institutional research ethics committee of Wuhan Third Hospital, Tongren Hospital of Wuhan University.

2.2. Human chondrocytes culture and IL-1β treatment

The immortalized human juvenile chondrocyte cell line C28/I2 was obtained from Beina Chuanglian biotechnology institute (Beijing, China). Chondrocytes were seeded into culture plates and cultured in a CO2 incubator at 37 °C with a frequency of changing fresh DMEM/F12 medium every two days. For IL-1β-induced chondrocyte injury, cells were cultured in a medium containing 10 ng/ml for 24 h.

2.3. Vector construction and transfection

Human PUM2-specific siRNA cloned into lentiviral vector pLL3.7 (Addgene) and PUM2 overexpression sequence (open reading frame) into pHAGE vectors were made according to the manufacturer's introduction. The constructs and packaging plasmids were transfected with 293 T cells. Lentiviral containing target gene siRNA (lenti-si-PUM2) and PUM2 overexpression sequence (lenti-PUM2) were collected and used to transfect chondrocytes. FOXO3 open reading frame was amplified and inserted into pcDNA3.0 (Invitrogen, USA) to produce an overexpression recombinant vector (pcDNA-FOXO3). The siRNA targeting FOXO3 (si-FOXO3) and control sequence were provided by Sigma-Aldrich. For plasmid transfection, chondrocytes were seeded at plates, and transfection was performed when cells were grown to 80 % influence using Lipofectamine 2000 (Invitrogen, USA). Cells were then collected for subsequent analysis after 24 h or 48 h. For lentiviral infection, chondrocytes were seeded at plates and infected with diluted lentiviral supernatant containing polybrene. Cells were scored at 48 h post-infection.

2.4. Quantitative real-time PCR (qRT-PCR)

For determine mRNA expression, real-time PCR was conducted as described before [17]. Total RNA was extracted from cartilage tissue or chondrocytes using Trizol reagent (Qiagen) and stored in RNase-free DEPC water at −80 °C. For the qRT-PCR procedure to analyze the mRNA level of PUM2 and FOXO3, RNA was primarily converted to cDNA using a High Capacity cDNA Reverse Transcription Kit (Applied Biosystems, CA, USA) according to the instruction of the manufacturer. The cDNA product was then subjected to real-time RNA procedure with the specific primers using Power SYBR Green PCR Master Mix (Applied Biosystems). Primers used were as follows: hPUM2 : F-AACATTGGCTTTTGGTCAGG, R-CCAACCACTAAGGCACCAGT; hFOXO3 : F-CTTCAAGGATAAGGGCGACA, R-CGACTATGCAGTGACAGGTTG; hGAPDH: F-TTGGTATCGTGGAAGGACTC, R-ACAGTCTTCTGGGTGGCAGT; mPUM2:

F-ACAGCAGCTCTTTCAGAGGAC, R-GGCCTGCTGAGAATACACCA; mGAPDH: F-CAAGGTCATCCATGACAACTTTG, R-GGCCATCCACAGTCTTCTGG. GAPDH was used to normalize gene expression.

2.5. Western blot

To determine protein expression of those genes, Western blot was carried out as described before [17]. Total protein samples were obtained from tissue homogenate or cell lysis supernatant using the RIPA buffer (Beyotime, China) supplementary with a mixture of proteinase inhibitor and storage in loading buffer at −20 °C. For detecting the relative level of PUM2 and FOXO3, an equal protein sample (30 μg) was resolved by 10 % sodium dodecyl sulfate-polyacrylamide gel electrophoresis and transferred onto a PVDF membrane (Millipore). After blocking, incubation of primary antibodies against PUM2, FOXO3, and β-actin (1: 800–1:2000, Abcam), and incubation of horseradish enzyme-labeled secondary antibodies, protein blotting in the membrane was visualized using ECL reagent (Beyotime, China).

2.6. MTT assay analyzes cell viability

Cell viability was analyzed using the MTT Cell Proliferation Kit (Sangon Biotech, China) according to the instruction of the manufacturer, and the experiments were conducted as before [18]. In brief, chondrocytes at 2×10^3 cells/well were seeded on a 96-well plate and treated by IL-1β or cell transfection. After 24 h treatment, a final concentration of 0.5 mg/ml MTT reagent was added to each well and incubated for 4 h at 37 °C in a 5 % CO_2 atmosphere. A volume of 100 μl of Formazan Solubilization Solution was added to each well to dissolve the formazan crystals shakily. The absorbance of each well was detected using a microplate reader at 570 nm.

2.7. Cell apoptosis analysis by flow cytometry

The percentage of apoptotic cells was analyzed by flow cytometry using a FITC Annexin V apoptosis Detection Kit (Sigma-Aldrich, USA) as the protocol of the manufacturer. In brief, chondrocytes were washed twice with ice-cold PBS buffer and resuspended in the buffer. A total of 1×10^5 chondrocytes were incubated with 8 μL of Annexin V-fluorescein isothiocyanate (FITC) and propidium iodide (PI) for 15 min at room temperature [19]. The apoptotic cell in the mixture sample was detected using a flow cytometer and analyzed.

2.8. ROS generation analysis by flow cytometry

ROS generated in chondrocytes was detected by 2′,7′ -dichlorofluorescin diacetate (DCFH-DA) as described before [20]. A compound that can be oxidized into the fluorescent dichlorofluorescein (DCF) in a cellular ROS environment using ROS detection kit (Beyotime, China; S0033S). In Brief, the DCFH-DA kit was added to chondrocytes at a concentration of 1×10^4. Incubation was sustained for 30 min at 37 °C avoiding light. Chondrocytes were harvested and washed with ice-cold PBS buffer for the following analysis in flow cytometry (excitation wavelength/emission wavelength is 488 nm/525 nm).

2.9. RNA immunoprecipitation

The combination of PUM2 and FOXO3 mRNA in chondrocytes was analyzed using RNA immunoprecipitation as before [21]. Briefly, chondrocytes were lysed and the supernatant was incubated with Protein A/G beads that have coated with PUM2 antibody or the control IgG (Sigma, USA) overnight at 4 °C. On the next day, the immunoprecipitation complex was eluted from Protein A-G beads and subjected to RNA isolation using miRNeasy kit (Qiagen, German). The qRT-PCR assay was performed to detect the enrichment of the FOXO3 mRNA level.

2.10. RNA pull-down

The typical binding site of PUM2 to mRNA is 5′-UGUAXAUA-3′ [22] and we found there is a binding site on FOXO3 3′-UTR for PUM2 at positions 5284. To detect the binding between PUM2 and FOXO3 3′-UTR, RNA pull-down was performed using Pierce Magnetic RNA-Protein Pull-Down Kit (Thermo Fisher, USA) as instructed and suggested by the manufacturer, and the experiments procedures were as before [23]. Biotin-labeled probe on FOXO3 3′-UTR containing binding site (5′-UGUAUAUA-3′) along with probe on the same sequence with a mutated binding site (5-′UCCCGUUA-3′) or negative control probe on the scrambled sequence was co-incubated with cell lysate of chondrocytes in the presence of magnetic beads. The bead–RNA–protein complex was collected. Proteins in the complex pulled down were eluted and boiled in SDS buffer for subsequent PUM2 expression analysis by Western blot.

2.11. Dual luciferase reporter analysis

Luciferase reporter plasmid containing FOXO3 3′-UTR sequence (wild) and along with similar constructs with mutated PUM2 binding site was amplified by PCR procedure and subcloned into luciferase reporter pGL3 vector (Promega, USA). To investigate the effect of the combination of PUM2 and FOXO3 3′-UTR on the translation of FOXO3, HEK293T cells were co-transfected with PUM2 overexpression plasmid and either pGL3-promoter or pGL3-*FOXO3* reporter along with pRL-CMV Renilla-expressing vector using lipofectamine 2000 (Invitrogen). After 48 h transfection, cells were harvested for analysis of firefly and Renilla luciferase activities

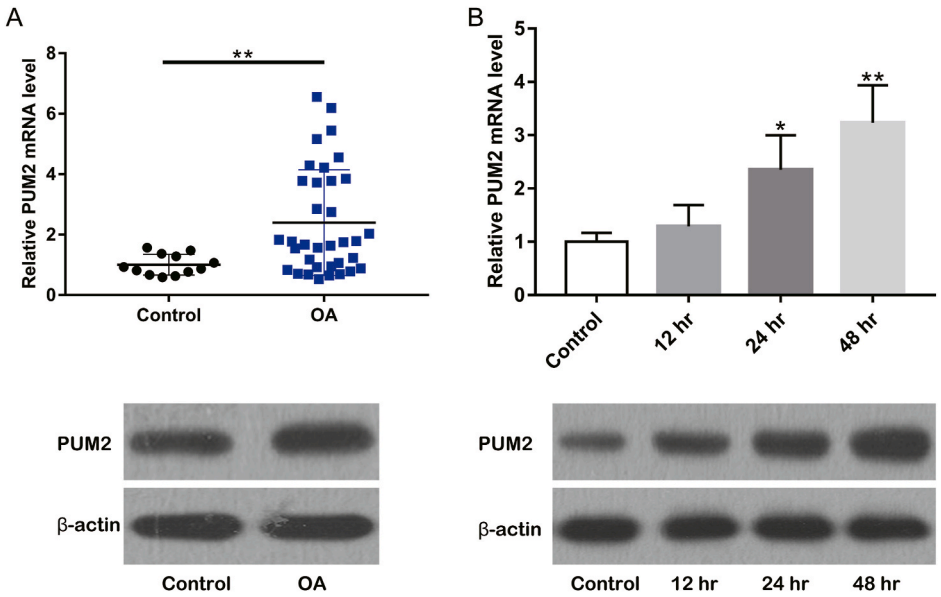

Fig. 1. PUM2 is upregulated in the cartilage tissue of osteoarthritis patients. (A–B), PUM2 expression was compared between osteoarthritis patients (n = 35) and healthy control (n = 12) (A) and between (B) IL-1β-stimulated chondrocytes and control chondrocytes using qRT-PCR and Western blot. *p < 0.05, **p < 0.01 compared with the matched group.

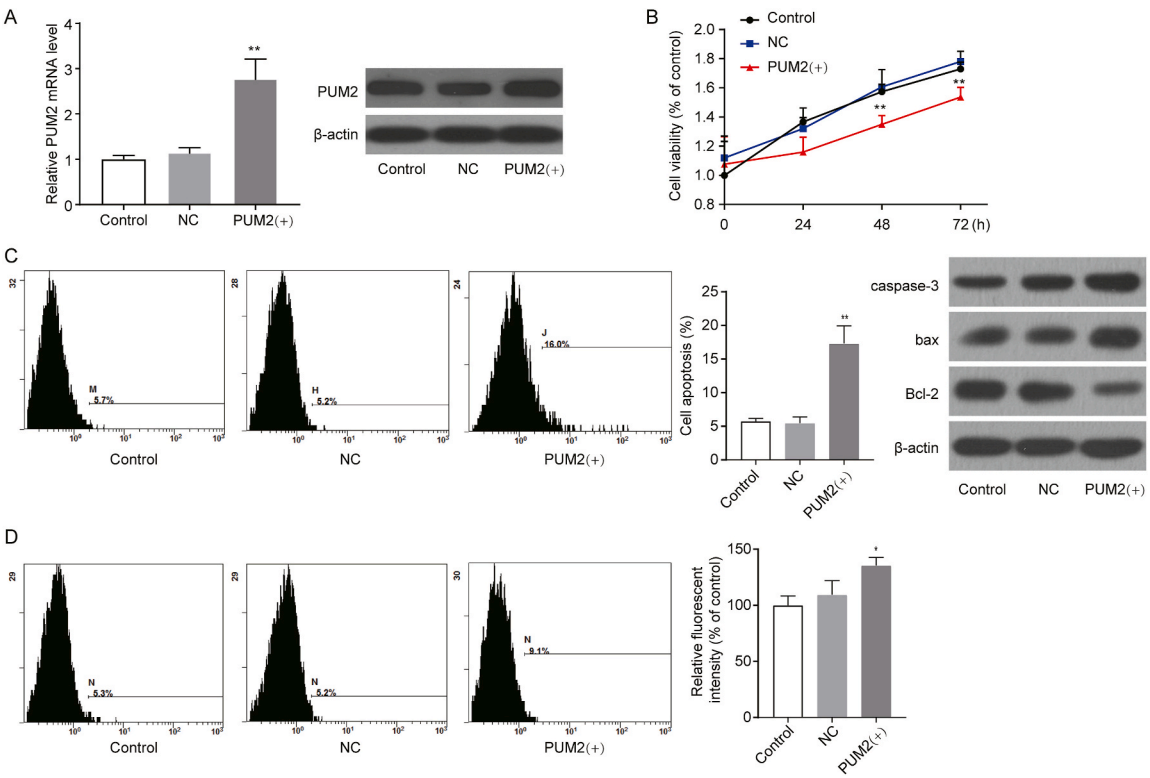

Fig. 2. PUM2 reduces cell viability and promotes cell apoptosis of human primary chondrocytes. PUM2 was overexpressed by lentivirus expression vector-mediated cell transduction with an empty lentivirus vector as the negative control (lenti-NC). (A) The PUM2 expression level was determined by qRT-PCR and Western blot. (B) Cell viability was examined by MTT assay. (C) Cell apoptosis and (D) ROS generation were analyzed by flow cytometry. **p < 0.01 compared with lenti-NC.

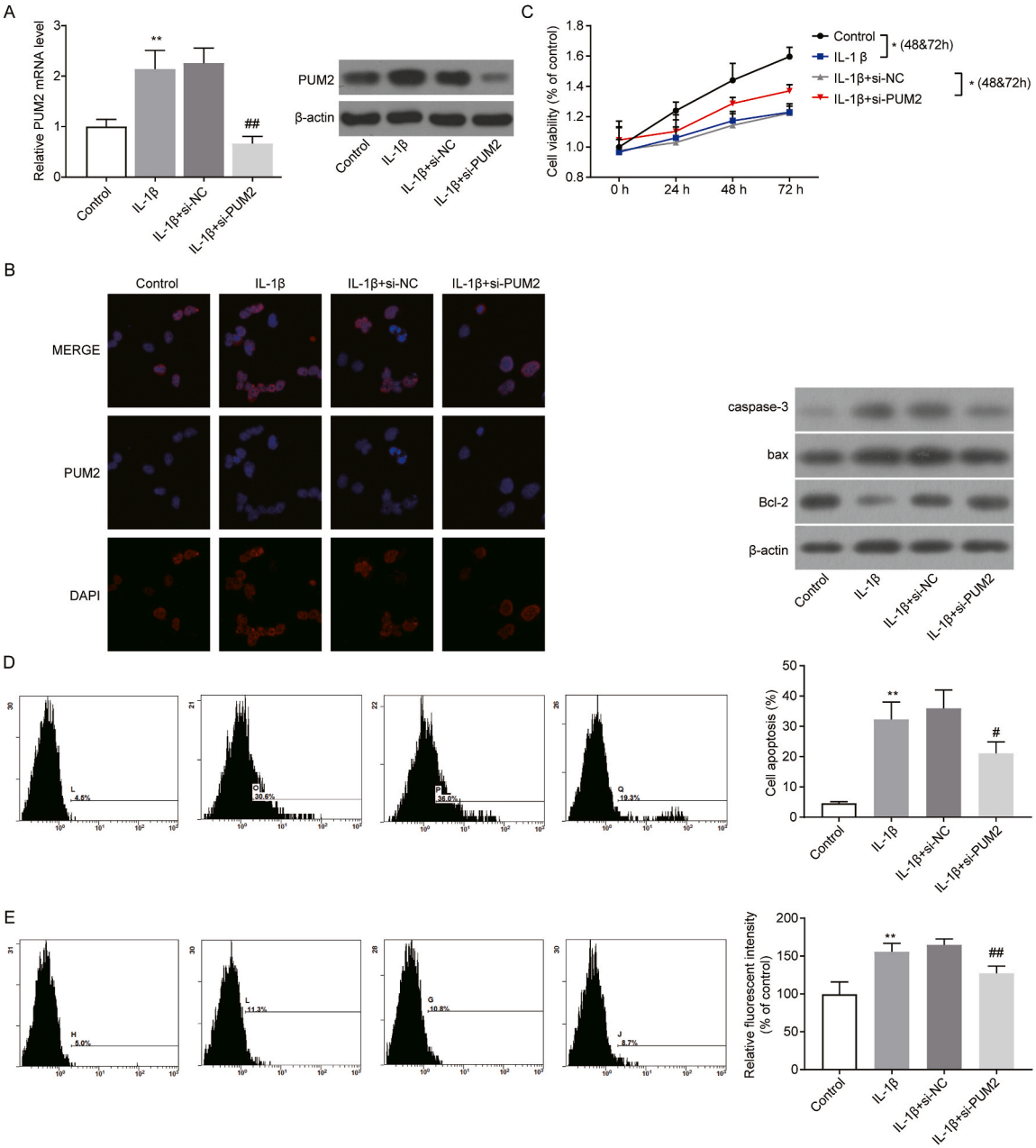

Fig. 3. PUM2 silencing increases cell viability and ameliorates cell apoptosis in chondrocytes induced by IL-1β. Cells were transduced by siRNA interfering lentivirus vector of PUM2 under the treatment of 10 ng/ml of IL-1β. (A) The PUM2 expression level was determined by qRT-PCR and Western blot. (B) PUM2 expression was detected by immunofluorescence. (C) Cell viability was examined by MTT assay. (D) Cell apoptosis were analyzed by flow cytometry and the apoptosis related biomarkers were detected by Western blot. (E) ROS generation were analyzed by flow cytometry. **p < 0.05, **p < 0.01 compared with control. #p < 0.05, ##p < 0.01 compared with IL-1β+lenti-si-NC.

using Dual-Luciferase™ reporter assay system (Promega, USA) based on the manufacturer's protocols. The experiments was conducted as described before [24].

2.12. Statistical analysis

Data are presented as mean with standard deviation (mean ± SD). GraphPad Prism 7.0 version was used for statistical analysis and significant analysis between groups was analyzed by T-test or one-way analysis of variance. Significance was when P-value is less than 0.05.

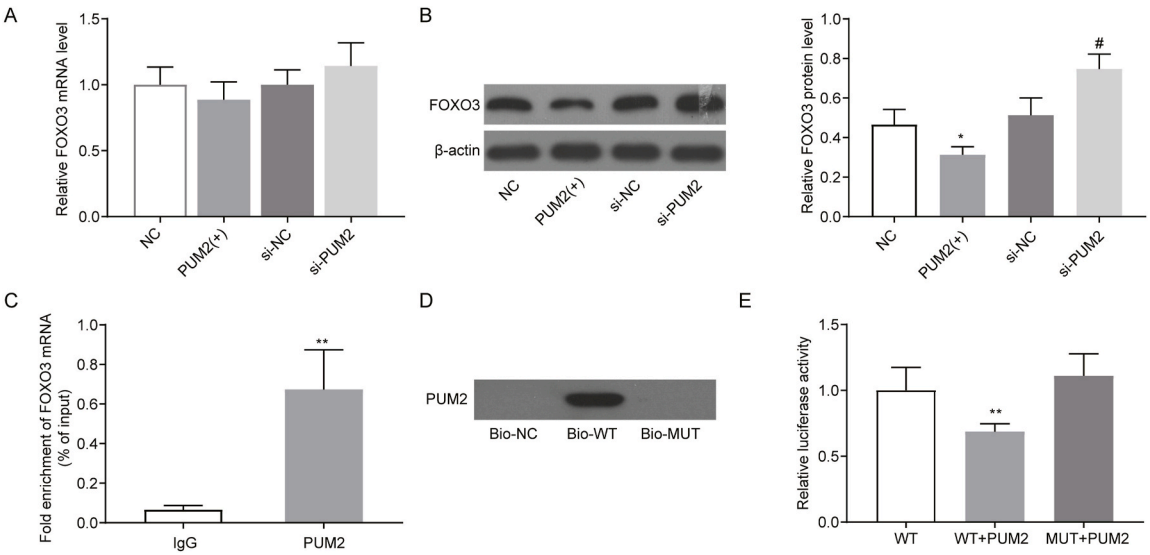

Fig. 4. PUM2 inhibits FOXO3 expression. (A & B) FOXO3 expression in chondrocytes transfected by PUM2 expression lentivirus vector and PUM2 interfering lentivirus vector was determined by qRT-PCR and Western blot. (C) FOXO3 mRNA enriched in the precipitation complex of PUM2 was analyzed by RNA immunoprecipitation. (D) Biotin-labeled wild-type probe and mutant probe of FOXO3 3′-UTR were incubated with cell lysates of chondrocytes and PUM2 pulled down by these probes was determined by Western blot. (E) Luciferase gene reporter analysis detected the combination of PUM2 and FOXO3 3′-UTR. $^{**}p < 0.05$, $^{**}p < 0.01$ compared with lenti-NC or luciferase reporter containing wild FOXO3 3′-UTR. $^{\#}p < 0.05$ compared with lenti-si-NC.

3. Results

3.1. PUM2 is upregulated cartilage tissue of osteoarthritis

The PUM2 level of cartilage tissue was compared between osteoarthritis patients and healthy controls and the result showed that osteoarthritis patients had a remarkably higher level of PUM2 (Fig. 1A). Further exploration of PUM2 level change related to chondrocyte inflammation, PUM2 expression was examined in IL-1β-stimulated chondrocytes. Consistent with changes in expression level in cartilage tissues, the PUM2 protein level was significantly increased after 12 h of IL-1β treatment (Fig. 1B).

3.2. PUM2 overexpression reduces cell viability and promotes cell apoptosis of chondrocytes

To ascertain the function of PUM2 on chondrocyte injury, we analyzed cell viability and apoptosis after chondrocytes transduced by the PUM2 expression vector. The transduction of the PUM2 expression vector induced the overexpression of PUM2 mRNA and protein (Fig. 2A). In these chondrocytes with PUM2 overexpression, cell viability was gradually reduced along with the transfection time (Fig. 2B), and the percentage of the apoptotic cells was significantly increased at 48 h-post transfections (Fig. 2C). Moreover, PUM2 overexpression caused an increase in ROS generation (Fig. 2D). These data suggest that abnormal upregulation of PUM2 may positively affect the progression of osteoarthritis by inducing chondrocyte damage.

PUM2 silencing increases cell viability and ameliorates cell apoptosis in chondrocytes induced by IL-1β.

To further define the damaging effect of PUM2 on chondrocytes, an experiment was performed on chondrocytes with the combined treatment of PUM2 silencing and IL-1β. PUM2 expression was upregulated by IL-1β alone and significantly silenced after the combined treatment of siRNA interference of PUM2 (Fig. 3A). The same expression trend of PUM2 was visualized by the result of the immunofluorescence assay with the PUM2 antibody (Fig. 3B). As for chondrocytes' damage, both the decreased cell viability and the increased cell apoptosis induced by IL-1β were relieved by PUM2 silencing to a certain extent (Fig. 3C and D). Besides that, IL-1β-induced increased ROS generation was also significantly lowered by PUM2 silencing suggesting that PUM2 affects chondrocyte damage via enhancing oxidative stress (Fig. 3E).

3.3. PUM2 inhibits FOXO3 expression

As reported, PUM2 can regulate target gene expression via binding to its mRNA 3′-UTR [6]. We confirmed the potential binding of PUM2 to FOXO3 mRNA by the bioinformatics analysis (data not shown). Therefore, the regulatory function of PUM2 on FOXO3 expression was investigated primarily. PUM2 was overexpressed and silenced respectively in chondrocytes to observe their impacts on FOXO3 expression. As shown in Fig. 4A and B, FOXO3 mRNA levels were not affected by PUM2 overexpression or silencing but the FOXO3 protein level was reduced by PUM2 overexpression and increased by PUM2 silencing suggesting the negative regulating

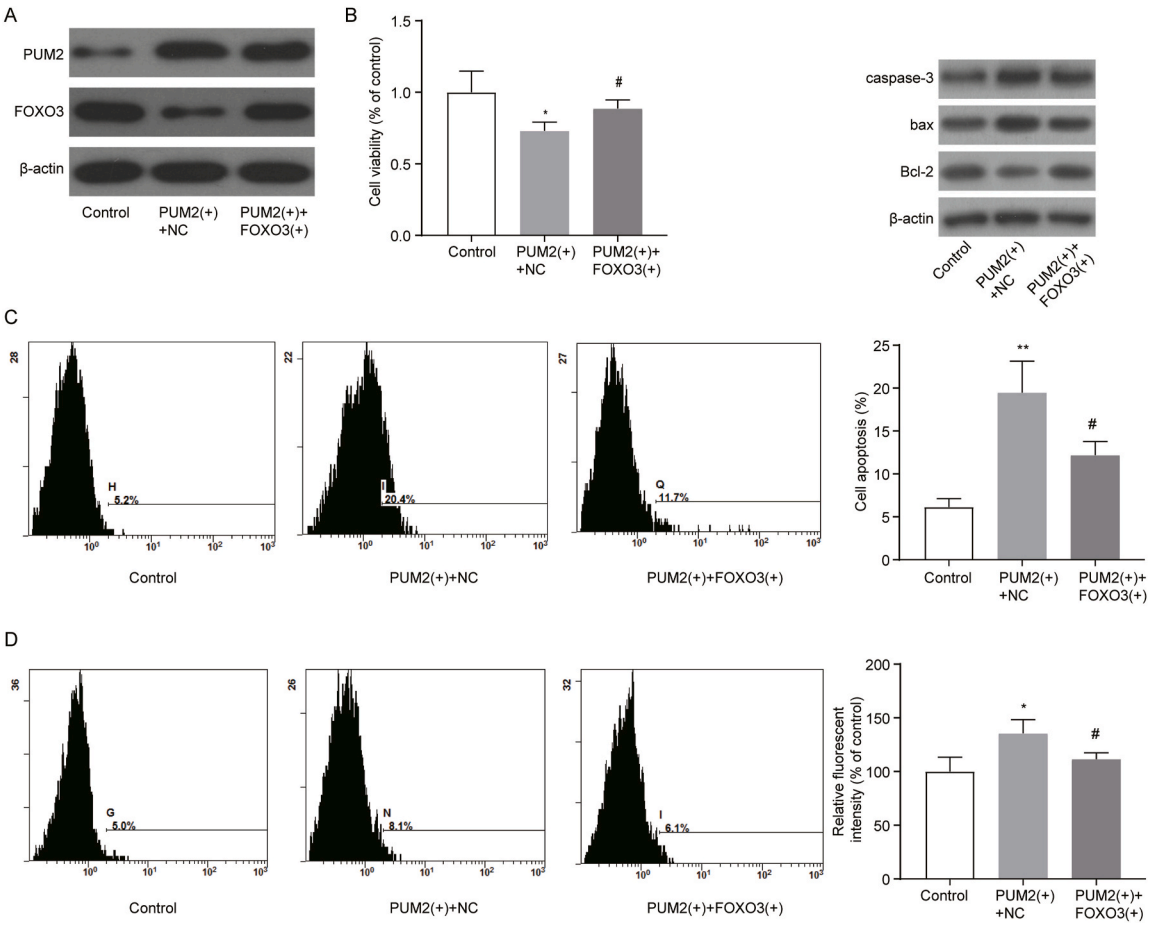

Fig. 5. FOXO3 overexpression reduces chondrocytes injury induced by PUM2. chondrocytes were co-transfected with the PUM2 expression lentivirus vector and FOXO3 expression pcDNA vector. (A) PUM2 expression was determined by Western blot. (B) Cell viability was analyzed by MTT assay. (C) Cell apoptosis were analyzed by flow cytometry, and the apoptosis related markers were analyzed by Western blot. (D) ROS generation were analyzed by flow cytometry. *$p < 0.05$, **$p < 0.01$ compared with control. $^{\#}p < 0.05$ compared with lenti-PUM2+pcDNA-NC.

function of PUM2 on FOXO3 translation. Followingly, the binding of PUM2 to FOXO3 mRNA was further demonstrated by the RNA immunoprecipitation assay where FOXO3 mRNA was enriched in the precipitation complex of PUM2 (Fig. 4C). To further verify PUM2 regulates FOXO3 expression by binding its mRNA 3′-UTR, a biotin-labeled FOXO3 3′-UTR probe was incubated with cell lysates of chondrocytes to determine the higher PUM2 expression level in pull-down complex, and luciferase reporter containing FOXO3 3′-UTR was co-transfected with PUM2 into chondrocytes to observe the stronger translational activity of FOXO3 than the corresponding control (Fig. 4D and E).

3.4. PUM2 impairs chondrocytes via FOXO3

To demonstrate that PUM2 forms a signaling axis with FOXO3, we primarily determined the role of FOXO3 overexpression in chondrocytes with PUM2 overexpression-induced damage. Co-overexpression of PUM2 and FOXO3 caused the upregulation of FOXO3 in chondrocytes (Fig. 5A). In these transducer chondrocytes, cell viability was increased and cell apoptosis was attenuated compared with PUM2 overexpression alone (Fig. 5B and C) suggesting the mitigative effect of FOXO3 on PUM2-induced chondrocytes damage. Except that, ROS generation induced by PUM2 overexpression was relieved by FOXO3 overexpression (Fig. 5D).

Next, PUM2 and FOXO3 were co-silenced in chondrocytes with IL-1β induced damage. This co-transducer caused the down-regulation of FOXO3 compared with PUM2 silencing alone (Fig. 6A). As for their effect on chondrocytes' damage, FOXO3 silencing ameliorated the protective effect of PUM2 silencing on cell viability and the inhibiting effect on cell apoptosis in the presence of IL-1β (Fig. 6B and C). The same mitigative role of FOXO3 silencing in ROS generation was also observed (Fig. 6D).

4. Discussion

Osteoarthritis is a kind of chronic inflammatory disease, with a chronic and progressive condition responding to the accumulation

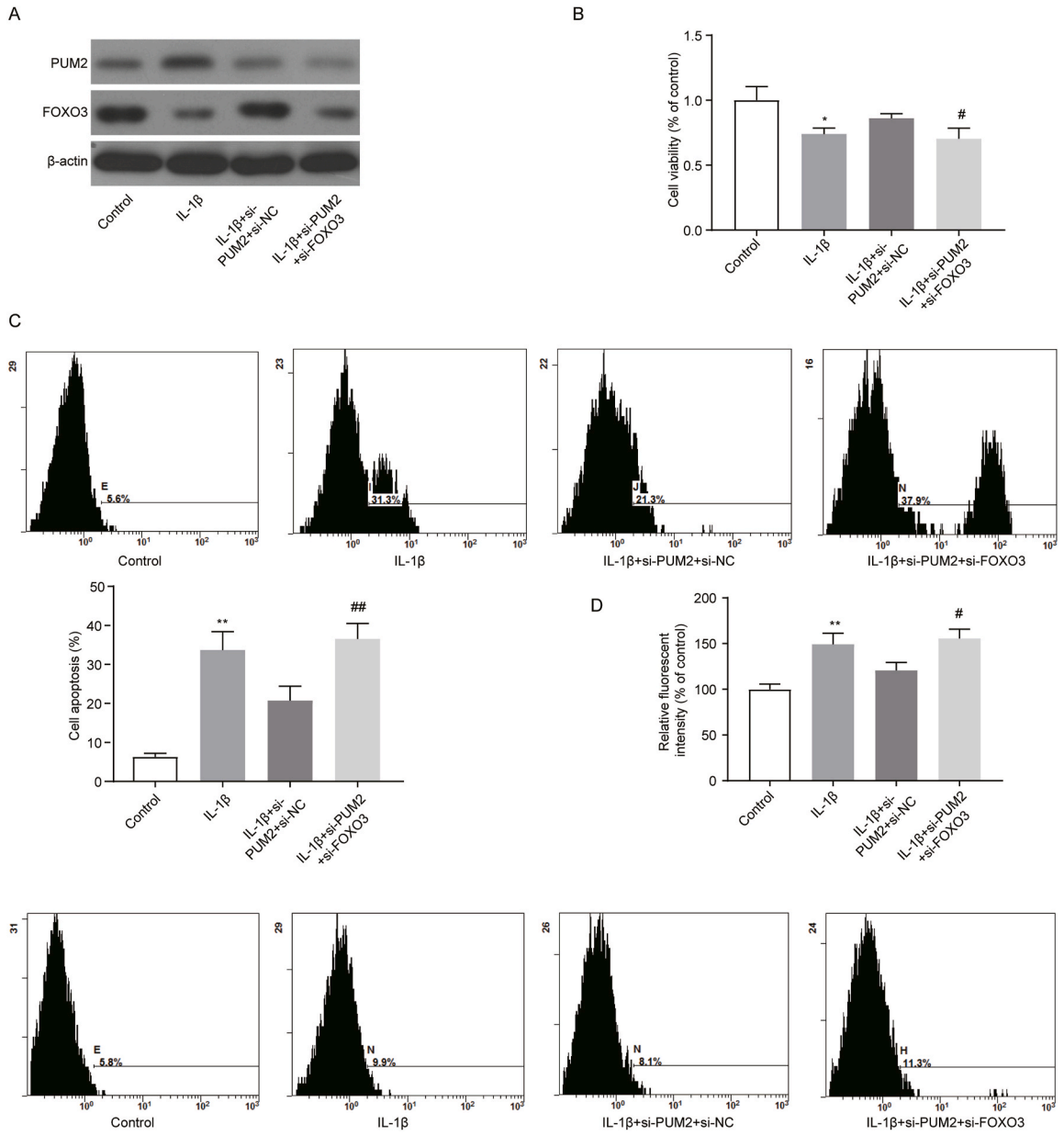

Fig. 6. FOXO3 silencing reverses the protective effect of PUM2 silencing on cell survival in IL-1β-injured chondrocytes. Chondrocytes were divided into four groups: control, IL-1β, IL-1β+Lenti-si-PUM2+si-NC, IL-1β+Lenti-si-PUM2+si-FOXO3. After the treatment, cells were collected for the analysis of (A) PUM2 and FOXO3 expression, (B) cell viability, (C) cell apoptosis, and (D) ROS generation. *$p < 0.05$, **$p < 0.01$ compared with control. $^{\#}p < 0.05$ compared with IL-1β+lenti-si-PUM2+si-NC.

of cytokines and dysfunction of cartilage tissue-resident cells. Increased chondrocyte apoptosis is a dominant cell injury event in this pathogenesis; therefore, it is more relevant to understand what underlying responding gene and its molecular mechanism are involved to prevent or restrain osteoarthritis. RBPs-PUM2 is interesting in the present study. Our data particularly points out that PUM2 is an important responding gene, significantly upregulates in cartilage tissue of patients with severe osteoarthritis, and accelerates apoptosis of chondrocytes with a molecular mechanism of regulating FOXO3 expression.

Apoptosis of chondrocytes accounts for the primarily responsible for osteoarthritis under the influence of inflammatory cartilage tissue. Multiple inflammatory factors including IL-1β have been proven certainly to be largely aggregated at cartilage tissue and exacerbated the inflammatory processes of chondrocytes to a great extent [16,25]. Of particular note is that IL-1β was considered to be involved in the key event cartilage damage and subchondral bone destruction relevant to osteoarthritis [26], as well as exerting an active role in chondrocyte loss [27]. In the present study, our results reveal that PUM2 silencing protects chondrocytes from apoptosis induced by IL-1β suggesting that PUM2 serves as a critical molecule in IL-1β-induced chondrocyte loss. As previous study demonstrated

that PUM2 knockdown enhanced mesenchymal stem cell osteogenic potential, and promoted bone regeneration [28], which also indicating the potential role of PUM2 on osteogenesis. For the knowledge of IL-1β function, previously, it is a well-known inflammatory factor that's involved in all kinds of cellular inflammatory responses through activating inflammation-related gene expression [29,30]. RBPs are the proteins that mediate IL-1β-induced gene expression and cell survival in various kinds of cells [31,32]. Our data for the first time defines that PUM2 acts as a mediator for IL-1β in chondrocytes. The role of PUM2 in osteoarthritis still needs to be further investigated in the following studies.

Increasing ROS level was detected in chondrocytes with PUM2 overexpression in our experiments. It has been reported that the excessive accumulation of ROS represents the intracellular oxidative stress, and maybe mediate IL-1β-induced cell apoptosis of chondrocytes [33,34]. Oxidative stress has been demonstrated to participate in the impairment of chondrocytes [35,36]. We, therefore, speculate that PUM2 mediates IL-1β-induced cell injury by promoting oxidative stress in chondrocytes. Moreover, FOXO3, a protein that is strongly associated with aging-related diseases and mainly affects oxidative stress [37,38], was investigated in the process of PUM2-induced chondrocyte apoptosis. In cartilage tissue, FOXO3 acts as a protector for chondrocytes against oxidative stress-induced damage [39]. Our data support these functions of FOXO3 and showed that FOXO3 overexpression reduced the increased ROS level induced by PUM2 overexpression accompanied by chondrocytes survival, and FOXO3 silencing reversed the decreased ROS level induced by PUM2 silencing in presence of IL-1β accompanied by chondrocytes apoptosis. The results were consistent with the previous study that knockdown of FOXO3 increased Dex-induced apoptosis as well as ROS levels [40], indicating the validity of our results. Finally, we can conclude that FOXO3 is an intermediate molecule that mediates PUM2-induced apoptosis of chondrocytes.

PUM2 is recognized as a membrane of RBPs, with the common characteristic of low-complexity domains that enable their condensation in ribonucleoprotein granules and play a part in the homeostasis and function of mRNA [4]. This protein functions in response to cellular stress, when the RBP is bound to the target mRNA in stress granules, resulting in protein translation being blocked [4]. The abnormal function of RBPs correlates to joint disorders [41,42]. However, the role of RBPs in osteoarthritis is largely unexplored. In this study, the gene regulation by PUM2 in FOXO3 was elaborated in chondrocytes. Previously, the modulation of PUM2 on gene expression referring to translation, metabolism, and transport of mRNA has been observed in several diseases [6,43]. Through the literature, we know that PUM2 can response to cellular stress to affect cell survival and inflammation [44,45] and also participates in cellular stress-related gene expression. Our data delightedly find that the oxidative stress-related gene FOXO3 expression is controlled by PUM2 where PUM2 binds to the 3′-UTR of FOXO3 mRNA and affects translation activity rather than change the mRNA expression of PUM2.

5. Conclusions

The data of the present study defined that PUM2, a kind of RBP, functions as a positive regulatory in IL-1β-induced cell apoptosis of chondrocytes in osteoarthritis, with an underlying molecule mechanism of affecting oxidative stress and controlling FOXO3 expression. Our findings may reveal a prospective therapeutic target for preventing progression of the osteoarthritis.

CRediT authorship contribution statement

Du Wang: Writing – review & editing, Writing – original draft, Validation, Supervision, Software, Methodology. **ZhiLi Zhang:** Writing – review & editing, Visualization, Supervision, Methodology. **Ling He:** Writing – review & editing, Writing – original draft, Methodology. **Xili Li:** Writing – original draft, Validation, Methodology, Conceptualization.

Declaration of competing interest

Authors declare there is no conflict of interest.

Acknowledgment

This study was supported by Science and Technology Project of Wuhan Health Commission (No.WX20D11).

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