Knockdown of lncRNA JPX suppresses IL-1β-stimulated injury in chondrocytes through modulating an miR-25-3p/PPID axis

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Abstract. The aim of this study was to investigate the potential mechanisms of long noncoding (lnc) RNA Just proximal to X-inactive specific transcript (JPX) in interleukin (IL)-1β-stimulated chondrocytes. Human C28/I2 chondrocytes were treated with IL-1β to simulate osteoarthritic (OA) injury. The expression levels of JPX, microRNA (miRNA/miR)-25-3p, and peptidylprolyl isomerase D (PPID) were measured using reverse transcription-quantitative PCR or western blotting. The IL-1β-stimulated injury was assessed using a Cell Counting Kit-8 assay, flow cytometry, and western blot analysis. The targeted relationship between miR-25-3p, JPX, and PPID was verified using a dual-luciferase reporter and RNA immunoprecipitation (RIP) assays. The results showed that JPX expression was upregulated in OA patients and IL-1β-stimulated chondrocytes. JPX knockdown enhanced cell viability and suppressed apoptosis of IL-1β-stimulated chondrocytes. miR-25-3p inhibition rescued the inhibitory effect of JPX knockdown on IL-1β-stimulated injury. PPID overexpression eliminated the effects of JPX knockdown on IL-1β-stimulated chondrocytes. In conclusion, JPX knockdown increased cell viability and reduced apoptosis in IL-1β-stimulated chondrocytes, and this involved modulation of a miR-25-3p/PPID axis.

Introduction

Osteoarthritis (OA) is a complex chronic disease characterized by stiffness, arthralgia, and swelling, which is the primary cause of disability amongst the elderly (1,2). Chondrocytes are a type of cell in cartilage tissues that primarily function to generate and maintain the extracellular matrix (ECM) components (3). During OA, chondrocytes undergo multiple changes, such as in their secretory profiles and regarding their cell viability (4). Aberrant apoptosis and inflammatory responses in chondrocytes are related to cartilage degradation in OA (5,6). Therefore, probing the mechanism of chondrocyte dysfunction may be useful in understanding OA pathogenesis.

Long noncoding RNAs (lncRNAs) exert important roles in a variety of biological processes, including metabolism, immunity, differentiation, and apoptosis (7,8). A growing number of studies have shown that lncRNAs play vital effects in osteogenesis, chondrogenesis, and OA (9,10). More recently, the dysregulation of lncRNAs has been studied in OA (11,12). LncRNA ZNFX1 antisense RNA 1 was shown to inhibit apoptosis and matrix synthesis, and facilitate chondrocyte growth and migration in OA (13). In human chondrocytes, lncRNA urothelial cancer associated 1 increased matrix metallopeptidase 13 expression by suppressing microRNA (miRNA/miR)-204-5p (14). Reports on Just proximal to X-inactive specific transcript (JPX), a lncRNA, have primarily focused on its regulatory effects in various cancers, including hepatocellular carcinoma, colorectal cancer, lung cancer, oral squamous cell carcinoma, and myeloid malignancies (15-19). Interestingly, Gál *et al* (20) found that JPX expression was upregulated in adult patients with allergic rhinitis compared with the adult control group. Chen *et al* (21) showed that JPX was upregulated in allergic rhinitis and knockdown of JPX improved the imbalance between Treg/Th17 observed in allergic rhinitis. Nevertheless, whether JPX participates in OA development and the underlying molecular mechanisms involved remain to be determined.

An increasing number of studies have demonstrated that miRNAs are involved in the regulation of cell viability, differentiation, inflammation, lipid metabolism, apoptosis, oncogenesis, and other core cellular activities (22). It has been shown that miRNAs may serve as novel therapeutic targets for OA (23). LncRNAs can act as competitive endogenous (ce)RNAs by functioning as miRNA sponges, leading to the suppression of miRNAs (24,25). For example, lncRNA plasmacytoma variant translocation 1 aggravated ECM degradation by inhibiting miR-140 expression in OA (26). LINC00461 enhanced chondrocyte proliferation and cell cycle progression by downregulating miR-30a-5p

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Abbreviations: lncRNAs, long noncoding RNAs; OA, osteoarthritis; PPID, peptidylprolyl isomerase D; mir/miRNA, microRNA; CCK-8, Cell Counting Kit-8; RIP, RNA immunoprecipitation; ECM, extracellular matrix; JPX, Just proximal to X-inactive specific transcript; ceRNA, competitive endogenous RNA; PPIase, peptidyl cis-trans prolyl isomerase; TNF-α, tumor necrosis factor-α

Key words: osteoarthritis, long non-coding RNA JPX, microRNA-25-3p, peptidylprolyl isomerase D, IL-1β

expression in OA (27). LncRNA HOX transcript antisense RNA (HOTAIR) knockdown promoted proliferation and inhibited ECM degradation of OA chondrocytes by increasing the activity of the miR-107/C-X-C motif chemokine ligand 12 axis (28). Kurowska *et al* (29) found that miR-25-3p expression was downregulated in patients with rheumatoid arthritis. Wang *et al* (30) found that miR-25-3p expression was downregulated in osteoarthritic cartilage compared with healthy cartilage. Moreover, miR-25-3p expression was decreased in TNF-α-induced rat chondrocytes, and miR-25-3p negatively regulated IGFBP7 to promote chondrocyte proliferation and reduce chondrocyte apoptosis in OA (31). However, whether lncRNA JPX regulates miR-25-3p in OA remains to be assessed.

The first peptidyl cis-trans prolyl isomerase (PPIase) was isolated in 1984 by Fischer *et al* (32). The inhibition of PPIase activity protects cells from apoptosis (33). Lebedev *et al* (34) reported that PPIF was involved in mitochondrial permeability transition. PPIF-mediated necrosis participates in the pathological process of myocardial and/or cerebral ischemia/reperfusion injury (35). Inhibition of PPIF increases cell viability of TNF-α treated osteoblast-like cells (36). However, as a member of the PPIase family, the potential regulatory role of peptidylprolyl isomerase D (PPID) has not been elucidated.

In the present study, we investigated the potential effects of lncRNA JPX in OA. The function of JPX in cell viability and apoptosis of chondrocytes, and the specific mechanism of modulating miR-25-3p activity were assessed.

Materials and methods

Specimen collection. A total of 20 OA patients and 16 non-OA patients (patients without symptoms of OA during total hip replacement) from Sunshine Union Hospital (Weifang, China) were collected in the present study. Patients provided signed consent to the collection of samples and the present study was approved by the Ethics Committee of Sunshine Union Hospital.

Cell culture and treatment. Human C28/I2 chondrocytes were obtained from BeNa Culture Collection. C28/I2 cells were cultured in DMEM/F12 (Thermo Fisher Scientific, Inc.) supplemented with 10% FBS and 1% penicillin-streptomycin solution (Thermo Fisher Scientific, Inc.) and maintained in a humidified incubator supplied with 5% CO_2 at 37˚C. To generate a model of OA, C28/I2 cells were treated with IL-1β recombinant protein (5, 10, and 20 ng/ml) for 24 h (37). C28/I2 cells without IL-1β treatment were used as the control.

Cell transfection. Small interfering (si)RNAs targeting JPX (50 nM; si-JPX#1, 5'-GTTGCAAGGCGTCCGAAGTAT-3' and si-JPX#2, 5'-GTCCGAAGTATGAGTCCACTA-3'), and negative control (50 nM; si-NC, 5'-TTCTCCGAACGTGTC ACGT-3'), miR-25-3p mimic (40 nM; sense, 5'-CAUUGCA CUUGUCUCGGUCUGA-3' and antisense, 5'-AGACCG AGACAAGUGCAAUGUU-3') and mimic negative control (40 nM; mimic NC, sense 5'-UUCUCCGAACGUGUCACG UTT-3' and antisense 5'-ACGUGACACGUUCGGAGA ATT-3'), miR-25-3p inhibitor (40 nM; 5'-UCAGACCGAGAC AAGUGCAAUG-3') and inhibitor negative control (40 nM; inhibitor NC, 5'-CAGUACUUUUGUGUAGUACAA-3') were purchased from Shanghai GenePharma, Co., Ltd. A PPID overexpression vector pcDNA™ 3.1/V5-HisB-PPID (pc-PPID, 75 ng) and an empty pcDNA3.1 vector (pc-NC, 75 ng) were obtained from Thermo Fisher Scientific, Inc. After addition of the transfection reagent [Lipofectamine® 2000 (Thermo Fisher Scientific, Inc.)] and constructs, cells were cultured for 48 h, after which the subsequent experiments were performed.

Reverse transcription-quantitative (RT-q)PCR analysis. Total RNA was isolated from chondrocytes using TRIzol® reagent (Thermo Fisher Scientific, Inc.), and then converted to cDNA using a PrimeScript RT kit (Takara Bio, Inc.) or TaqMan™ MicroRNA Reverse Transcription Kit (Thermo Fisher Scientific, Inc.) according to the manufacturer's protocol. Subsequently, qPCR analysis was performed for miRNAs using a MiScript SYBR-Green PCR kit (Qiagen GmbH) or SYBR Premix Ex Taq™ Kit (Takara Bio, Inc.) on an ABI 7300HT system. The qPCR thermocycling conditions were as follows: Pre-denaturation at 95˚C for 2 min; followed by 40 cycles of denaturation at 95˚C for 15 sec, and annealing and extension at 60˚C for 30 sec. GAPDH was used as the internal control for JPX and PPID, and U6 was used as the internal control for miR-25-3p. The expression levels of miR-25-3p and lncRNA JPX were quantified using the $2^{-\Delta\Delta Cq}$ method (38). The sequences of the primers were: JPX forward, 5'-TTGCAA GGCGTCCGAAGTAT-3' and reverse, 5'-AGGCGATCAGCG AGAAAGAA-3'; miR-25-3p forward, 5'-ACACTCCAGCTG GGCATTGCACTTGTCTCG-3' and reverse, 5'-ACACTC CAGCTGGGCATTGCACTTGTCTCG-3'; PPID forward, 5'-GTGAAAAACCTGCTAAATTGTGCG-3' and forward, 5'-ATCCGCATCCTCAGGGAAATC-3'; U6 forward, 5'-GGA ACGATACAGAGAAGATTAGC-3' and reverse, 5'-TGGAAC GCTTCACGAATTTGCG-3'; GAPDH forward, 5'-GTCAAC GGATTTGGTCTGTATT-3' and reverse, 5'-AGTCTTCTG GGTGGCAGTGAT-3'.

Western blot assay. Total protein was extracted from chondrocytes using RIPA lysis buffer containing protease inhibitors. The concentration of proteins was measured using a BCA kit (Beyotime Institute of Biotechnology). Then, 50 μg protein lysate was loaded per lane on a 10% SDS-gel, resolved using SDS-PAGE, and transferred onto a PVDF membrane. After blocking with 5% fat-free milk for 1 h, the PVDF membranes were incubated with the following primary antibodies: Anti-PPID antibody (cat. no. ab3562; 1:1,000; Abcam), anti-Bax antibody (cat. no. ab32503; 1:1,000; Abcam), anti-Bcl-2 (cat. no. ab32124; 1:1,000; Abcam), anti-cleaved (c)-caspase-9 (cat. no. 20750; 1:1,000; Cell Signaling Technology, Inc.) and GAPDH (cat. no. ab9485; 1:1,000; Abcam) at 4˚C overnight. Subsequently, the membranes were incubated with goat anti-rabbit IgG H&L (HRP) secondary antibody (cat. no. ab205718; 1:10,000; Abcam) at room temperature for 1 h. The protein bands were visualized using the BeyoECL Plus kit (Beyotime Institute of Biotechnology) and were semi-quantified with ImageJ version 1.52v (National Institutes of Health).

Cell viability assay. Cell viability was analyzed using a Cell Counting Kit (CCK)-8 assay (Beyotime Institute of Biotechnology) according to the manufacturer's protocol.

After 48 h of transfection, cells were plated to a 96-well plate and cultured at 37˚C, and incubated until they had adhered. Then, the CCK-8 reagent was added, and cells were further incubated for 4 h. Finally, the absorbance was measured using a microplate reader at 450 nm.

Apoptosis analysis. Apoptosis was measured using an Annexin V-FITC Apoptosis Detection Kit (Beyotime Institute of Biotechnology). After 48 h of transfection, cells were stained with Annexin V-FITC and PI for 15 min at 25˚C in the dark. Cell apoptosis was scanned using a BD FACSCalibur™ flow cytometer (BD Biosciences) and analyzed using FlowJo 10.0.6 software (FlowJo, LLC).

Dual-luciferase reporter assay. The binding sites between miR-25-3p and JPX or the 3'UTR PPID were predicted using starBase (https://starbase.sysu.edu.cn/). Cells were plated in a 24-well plate. When cell confluence reached 80%, the WT (or Mut) pmirGLO luciferase reporter gene vector of JPX (or PPID 3'UTR) and miR-25-3p mimic (or mimic NC) were co-transfected into cells with Lipofectamine 2000. After 48 h of transfection, the luciferase activity was measured using a dual luciferase assay kit according to the manufacturer's protocol (Promega Corporation) and normalized to *Renilla* luciferase activity.

RNA immunoprecipitation (RIP) assay. RIP assays were performed to verify the targeted relationship between miR-25-3p, JPX, and PPID using the RIP Kit (Millipore Sigma) according to the manufacturer's protocol. Cells were lysed using the lysis buffer and incubated with magnetic beads pre-coated with Ago2 antibody (cat. no. ab186733; 1:1,000; Abcam) for 6 h at 4˚C. IgG (cat. no. SAB5600195; 1:1,000; MilliporeSigma) was used as the control. Subsequently, beads were washed with RNA binding buffer, and the levels of JPX, miR-25-3p, and PPID were detected by RT-qPCR.

starBase database analysis. The starBase database is a widely-used open-source platform for studying ncRNA interactions from CLIP-seq, degradome-seq, and RNA-RNA interactome data (39,40). Here, starBase was used to predict the binding sites between lncRNA JPX, miR-25-3p, and PPID.

Statistical analysis. All data are presented as the mean ± SD. All experiments were performed in triplicate. A one-way ANOVA followed by a Tukey's post hoc test was used to analyze the differences between multiple groups. A Student's t-test was used to analyze the differences between two groups. $P<0.05$ was considered to indicate a statistically significant difference.

Results

Effect of lncRNA JPX on cell viability and apoptosis of IL-1β-treated chondrocytes. The RT-qPCR results showed that JPX was notably upregulated in OA cartilage tissues compared with the NC cartilage tissues (Fig. 1A). JPX expression was significantly increased in chondrocytes after treatment with 10 and 20 ng/ml IL-1β (Fig. 1B). Thus, 10 ng/ml IL-1β was used for the follow-up experiments. To probe the effect of JPX on chondrocytes, JPX siRNAs were transfected into chondrocytes. Transfection of JPX siRNAs markedly decreased JPX expression levels, especially si-JPX#1 (Fig. 1C). JPX knockdown significantly decreased JPX expression in IL-1β-treated chondrocytes (Fig. 1D). Thus, the effect of JPX knockdown on IL-1β-treated chondrocytes was assessed. IL-1β treatment reduced cell viability and increased cell apoptosis of chondrocytes (Fig. 1E-G); JPX knockdown significantly enhanced cell viability and inhibited cell apoptosis of IL-1β-stimulated chondrocytes (Fig. 1E-G). Additionally, the protein expression levels of Bcl-2, Bax, and c-caspase-9 were determined using western blotting. Bcl-2 expression levels in the IL-1β group were significantly decreased whereas the expression levels of Bax and c-caspase-9 were significantly increased (Fig. 1H and I). JPX knockdown increased Bcl-2 expression levels whilst decreasing the expression levels of Bax and c-caspase-9 in the IL-1β-stimulated chondrocytes (Fig. 1H and I).

miR-25-3p is a target of JPX in chondrocytes. starBase analysis predicted that JPX could target miR-25-3p (Fig. 2A). A dual-luciferase reporter assay and RIP assay were used to confirm the targeting relationship between JPX and miR-25-3p. miR-25-3p mimics decreased luciferase activity in the JPX-WT group (Fig. 2B). The results of RIP revealed that JPX and miR-25-3p were significantly enriched using anti-Ago2 (Fig. 2C). JPX knockdown significantly increased the expression levels of miR-25-3p (Fig. 2D). The results of RT-qPCR showed that miR-25-3p expression in OA patients was notably decreased compared with that in healthy individuals (Fig. 2E). The correlation analysis revealed that miR-25-3p levels in OA patients were negatively related with JPX levels (Fig. 2F). Additionally, miR-25-3p expression levels were significantly decreased by IL-1β (Fig. 2G). Together, these results suggested that lncRNA JPX could target miR-25-3p in chondrocytes.

Inhibition of miR-25-3p abrogates the effects of JPX knockdown on IL-1β-stimulated chondrocytes. Next, whether JPX regulated miR-25-3p to affect the activity and apoptosis of IL-1β-stimulated chondrocytes was assessed. miR-25-3p inhibitor notably reversed the JPX knockdown-induced increase in miR-25-3p expression (Fig. 3A). CCK-8 results showed that JPX knockdown increased cell viability of IL-1β-stimulated chondrocytes (Fig. 3B). JPX knockdown notably reduced cell apoptosis in the IL-1β-stimulated chondrocytes (Fig. 3C and D). However, cell viability was decreased and apoptosis was increased in the IL-1β+si-JPX#1+miR-25-3p inhibitor group compared with the IL-1β+si-JPX#1+inhibitor NC group (Fig. 3B-D). Western blotting results showed that JPX knockdown significantly increased Bcl-2 levels and decreased the levels of Bax and c-caspase-9 (Fig. 3E and F); the effect of JPX knockdown was abrogated by the miR-25-3p inhibitor (Fig. 3E and F). Together, these findings demonstrated that inhibition of miR-25-3p abrogated the effects of JPX knockdown on IL-1β-stimulated injury in chondrocytes.

PPID is a target of miR-25-3p in chondrocytes. starBase analysis predicted that PPID was a target of miR-25-3p (Fig. 4A). The results of the dual-luciferase reporter assay and RIP analysis confirmed the targeted relationship between

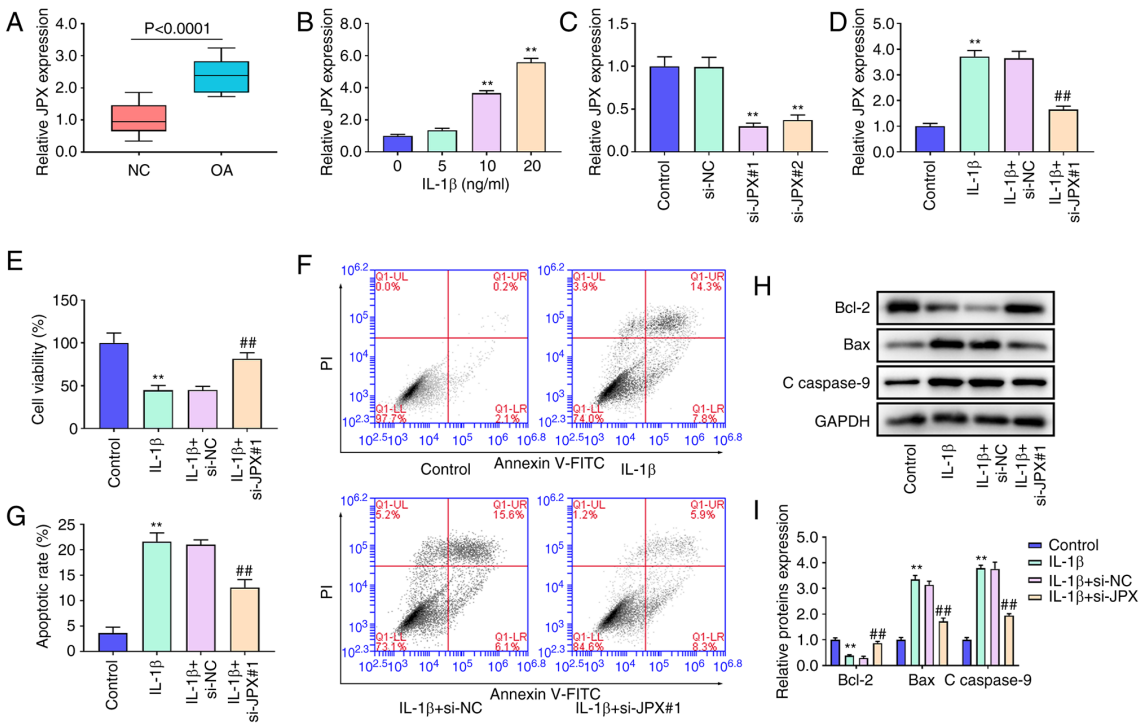

Figure 1. Effect of lncRNA JPX on cell viability and apoptosis in IL-1β-treated chondrocytes. (A) lncRNA JPX expression in OA and NC cartilage tissues were measured using RT-qPCR. (B) JPX expression in chondrocytes stimulated with 0, 5, 10, or 20 ng/ml IL-1β was detected using RT-qPCR. (C) JPX expression in chondrocytes transfected with the JPX siRNAs was detected using RT-qPCR. (D) JPX expression in IL-1β-stimulated chondrocytes after transfection with JPX siRNA was detected using RT-qPCR. (E) The cell viability of chondrocytes was assessed using a Cell Counting Kit-8 assay. (F and G) Cell apoptosis of chondrocytes was measured using flow cytometry. (H and I) The protein expression levels of apoptosis-related proteins Bcl-2, Bax and c-caspase-9 were detected using western blotting. **P<0.01 vs. control group; $^{\#\#}$P<0.01 vs. IL-1β+si-NC group.. lncRNA, long non-coding RNA; JPX, Just proximal to X-inactive specific transcript; RT-qPCR, reverse transcription-quantitative PCR; OA, osteoarthritic; NC, negative control; siRNA, small interfering RNA; c-caspase, cleaved-caspase.

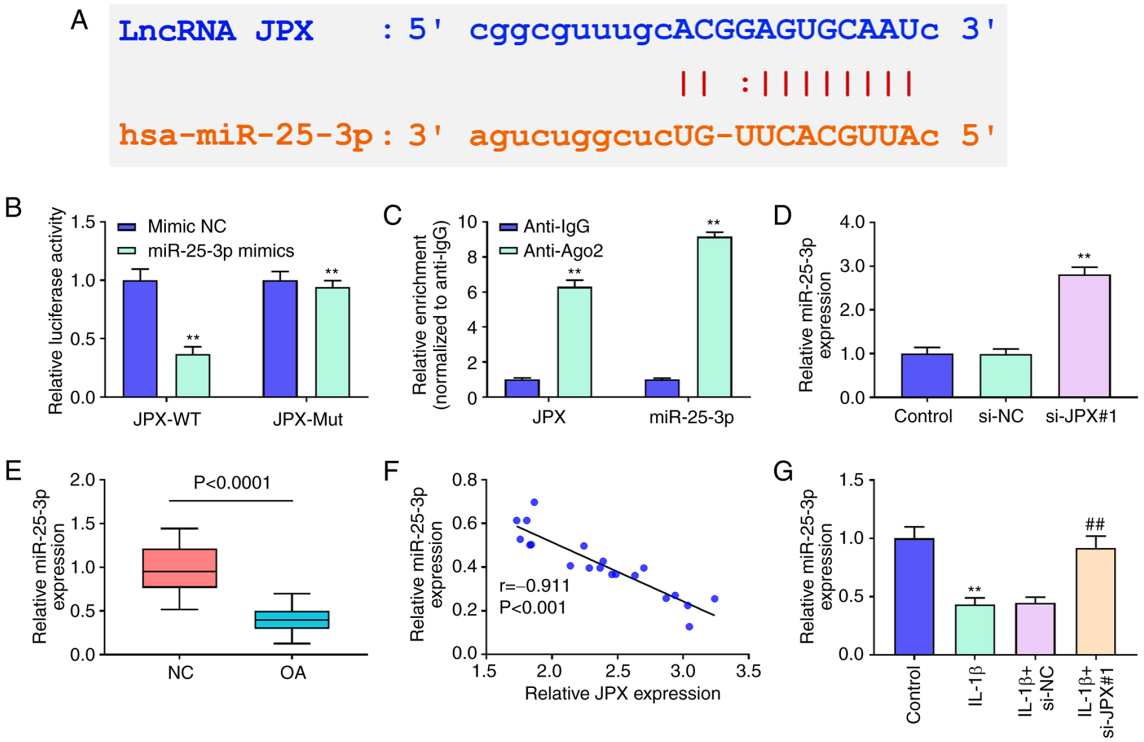

Figure 2. miR-25-3p is a target of JPX in chondrocytes. (A) starBase was used to predict the potential binding sites between JPX and miR-25-3p. (B) A dual-luciferase reporter assay was used to confirm the targeted relationship between JPX and miR-25-3p. (C) The targeted relationship between JPX and miR-25-3p was confirmed using RIP assays. (D and E) miR-25-3p expression was measured using RT-qPCR. (F) Correlation analysis between JPX and miR-25-3p in OA cartilage tissues. (G) miR-25-3p expression in IL-1β-stimulated chondrocytes transfected with JPX siRNA was measured using RT-qPCR. **P<0.01 vs. control group; $^{\#\#}$P<0.01 vs. IL-1β+si-NC group. miR, microRNA; JPX, Just proximal to X-inactive specific transcript; RT-qPCR, reverse transcription-quantitative PCR; OA, osteoarthritic; NC, negative control; siRNA, small interfering RNA; RIP, RNA immunoprecipitation.

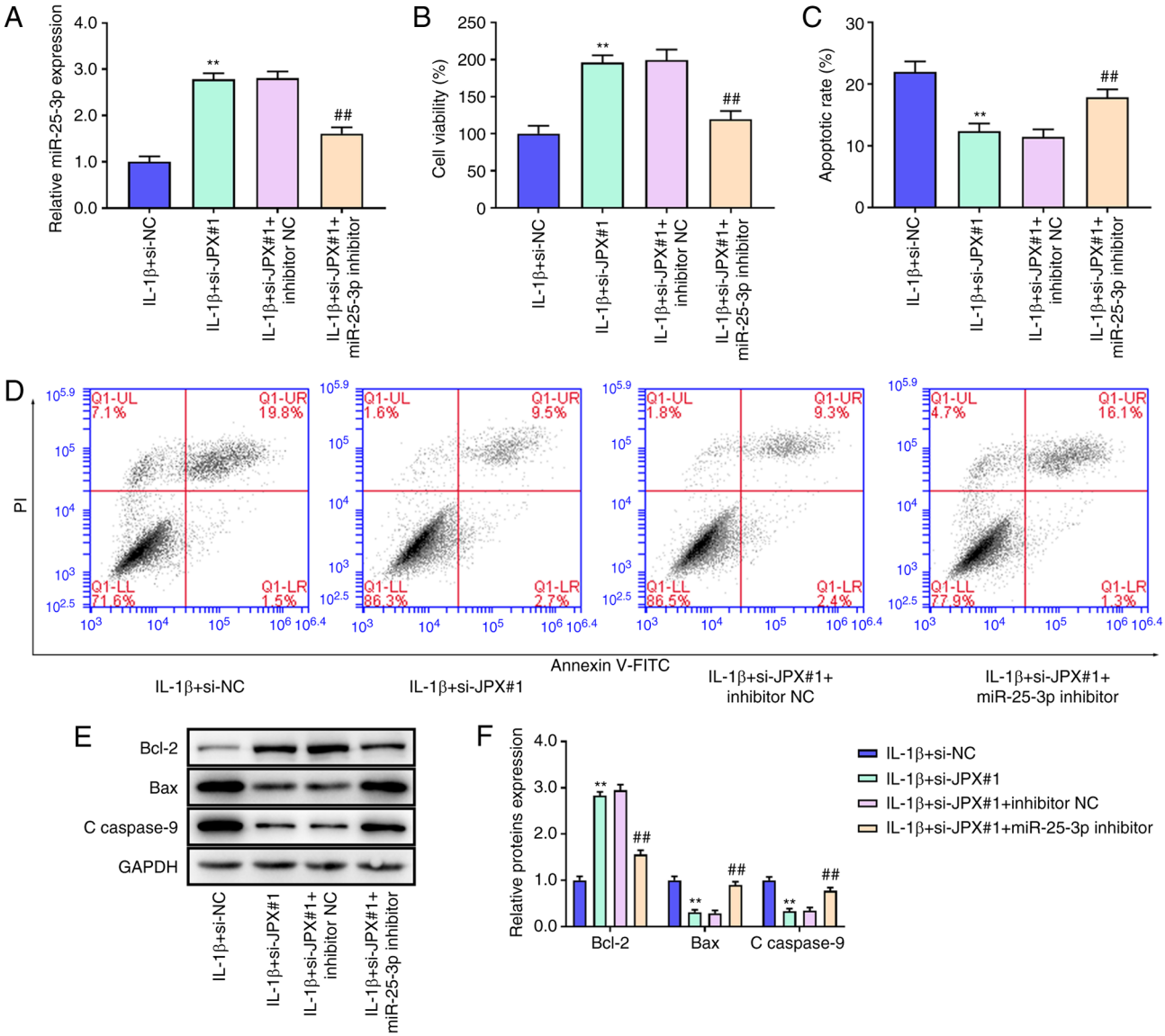

Figure 3. Inhibition of miR-25-3p eliminates the effects of JPX knockdown on IL-1β-stimulated chondrocytes. (A) miR-25-3p expression in IL-1β-stimulated chondrocytes after transfection with the JPX siRNA and/or miR-25-3p inhibitor was detected by RT-qPCR. (B) The cell viability of IL-1β-stimulated chondrocytes after transfection with JPX siRNA and/or miR-25-3p inhibitor was measured using a Cell Counting Kit-8 assay. (C and D) Cell apoptosis in the IL-1β-stimulated chondrocytes after transfection with JPX siRNA and/or miR-25-3p inhibitor was detected using a flow cytometer. (E and F) The protein expression levels of Bcl-2, Bax and c-caspase-9 in the IL-1β-stimulated chondrocytes after transfection with JPX siRNA and/or miR-25-3p inhibitor were detected using western blotting. **P<0.01 vs. IL-1β+si-NC group; ##P<0.01 vs. IL-1β+si-JPX#1+inhibitor NC group. miR, microRNA; JPX, Just proximal to X-inactive specific transcript; RT-qPCR, reverse transcription-quantitative PCR; NC, negative control; siRNA, small interfering RNA; c-caspase, cleaved-caspase.

miR-25-3p and PPID (Fig. 4B and C). The results of RT-qPCR showed that PPID expression in OA patients was significantly higher than that in the healthy individuals (Fig. 4D). The correlation analysis showed that the PPID levels in OA patients were negatively associated with miR-25-3p and positively related to JPX levels (Fig. 4E and F). Next, the effect of miR-25-3p and JPX on PPID expression was assessed. miR-25-3p inhibitor significantly decreased miR-25-3p expression levels (Fig. 4G). Additionally, IL-1β treatment significantly increased PPID expression in chondrocytes (Fig. 4I and K). The protein expression levels of PPID were significantly increased by transfection of the miR-25-3p inhibitor whereas JPX knockdown significantly decreased PPID expression irrespective of IL-1β treatment (Fig. 4H and J). These results suggested that PPID is a target of miR-25-3p and it can be regulated by lncRNA JPX in chondrocytes.

PPID overexpression abrogates the effect of JPX knockdown in IL-1β-stimulated chondrocytes. To confirm whether JPX affected cartilage damage via regulation of PPID, JPX siRNA and PPID overexpression plasmid were transfected into chondrocytes. PPID was notably increased in the pc-PPID group compared with the pc-NC group (Fig. 5A). In the IL-1β-stimulated chondrocytes, JPX knockdown markedly decreased PPID expression compared with the IL-1β+si-NC group. Compared with the IL-1β+si-JPX#1+pc-NC group, the expression levels of PPID were significantly enhanced in the IL-1β+si-JPX#1+pc-PPID group (Fig. 5B). CCK-8 analysis

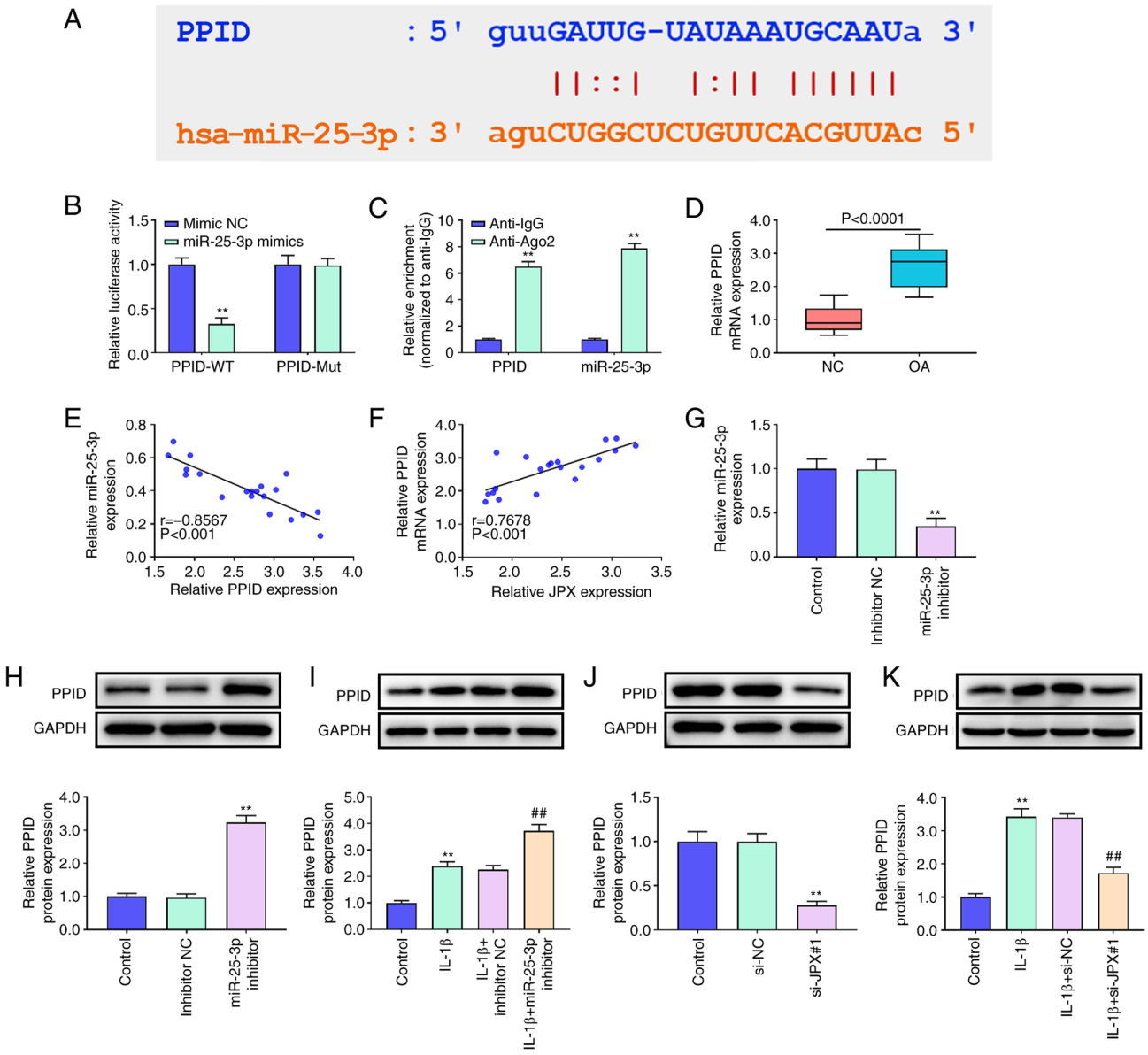

Figure 4. PPID is a target of miR-25-3p in chondrocytes. (A) starBase was used to predict the potential binding sites between miR-25-3p and the 3'UTR of PPID mRNA. (B and C) The targeted relationship between miR-25-3p and PPID was confirmed using a dual-luciferase reporter assay and RIP assay. **P<0.01. (D) PPID mRNA expression in OA and NC cartilage tissues were measured using RT-qPCR. (E) Correlation analysis between miR-25-3p and PPID in the OA cartilage tissues. (F) Correlation analysis between JPX and PPID in the OA cartilage tissues. (G) Expression of miR-25-3p in IL-1β-stimulated chondrocytes after transfection with the miR-318-3p inhibitor was measured using RT-qPCR. (H) PPID protein expression in chondrocytes after transfection with the miR-25-3p inhibitor. **P<0.01 vs. Control group. (I) PPID protein expression in chondrocytes after treatment with IL-1β and/or transfection with the miR-25-3p inhibitor was measured using western blotting. **P<0.01 vs. Control group; $^{\#\#}$P<0.01 vs. IL-1β+inhibitor NC group. (J) PPID protein expression in chondrocytes after transfection with JPX siRNA was detected using western blotting. **P<0.01 vs. Control group. (K) PPID protein expression PPID in chondrocytes after treatment with IL-1β and/or transfection with JPX siRNA was detected by western blotting. **P<0.01 vs. Control group; $^{\#\#}$P<0.01 vs. IL-1β+si-NC group. PPID, Peptidyl cis-trans prolyl isomerase D; miR, microRNA; UTR, untranslated region; RIP, RNA immunoprecipitation; OA, osteoarthritic; NC, negative control; siRNA, small interfering RNA.

showed that JPX knockdown increased cell viability in IL1β-stimulated chondrocytes, and the increase in cell viability following JPX knockdown was eliminated by PPID overexpression (Fig. 5C). As shown in Fig. 5D and E, JPX knockdown significantly decreased apoptosis of IL-1β-stimulated chondrocytes. Additionally, the inhibitory effect of JPX knockdown on apoptosis was eliminated by overexpression of PPID. JPX knockdown markedly increased Bcl-2 expression and reduced expression of Bax and c-caspase-9 in IL-1β-stimulated chondrocytes, and the effects of JPX knockdown on Bcl-2, Bax, and c-caspase-9 were eliminated by PPID overexpression (Fig. 5F and G). These results indicated that JPX knockdown may reduce cell injury in IL-1β-stimulated chondrocytes by regulating PPID expression.

Discussion

lncRNAs are associated with the function and inflammatory response of chondrocytes in OA (41,42). JPX has been reported to play an important role in myeloid malignancies (19). However, the underlying mechanism of JPX in OA remains unclear. In this study, C28/I2 cells were used to explore the effect of

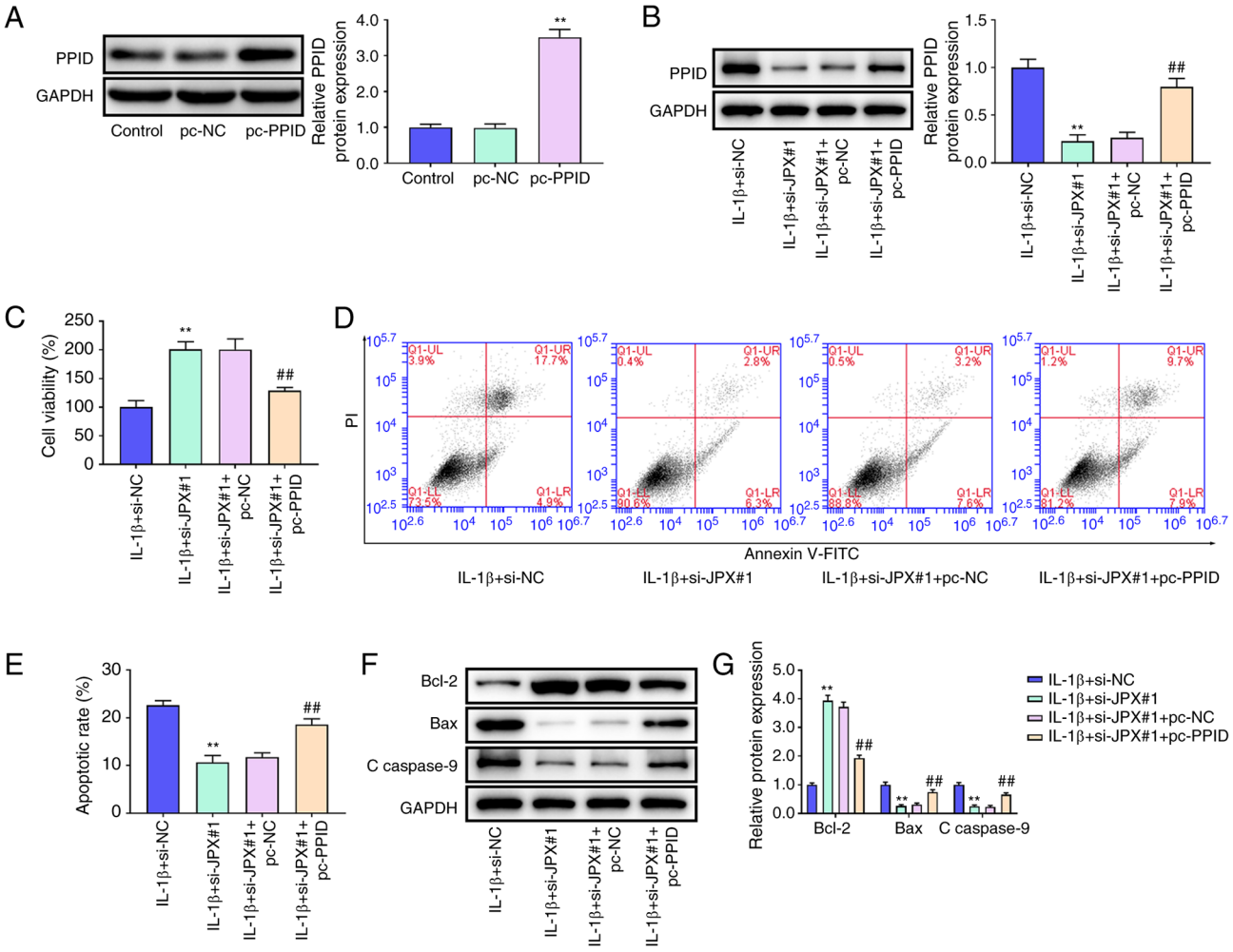

Figure 5. PPID overexpression eliminates the effect of JPX knockdown in IL-1β-stimulated chondrocytes. (A) PPID protein expression in chondrocytes after transfection with the PPID overexpression plasmid were measured using western blotting. (B) Protein expression of PPID in IL-1β-stimulated chondrocytes after transfection with the JPX siRNA and/or the PPID overexpression plasmid were measured using western blotting. (C) Cell viability of IL-1β-stimulated chondrocytes after transfection with JPX siRNA and/or the PPID overexpression plasmid was assessed using a Cell Counting Kit-8 assay. (D and E) Cell apoptosis of IL-1β-stimulated chondrocytes after transfection with JPX siRNA and/or the PPID overexpression plasmid was detected using a flow cytometer. (F and G) The expression of apoptosis-related proteins in IL-1β-stimulated chondrocytes after transfection with JPX siRNA and/or the PPID overexpression plasmid were detected using western blotting. **P<0.01 vs. IL-1β+si-NC group; $^{\#\#}$P<0.01 vs. IL-1β+si-JPX#1+pc-NC group. PPID, Peptidyl cis-trans prolyl isomerase D; JPX, Just proximal to X-inactive specific transcript; siRNA, small interfering RNA; NC, negative control.

JPX on IL-1β-stimulated injury. The results showed that JPX knockdown enhanced cell viability and reduced apoptosis of IL-1β-stimulated chondrocytes. Moreover, the results showed that JPX was associated with the miR-25-3p/PPID axis in OA.

The cartilage damage is a characteristic and defining feature of OA (5,43). IL-1β has been reported to participate in OA progression (44). Therefore, chondrocyte C28/I2 cells were treated with IL-1β to establish an *in vivo* OA model. The results showed that IL-1β treatment reduced the viability of C28/I2 cells. Apoptosis is an important process related to cell viability (45). Activation of apoptosis-related proteins, such as Bcl-2, Bax, and caspase-3, are reliable markers of cell apoptosis (46,47). In this study, it was shown that IL-1β treatment facilitated apoptosis, which was observed as an increase in Bcl-2 levels, and a decrease in the levels of Bax and cleaved-caspase-9 in C28/I2 cells. Additionally, it was found that JPX expression was upregulated in OA patients and IL-1β-stimulated chondrocytes, which indicated that JPX may be associated with OA pathogenesis. The experiments confirmed that JPX knockdown suppressed the cell injury stimulated by IL-1β in chondrocytes, highlighting the therapeutic potential of JPX suppression on the progression of OA.

It has been found that JPX can competitively bind to various miRNAs as a ceRNA, such as miR-33a-5p (17), miR-155-5p (48), and miR-944 (18). In intervertebral disc degeneration, JPX upregulates HIF-1α expression by inhibiting miR-18a-5p in nucleus pulposus cells (49). In the present study, it was confirmed that JPX could bind to miR-25-3p as a ceRNA in C28/I2 cells. Additionally, miR-25-3p expression was downregulated in OA tissues and IL-1β-stimulated chondrocytes. miR-25-3p inhibitor reduced cell viability, promoted apoptosis in chondrocytes, and reversed the effects of JPX knockdown in chondrocytes. Li *et al* (50) found that miR-25-3p has anti-apoptotic effects on cultured primary neurons. Suppression of miR-25-3p reduced cell proliferation in a mouse model of polycystic kidney disease (51). The results of the present study are in agreement with the previous studies;

the protective effects of JPX knockdown on chondrocytes were achieved by abrogating the effects of miR-25-3p.

As a member of the PPIase family, PPID knockdown protected HaCaT keratinocytes from death following UVA irradiation (52). In the present study, PPID was a target gene of miR-25-3p. PPID expression was upregulated in OA tissues and IL-1β-stimulated chondrocytes. Additionally, PPID expression was regulated by JPX, and JPX levels were positively correlated with PPID. Next, whether JPX could regulate PPID to affect IL-1β-stimulated chondrocytes was assessed. The data showed that PPID overexpression promoted apoptosis in chondrocytes and increased the expression of Bax and c-caspase-9. Moreover, PPID overexpression partly eliminated the influence of JPX knockdown on chondrocytes. These findings also resulted in accelerating the effect of PPID on OA progression, which was observed as the reversal of the protective effect of JPX knockdown in IL-1β-stimulated injury. Together, JPX can affect OA progression via actively modulating PPID through competitively sponging miR-25-3p.

The present study has some limitations. The role of JPX in OA was only explored *in vitro*, thus *in vivo* experiments should be performed to confirm the results in future studies. The effect of JPX on inflammatory response, oxidative stress, and other aspects associated with OA development/progression need further study. Finally, the number of patients included in the present study was low, thus the results should be confirmed in a larger cohort.

In conclusion, the results of the present study showed that lncRNA JPX increased the cell viability of chondrocytes and suppressed apoptosis in OA by modulating a miR-25-3p/PPID axis, thereby reducing the cell damage in OA. These findings highlight the JPX/miR-25-3p/PPID axis as a potentially novel therapeutic target in OA.

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Availability of data and material

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

Authors' contributions

ZR designed the study. LT and ZD performed the experiments. JS, HZ and DL analyzed the data. ZR wrote the manuscript. ZR, LT and ZD confirm the authenticity of all the raw data. All authors read and approved the final manuscript.

Ethics approval and consent to participate

The present study was approved by the Ethics Committee of the Sunshine Union Hospital. All patients provided written informed consent.

Patient consent for publication

Not applicable.

Competing Interests

The authors declare that they have no competing interests.

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