Targeting of CIRP attenuates osteoarthritis progression via suppressing TLR4/NF-κB/NLRP3 signaling axis

WEICHAO SUN[1,2*], YI LIAO[3*], JIANGUO FENG[4], JIANHUI LIANG[1], QIFEI HE[1], YINXING CUI[5], DIXI HUANG[1,5], HOUYIN SHI[6], WEI YOU[1], WEI SUN[1] and QIAN YI[5]

[1]Department of Orthopedics, Shenzhen Second People's Hospital/First Affiliated Hospital of Shenzhen University Health Science Center, Shenzhen, Guangdong 518035, P.R. China; [2]The Central Laboratory, Shenzhen Second People's Hospital/First Affiliated Hospital of Shenzhen University Health Science Center, Shenzhen, Guangdong 518035, P.R. China; [3]Department of Technical Support, The People's Hospital of Guangxi Zhuang Autonomous Region, Guangxi Academy of Medical Sciences, Nanning, Guangxi 530021, P.R. China; [4]Department of Anesthesiology, The Affiliated Hospital of Southwest Medical University, Luzhou, Sichuan 646099, P.R. China; [5]Department of Physiology, School of Basic Medical Science, Southwest Medical University, Luzhou, Sichuan 646099, P.R. China; [6]Department of Orthopedics, The Affiliated Traditional Chinese Medicine Hospital of Southwest Medical University, Luzhou, Sichuan 646000, P.R. China

Received April 6, 2025; Accepted September 16, 2025

DOI: 10.3892/ijmm.2025.5674

Abstract. Osteoarthritis (OA) is one of the most common joint diseases worldwide. Recently, cold-inducible RNA binding protein (CIRP), a novel identified pro-inflammatory cytokine, was reportedly increased in the synovial fluid of OA patients. However, its function and the underlying mechanism in OA progression remains unclear. Therefore, the current study investigated the role of CIRP in the progression of OA. It was observed that CIRP and matrix metalloproteinases were highly expressed in OA chondrocytes, whereas collagen II exhibited low expression levels. Additionally, CIRP was found to be secreted by OA human chondrocytes in the form of exosomes. CIRP treatment influenced inflammatory related signaling pathway, which in turn affected inflammatory response and extracellular matrix (ECM) degradation in human chondrocytes. Additionally, CIRP induced the nuclear translocation of p65 and promoted ECM degradation dependent the Toll-like receptor 4 (TLR4)/NF-κB signaling. The present study also reported that CIRP increased the IL-1β secretion via the NLR family pyrin domain containing 3 (NLRP3) inflammasome. Furthermore, targeting of CIRP by microRNA-145 or exosome loading with microRNA-145 attenuated its role in promoting OA both *in vitro* and *in vivo*. The findings indicated that CIRP acts as a pro-inflammatory factor and activates the TLR4/NF-κB/NLRP3 pathway, which promotes the inflammatory response, ECM degradation and the progression of OA and targeting CIRP could be a novel strategy for OA treatment.

Correspondence to: Professor Qian Yi, Department of Physiology, School of Basic Medical Science, Southwest Medical University, No. 1, Section 1, Xianglin Road, Luzhou, Sichuan 646099, P.R. China
E-mail: yiqian@swmu.edu.cn

Professor Wei Sun, Department of Orthopedics, Shenzhen Second People's Hospital/First Affiliated Hospital of Shenzhen University Health Science Center, 3002 Sungang West Road, Shenzhen, Guangdong 518035, P.R. China
E-mail: 414464705@qq.com

*Contributed equally

Abbreviations: OA, osteoarthritis; MMPs, matrix metalloproteinases; AGEs, advanced glycation end products; CIRP, cold-inducible RNA-binding protein; DAMP, damage-associated molecular pattern molecule; TLR4, Toll-like receptor 4; NLRP3, NLR family pyrin domain containing 3; ECM, extracellular matrix; COX-2, cyclooxygenase-2; iNOS, inducible nitric oxide synthase

Key words: cold-inducible RNA binding protein, exosome, osteoarthritis, microRNA-145, TLR4/NF-κB/NLRP3 signaling pathway

Introduction

Osteoarthritis (OA) is one of the most common joint diseases worldwide and its incidence in the world population >60-years old is ~20%. The typical features of OA including cartilage degeneration, osteophyte formation, subchondral bone sclerosis and synovial inflammation (1,2). Although a number of studies have shown that the sex, age and obesity are the main risks factors of OA, the pathophysiology of OA has not been fully demonstrated (3,4). Increasing number of studies reveal that inflammation and inflammatory cytokines seem to play a critical role in the initiation and progression of OA. Among these inflammatory cytokines, IL-1β is considered as the most important (5). IL-1β contributes to the cartilage degradation via increasing the expression of matrix metalloproteinases (MMPs), NO and prostaglandin E2 (6,7). Moreover, some small molecule also play a marked role, such as high mobility group box 1 (8), free fatty acids (9) and advanced glycation end products (AGEs) (10). For example, AGEs could induce the activation of NLR family pyrin domain containing 3 (NLRP3)

inflammasome to promote IL-1β production and inflammatory response in human chondrocytes (11). These results indicate that the pathophysiology of OA is complicated and the mechanism underlying the occurrence and development of OA remains to be elucidated.

Cold-inducible RNA-binding protein (CIRP) is a 172-amino acid protein and belongs to a family of cold-shock proteins. The mature CIRP peptide contains one RNA recognition motif domain in the N-terminal and one glycine-rich C-terminal domain. CIRP was first identified as an RNA binding protein and is lowly expressed in various tissues (12,13). It is a stress-inducible protein which responds to a number of stresses, including hypoxia and UV radiation. The stress-induced CIRP regulates target genes expression through binding and stabilizing them (14,15). It has been reported that CIRP is a new damage-associated molecular pattern molecule (DAMP) and can function as an inflammatory mediator (16). Qiang *et al* (17) found that hypoxia stress could induce CIRP secretion, the extracellular CIRP working as a DAMP to promote inflammatory response in shock and sepsis. Sakurai *et al* (18) reported that CIRP increases the expression of TNF-α and IL-32, linking inflammation and tumorigenesis in colitis-associated cancer. In cerebral ischemia, CIRP activates the NF-κB signaling pathway, promotes the TNF-α expression and mediates neuroinflammation (19). NLRP3 inflammasomes are key regulators of inflammatory response in the pathogenesis of OA (20-22). The activation of the NLRP3 inflammasome leads to the cleavage of caspase-1, which processes pro-IL-1β into its mature form, IL-1β. This mature cytokine is then secreted and further amplifies the inflammatory response (23). It has been reported CIRP can trigger an inflammatory response through the Toll-like receptor 4 (TLR4)/NF-κB signaling pathway (17,24). The assembly of TLR4/NF-κB and NLRP3/ASC/procaspase-1 complexes contributes to the activation of canonical NLRP3 inflammasome signaling (25). Furthermore, CIRP has been evaluated in the synovial fluid of OA and rheumatoid arthritis, indicating a positive relationship between CIRP and OA (26,27). However, the roles of CIRP in OA progression and the underlying mechanism remain to be elucidated.

MicroRNAs (miRNAs) are a cluster of short non-coding RNAs with an average length of 22 nucleotides. They suppress target genes expression by binding to their 3'-UTRs. Studies have indicated that miRNAs play critical roles in maintaining cartilage homeostasis as well as clinical treatment of OA (28,29). For example, miR-92a-3p enhances chondrogenesis and suppresses cartilage degradation by targeting WNT5A (30). As cell-secreted nanovesicles, exosomes contain multiple types of cargo, including non-coding RNA molecules, DNA, lipids and functional proteins. Recent evidence has suggested that exosomes play a novel function as attractive carriers for gene therapy or drug delivery (31). For example, exosome-mediated miR-144-3p promotes ferroptosis to inhibit osteosarcoma proliferation, migration and invasion by regulating ZEB1 (32). Moreover, umbilical cord blood-derived M1 macrophage exosomes loaded with cisplatin reversed cisplatin resistance of ovarian cancer *in vivo* (33). These studies indicate that exosomes are novel carriers capable of delivering RNA molecules or drugs to target cells, representing a potential strategy for OA treatment.

The present study aimed to investigate the function and molecular mechanisms of CIRP in OA progression. It also sought to explore how CIRP regulates the inflammatory response and extracellular matrix (ECM) degradation in human chondrocytes through the TLR4/NF-κB/NLRP3 inflammasome. Additionally, it identified CIRP as a target of miRNA-145-5p (miR-145-5p), a microRNA previously shown to have protective effects in chondrocytes and nucleus pulposus cells (34-36). This led the present study to further examine whether miR-145-5p could prevent OA development by targeting CIRP.

Materials and methods

Chemicals and reagents. Human recombinant CIRP peptides were purchased from Abcam. NF-κB inhibitor BAY 11-7082 and cell counting kit-8 (CCK-8) was purchased from Beyotime Institute of Biotechnology. TLR4 inhibitor TAK-242 was purchased from Shanghai Xianding Biotechnology Co., Ltd. Primary antibodies directed against inducible nitric oxide synthase (iNOS), cyclooxygenase-2 (COX-2), MMP1, MMP3, MMP13, Lamin B1, CD63, CD9, 130 kDa cis-Golgi matrix protein 1 (GM130) and GAPDH were purchased from Proteintech Group, Inc., ADAM metallopeptidase with thrombospondin type 1 motif 5 (ADAMTS5) was purchased from ABclonal. Human Reactive Inflammasome Antibody Sampler Kit II, CIRP, IκBα and p65 were purchased from Cell Signaling Technology, Inc. Collagen II was purchased from Beyotime Institute of Biotechnology. Anti-rabbit IgG, HRP-linked antibody and Anti-mouse IgG, HRP-linked antibody were purchased from Beyotime Institute of Biotechnology.

Preparation and culture of human primary chondrocytes. The collection of cartilage tissues from patients was approved by the Ethics Committee of Shenzhen Second People's Hospital (2023-157-01YJ). Samples used in this study were collected after all patients received detailed explanation of the research and submitted written informed consents. Human articular cartilage tissues were obtained from volunteer donors who had undergo joint replacement or joint surgery (December 2023-September 2024). Normal chondrocytes were obtained from the articular cartilage of patients who underwent joint replacement surgery due to femoral neck fractures. Notably, these patients did not have a diagnosis of OA. The control group comprised 11 patients (5 males and 6 females), all aged from 60-74 years old. By contrast, OA chondrocytes were sourced from the articular cartilage of individuals diagnosed with OA, comprising 11 patients (3 males and 8 females), also aged from 60 to 81 years old. Washing the cartilage samples 3 times with phosphate buffer saline (PBS) containing penicillin and streptomycin and then cutting them into small fragments (1-3 mm^3). The minced samples were placed in 1 mg/ml collagenase II (MilliporeSigma) for digestion at 37˚C for 16 h in a shaker, digesting the samples multiple times until collagen was completely dissolved. After digestion, the suspension was filtered with a 100-mm nylon cell strainer to remove matrix debris and the cells collected via centrifugation at 108 x g for 10 min at 24˚C. After centrifugation, cells were resuspended and subcultures with chondrocyte growth medium (DMEM/F12; 10% FBS (Gibco; Thermo

Table I. Sequences of primers for PCR.

Gene	Forward primer (5'-3')	Reverse primer (5'-3')
iNOS	CCTTACGAGGCGAAGAAGGACAG'	CAGTTTGAGAGAGGAGGCTCCG
COX-2	GAGAGATGTATCCTCCCACAGTCA	GACCAGGCACCAGACCAAAG
IL-6	ACTCACCTCTTCAGAACGAATTG	CCATCTTTGGAAGGTTCAGGTTG
TNF-a	GTCAGATCATCTTCTCGAACC	CAGATAGATGGGCTCATACC
IL-1b	ATGATGGCTTATTACAGTGGCAA	GTCGGAGATTCGTAGCTGGA
MMP1	CTGTTCAGGGACAGAATGTGC	TTGGACTCACACCATGTGTT
MMP3	TGCGTGGCAGTTTGCTCAGCC	GAATGTGAGTGGAGTCACCTC
MMP13	GGCTCCGAGAAATGCAGTCTTTCTT	ATCAAATGGGTAGAAGTCGCCATGC
Adamts5	GAACATCGACCAACTCTACTCCG	CAATGCCCACCGAACCATCT
Aggrecan	GTGCCTATCAGGACAAGGTCT	GATGCCTTTCACCACGACTTC
Collagen II	CTGTCCTTCGGTGTCAGGG	CGGCTTCCACACATCCTTAT
CIRP	CAAGTACGGACAGATCTCTG	CGGATCTGCCGTCCATCTA
miR-145-5p	GTCCAGTTTTCCCAGGAATC	AGAACAGTATTTCCAGGAAT
U6	CTCGCTTCGGCAGCACATATACT	ACGCTTCACGAATTTGCGTGTC
GAPDH	GGAGCGAGATCCCTCCAAAAT	GGCTGTTGTCATACTTCTCATGG

Fisher Scientific, Inc.) and 100 IU/ml penicillin and 100 μg/ml streptomycin (Gibco; Thermo Fisher Scientific, Inc.). The cells were cultured in an incubator with 5% CO_2 atmosphere at 37˚C. The medium was changed when the cell was attached and the cells were passaged at 80% confluence. Only the passage 1(P1) and passage 2 (P2) chondrocytes were used for the experiments. Toluidine blue staining was used to assess chondrocyte morphology. Briefly, the samples were stained with toluidine blue solution for 5 min at room temperature, followed by the addition of an equal volume of distilled water and mixed thoroughly. After allowing the mixture to stand for 10 min at room temperature, images were captured using an Olympus microscope (Olympus Corporation).

Cell viability. Human primary chondrocytes cells were seeded in 96-well cell culture plates at a density of $1x10^4$/well and cultured overnight. The concentration range of BAY 11-7082 and TAK-242 used in this assay was based previous studies (37,38). The concentration range of CIRP used in this assay was from 0-8 μg/ml. After incubation with CIRP for 48 h at 37˚C, the cell viability was measured using CCK-8 following the standard protocol. Briefly, the culture medium and CCK-8 solution were mixed at a ratio of 10:1 and 100 μl mixture was added into each well. After 1 h incubation at 37˚C in a cell culture incubator, the absorbance at 450 nm was detected using a microplate reader.

RNA isolation, reveres transcription and reverse transcription-quantitative (RT-q) PCR. Total RNA was extracted from human chondrocytes using TRIzol® reagent (Invitrogen; Thermo Fisher Scientific, Inc.) according to the manufacturer's instructions. In brief, the cells were first lysed in TRIzol® to release the RNA, and then chloroform was added to facilitate phase separation. After mixing and centrifugation at 12,000 x g for 15 min at 4˚C, the upper aqueous phase containing the RNA was transferred to a new tube. Isopropanol was subsequently added to precipitate the RNA. The RNA pellet was washed with 75% ethanol, dried and finally resuspended in RNase-free water. Then cDNA was synthesized using PrimeScript RT Reagent Kit with gDNA Eraser (Takara Biotechnology Co., Ltd.) according to the manufacturer's instructions. RT-qPCR was conducted with SYBR Green Supermix (Beyotime Institute of Biotechnology) using aViiA 7 Real-Time PCR System (Applied Biosystems; Thermo Fisher Scientific, Inc.) according to the manufacturer's instructions. The PCR cycling conditions consisted of an initial denaturation at 95˚C for 10 min, followed by 35 cycles of denaturation at 95˚C for 15 sec, annealing at 60˚C for 1 min, and extension at 72˚C for 30 sec. Transcript levels were normalized to GAPDH (for mRNA) or the small U6 RNA (for miRNA). The specific primers used for these analyses are listed in Table I. Gene expression was calculated using the $2^{-\Delta\Delta Cq}$ method (39) and each experiment was performed in triplicate.

Western blotting. The whole cell proteins were extracted from chondrocytes using lysis buffer (50 mM Tris-HCl, pH 7.4, 150 mM NaCl and 0.1% sodium dodecyl sulfate) with protease inhibitors supplement. Separate extraction of nuclear and cytoplasmic proteins was conducted using a Nuclear and Cytoplasmic Protein Extraction Kit (Beyotime Institute of Biotechnology). The determination of protein concentration was conducted using the BCA protein concentration detection kit (Thermo Fisher Scientific, Inc.). Denatured protein solution (40 μg) was subjected to 12% SDS-PAGE followed by immunoblot analysis. The sample was then transferred to a PVDF membrane (Merck KGaA) that had been incubated in methanol for 1 min at room temperature. After removing the methanol, the membrane was equilibrated in transfer buffer until it was ready for use. Following a transfer at 100 V for 1 h at 4˚C, the membrane was blocked with 5% skimmed milk for 1 h at room temperature before being incubated with primary antibodies against GADPH (1:50,000; cat. no. 60004-1-Ig; ProteinTech Group, Inc.), MMP1 (1:1,000, 10371-2-AP, ProteinTech Group, Inc.), MMP3 (1:5,000; cat. no. 66338-1-Ig; ProteinTech Group,

Inc.), MMP13 (1:1,000; cat. no. 18165-1-AP; ProteinTech Group, Inc.), a disintegrin and metalloproteinase with thrombospondin motif-5 (Adamts5; 1:500; cat. no. A2836; ABclonal Biotech Co., Ltd.), iNOS (1:500; cat. no. 18985-1-AP; ProteinTech Group, Inc.), cyclooxygenase-2 (COX-2; 1:500; cat. no. 12375-1-AP; ProteinTech Group, Inc.), lamin B1 (1:5,000; cat. no. 12987-1-AP; ProteinTech Group, Inc.), CD63 (1:500; cat. no. 32151-1-AP, ProteinTech Group, Inc.), CD9 (1:500; cat. no. 84801-13-RR, ProteinTech Group, Inc.), GM130 (1:500; cat. no. 11308-1-AP, ProteinTech Group, Inc.), Human Reactive Inflammasome Antibody Sampler Kit II (1:1,000; cat. no. 25620T; Cell Signaling Technology Inc.), CIRP (1:1,000; cat. no. 68522; Cell Signaling Technology Inc.), p65 (1:1,000; cat. no. 8242; Cell Signaling Technology Inc.), IκBα (1:1,000, cat. no. 4814; Cell Signaling Technology Inc.), and collagen II (1:500; cat. no. AF6528, Beyotime Institute of Biotechnology) overnight at 4˚C. Subsequently, the membranes were incubated with a HRP-conjugated goat anti-rabbit (1:1,000; cat. no. A0208; Beyotime Institute of Biotechnology Inc.) or goat anti-mouse IgG (1:1,000; cat. no. A0216; Beyotime Institute of Biotechnology Inc.) secondary antibody for 2 h at room temperature. ECL reagent (Thermo Fisher Scientific, Inc.) was used for development according to the manufacturer's protocol. The semi-quantitatively analysis of immunoreactive bands was performed using ImageJ software 1.53e (National Institutes of Health).

Immunofluorescence. For immunofluorescence, cells were treated with CIRP for 24 h. The cells were fixed with 4% paraformaldehyde for 15 min at room temperature. Fixed samples were permeabilized with 0.3% Triton X-100 for 10 min at room temperature and then blocked with 5% BSA for 30 min. Chondrocytes were incubated with p65 (1:50) primary antibodies overnight at 4˚C. Afterwards, they were washed with PBS 3 times and incubated with Cy3-conjugated secondary antibodies for 1 h at room temperature in the dark. Finally, the cells were incubated with DAPI for nucleuses stain at room temperature for 10 min. A fluorescence microscope was used to acquire the images at a magnification of 400x. Collagen II (1:50) and MMP13 (1:50) primary antibody were used to detect its expression in human chondrocytes using the same immunofluorescence protocol, and a fluorescence microscope was used to acquire the images at a magnification of 100x. The cell density seeded onto the slide was $1x10^5$ cells per well.

Purification of exosomes. Exosomes were harvested through a sequential centrifugation method. Cells were cultured in serum-free medium for 48 h. The medium was collected and centrifuged sequentially at 300 x g for 10 min, at 2,000 x g for 15 min and at 10,000 x g for 30 min at 4˚C. The supernatant was filtered using a 0.22 mm filter (Beyotime Institute of Biotechnology) to remove remaining cells and debris and then centrifuged at 120,000 x g for 70 min at 4˚C to harvest exosomes. PBS was used to re-suspend the purified exosomes. Exosomes were kept either at -80˚C for long term preservation or at -20˚C for short term preservation.

Characterization of exosomes. Exosomes were entrusted to Wuhan Servicebio Technology Co., Ltd. for transmission electron microscopy. In brief, fix the exosomes with 1 ml of 2% paraformaldehyde for 5 min at room temperature. Then, 10 μl of exosomes was applied to carbon-coated copper grids for 1 min, then washed with double-distilled water and dried using filter paper. Afterwards, ~15 μl of 2% uranyl acetate staining solution was added for 1 min at room temperature. The grids were examined using a Hitachi HT7700 transmission electron microscope (Hitachi High-Technologies Corporation). The particle size and particle concentration were determined by a Nano sight Tracking Analysis (NTA) system NanoSight NS300 (Malvern Panalytical Instruments Corp.). Briefly, exosomes were re-suspended in PBS at a concentration of ~3 μg protein content/ml. Then the solution was further diluted by 100 to 500 hundred folds for analysis. A 1 min video was recorded by the NTA software (NTA 3.4-Sample Assistant Build 3.4.003-SA, Malvern Panalytical Ltd.) for further analysis. Western blotting was used to detect the exosome marker protein expression.

Construction of a Venn diagram of CIRP targeting micro-RNAs. To predict the miRNAs that could potentially regulate CIRP expression, the miRWalk (http://mirwalk.umm.uni-heidelberg.de/), TargetScan (http://www.targetscan.org/), StarBase (http://starbase.sysu.edu.cn/), miRDIP (http://ophid.utoronto.ca/mirDIP/) and a chondrogenesis-related microRNA databases (GSE135588) were used to find CIRP targeting miRNAs. A Venn diagram (http://bioinformatics.psb.ugent.be/cgi-bin/liste/Venn/calculate_venn.htpl) was created based on comparisons of these miRNAs.

Dual-luciferase reporter assay. To study the interaction between miR-145-5p and the 3' untranslated region (UTR) of the CIRP gene, plasmid vectors containing the wild-type CIRP 3' UTR that included the predicted binding sites for miR-145-5p were constructed. These plasmids were transfected into 293 cells using GP-transfect-Mate (GenePharma, Inc.). To evaluate transfection efficiency, a *Renilla* luciferase vector was co-transfected in each experiment. After transfecting the cells, incubate them in a 37˚C incubator for 24 h, the luciferase activity was measured in relative light units, with the firefly luciferase activity normalized against the *Renilla* luciferase activity using Dual-Luciferase Reporter Assay System (Promega Corporation). In brief, 250 μl of 1X cell lysis buffer was added to each well, and 50 μl of the lysate transferred to a microplate, setting up three replicates. Then, 10 μl of firefly luciferase reaction solution was added, gently mixed, and the firefly luciferase activity measured within 30 min. Finally, 30 μl of *Renilla* luciferase reaction solution was added, mixed immediately and the *Renilla* luciferase activity measured.

Loading exosomes with miRNA mimic. Electroporation method was used to load miR-145-5p mimics into exosomes. Exosomes at a final concentration of 100 μg/μl protein (measured by BCA) were mixed with electroporation buffer (in a 1:1 ratio) and 100 pmol of synthetic miR-145-5p mimics (sense 5'-GUC CAGUUUUCCCAGGAAUCCCU-3', antisense 5'-GGAUUC CUGGGAAAACUGGACUU-3') or negative control (mimics NC, sense 5'-UUCUCCGAACGUGUCACGUTT-3', antisense 5'-ACGUGACACGUUCGGAGAATT-3') and electroporated at 0.400 kV using an electroporation instrument. After the loading of miR-145-5p mimics, the exosome samples were diluted with PBS and centrifuged at 10,000 x g for 30 min and

100,000 x g for 70 min at 4˚C to remove unloaded miR-145-5p mimics.

Establishment of a mice model of OA. All procedures were approved by the Animal Research Committee of Southwest Medical University (approval no. 20220307-004). A total of 20 six-week-old male C57BL/6J mice were randomized into four groups: normal group, OA group, miR-NC-Exos OA group and miR-145-Exos OA group, with each group comprising five mice. The OA model was built on the instability of the medial meniscus (DMM) caused by surgical therapy (40). The normal and OA group were given articular cavity injections of PBS. MiR-NC-Exos OA group were given articular cavity injections of 100 μg exosomes from cells overexpressed miR-NC mimics and miR-145-Exos OA group were given articular cavity injections of 100 μg exosomes from cells overexpressed miR-145 mimics. The injections were carried out twice a week for four consecutive weeks. All animals were sacrificed via cervical dislocation while under anesthesia induced by an intraperitoneal injection of pentobarbital sodium (50 mg/kg). Mortality was confirmed through the observation of respiratory arrest. a total of 10 cartilage samples were collected for safranin-O staining, hematoxylin and eosin staining and immunohistochemical (IHC) staining.

Histopathological analysis. The calcification of the knee joint was removed with a 10% EDTA solution after it had been fixed with 4% paraformaldehyde (24 h; 4˚C). The tissue was then dehydrated by immersing it in 70% ethanol for 30 min, followed by 80% ethanol for 30 min, 95% ethanol for 30 min, and finally 100% ethanol for 1 h to ensure complete dehydration. After dehydration, the tissue was cleared and placed in melted paraffin wax at approximately 60˚C for 3 h, which was then transferred into a mold filled with fresh paraffin wax to cool and solidify. The paraffin blocks were sliced into frontal serial slices with a thickness of 5 mm. Hematoxylin and eosin (H&E) staining was performed at room temperature, with hematoxylin applied for 10 min, and eosin applied for 3 min. Safranin O-fast green (S-O) staining was also performed at room temperature, with safranin O staining lasting 5 min, and fast green staining for 15 sec. And a light microscope was used to identify the degree of cartilage degeneration.

Immunohistochemical assay. MMP3 and collagen II expression levels were detected in the cartilage by immunohistochemistry. Briefly, the slides were dewaxed and rehydrated. The slide was sealed with 5% BSA for 30 min after a 20 min incubation with 0.4% pepsin (Sangon Biotech Co., Ltd.) in 5 mM HCl to repair the antigen. Then, with primary antibodies specific to MMP3 (1:500) and collagen II (1:50) overnight (at 4˚C), incubation of the slides was performed. After 50 min (room temperature) of incubation, the slides were subjected to incubation with HRP-conjugated goat anti-rabbit (1:50; cat. no. A0208; Beyotime Institute of Biotechnology, Inc.) and images were captured with an Olympus BX51 microscope (Olympus Corporation).

RNA sequencing (RNA-seq). RNA-seq was conducted and analyzed by Genedenov Co., Ltd. Briefly, total RNA extraction from the cells was performed using TRIzol® Reagent (Invitrogen; Thermo Fisher Scientific, Inc.) and genomic DNA removal was performed with DNase I (Takara Biotechnology Co., Ltd.). After preparation of the RNA-seq transcriptome library, the Illumina HiSeq xten/NovaSeq 6000 sequencer (Illumina, Inc.) was used to perform RNA-seq. Gene Ontology (GO) functional enrichment and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis were conducted using omicshare (https://www.omicshare.com). The RNA-seq dataset has been deposited in the BioProject database under the accession number PRJNA1302182 and the URL is https://www.ncbi.nlm.nih.gov/bioproject/PRJNA1302182.

Statistical analysis. The data were expressed as the mean ± SD and all experiments were repeated three times with independent cultures. Prior to statistical analysis, the normality of the data distribution was verified using the Shapiro-Wilk test. The statistical significance of differences for the mean values between two groups was determined with an unpaired two-tailed Student's t-test and multiple groups compared using one-way analysis of variance (ANOVA) with Bonferroni post hoc test. $P<0.05$ was considered to indicate a statistically significant difference.

Results

Overexpression and secretion of CIRP in OA human chondrocytes. To assess the effect of CIRP on OA progression, chondrocytes were first collected from patients with fractures or OA undergoing joint replacement surgery to evaluate its expression. As shown in Fig. 1A, the extracted human chondrocytes were stained with toluidine blue, confirming that the isolated cells were indeed human chondrocytes and suitable for further study. In Fig. 1B, qPCR analysis revealed that the expression of CIRP was markedly increased, while collagen II levels decreased in OA chondrocytes compared with normal chondrocytes. Additionally, the expression of MMP1, MMP3, MMP13 and ADAMTS5 were also overexpressed in OA chondrocytes. The present study then analyzed the protein levels of these genes and the data presented in Fig. 1C and D supported the mRNA findings. These results indicated that CIRP is overexpression in OA chondrocytes and may contribute to the progression of OA.

Secretion of CIRP in OA human chondrocytes via exosomes. Recent studies revealed that CIRP can be released into extracellular space by various cell types, including cardiomyocytes (41-43). The exosomes released by OA chondrocytes were next collected and analyzed using TEM (Fig. 2A). The observations indicated that the exosomes derived from chondrocytes were round or oval-shaped with uneven sizes and ranging in diameter from 150-200 nm (Fig. 2B). Western blotting revealed positive expression of CD63 and CD9, along with negative expression of GM130 in the exosomes (Fig. 2C). It was then examined whether CIRP was present in chondrocyte-derived exosomes. The western blotting results showed that chondrocyte exosomes indeed contained CIRP and its expression was markedly increased in OA chondrocytes compared with normal chondrocytes (Fig. 2D and E). Moreover, treatment of chondrocytes with 10 ng/ml IL-1β also resulted in an increase in CIRP expression (Fig. 2F-H). Furthermore, the exosome

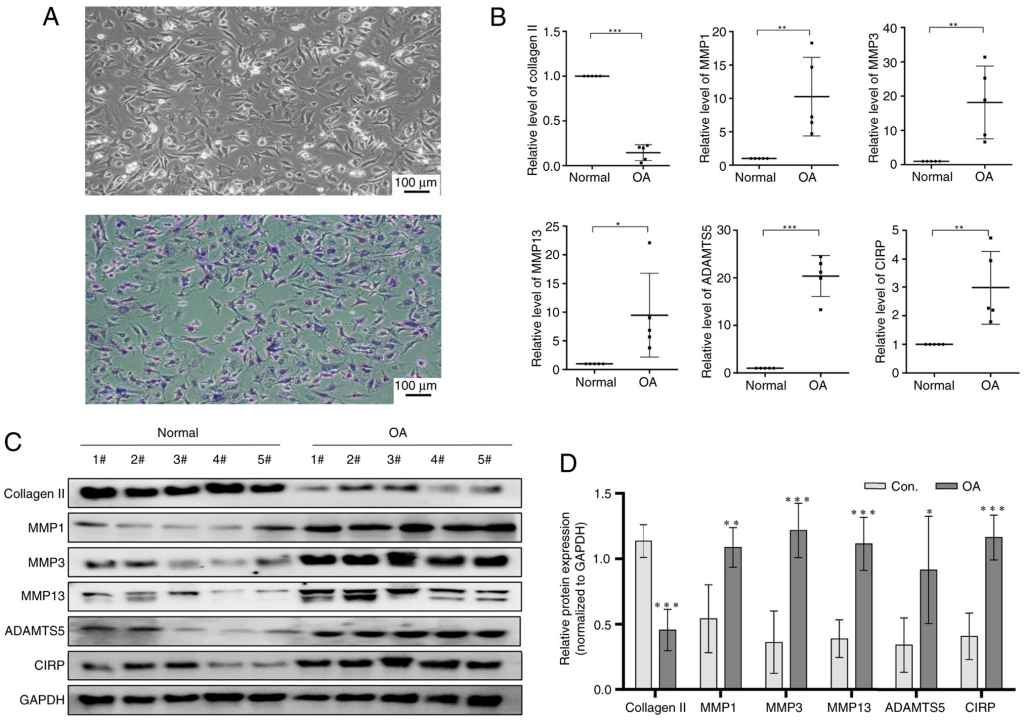

Figure 1. CIRP expression in chondrocytes from healthy individuals and OA patients. (A) Trypan blue staining of chondrocytes. (B) Representative quantitative PCR showing the expression of CIRP, collagen II, MMP1, MMP3, MMP13 and ADAMTS5 in chondrocytes from healthy individuals and OA patients. (C and D) Western blotting showing the expression of CIRP, collagen II, MMP1, MMP3, MMP13 and ADAMTS5 in chondrocytes from healthy individuals and OA patients. (n=5), *P<0.05, **P<0.01, ***P<0.001. CIRP, cold-inducible RNA-binding protein; OA, osteoarthritis; MMPs, matrix metalloproteinases; ADAMTS5, ADAM metallopeptidase with thrombospondin type 1 motif 5.

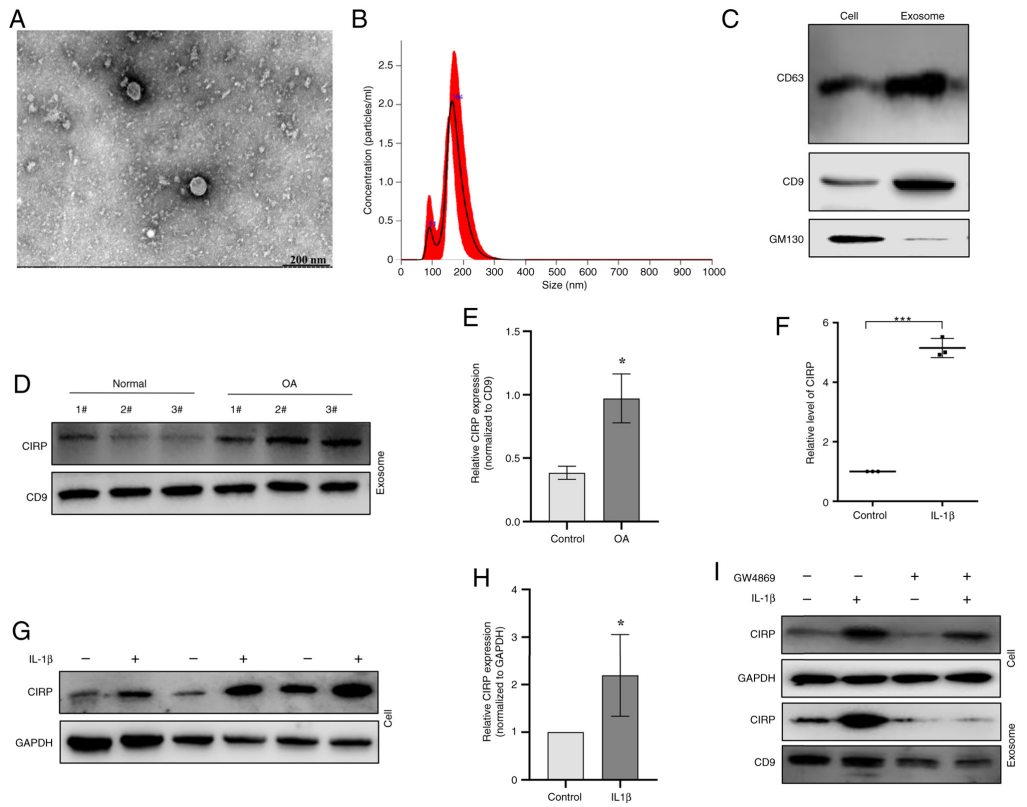

Figure 2. CIRP secretion in OA human chondrocytes via exosomes. (A) Morphology of exosomes observed using transmission electron microscopy. (B) Particle size distribution of exosomes measured by nanoparticle tracking analysis. (C) Western blotting analysis showing the expression of the exosomal markers CD63, CD9 and GM130 in both cells and exosomes. (D and E) Western blotting showing the protein expression of CIRP in exosomes. (F) Quantitative PCR analysis showing the mRNA expression of CIRP in chondrocytes treated with IL-1β. (G and H) Western blotting analysis showing the protein expression of CIRP in chondrocytes treated with IL-1β. (I) Western blotting analysis showing the protein expression of CIRP in chondrocytes following treatment with the exosome release inhibitor GW4869. (n=3), *P<0.05, ***P<0.001. CIRP, cold-inducible RNA-binding protein; OA, osteoarthritis

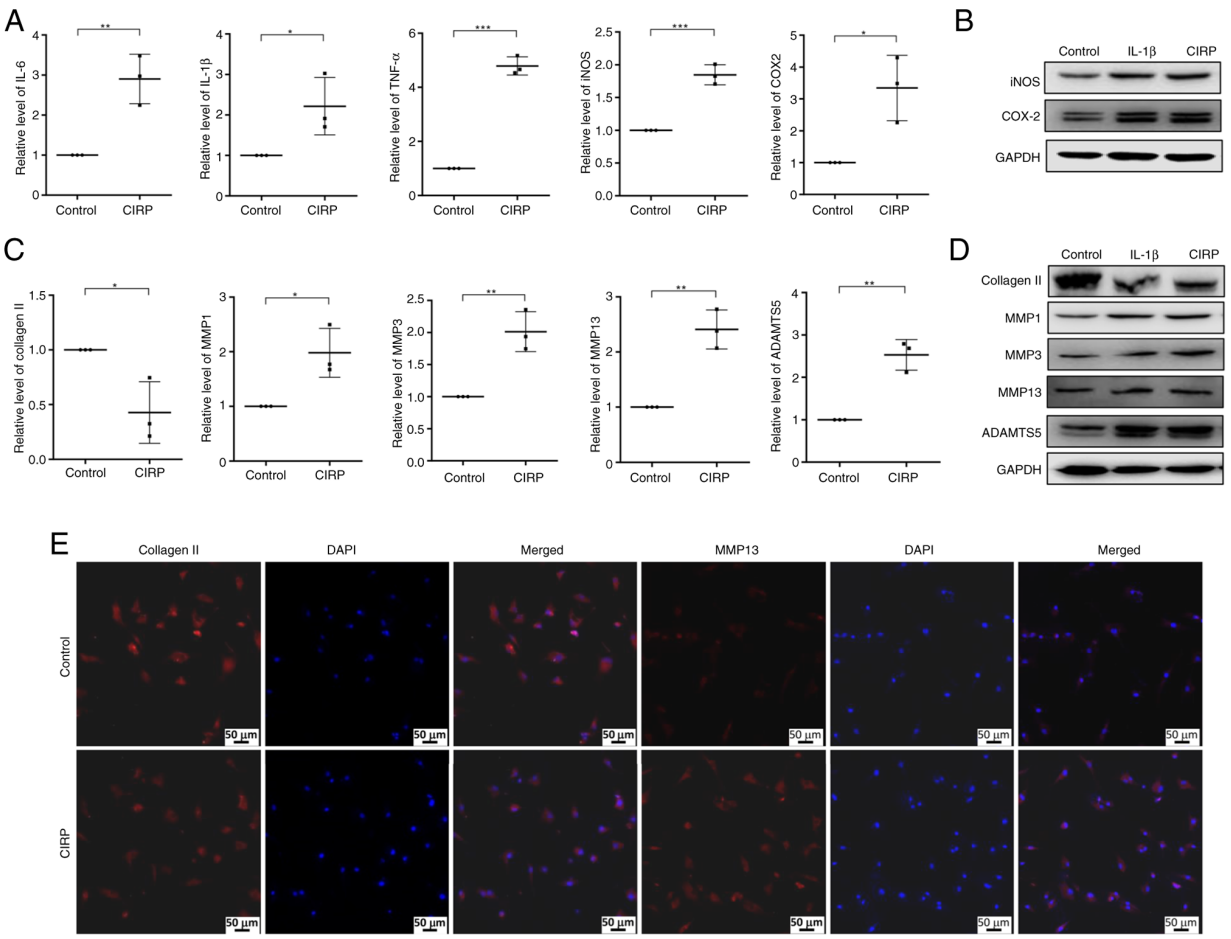

Figure 3. CIRP promotes inflammatory response and ECM degradation in human chondrocytes. (A) Quantitative PCR analysis showing the effect of CIRP on the expression of inflammatory cytokines, including IL-6, IL-1β, TNF-α, iNOS and COX-2. (B) Western blotting showing the protein expression of iNOS and COX-2. (C and D) Quantitative PCR and western blotting showing the effect of CIRP on the expression of MMP1, MMP3, MMP13, ADAMTS5 and collagen II. (E) Immunofluorescence analysis of collagen II and MMP13 expression following CIRP treatment, with DAPI staining for nuclei (scale bar, 50 μm). n=3, *P<0.05, **P<0.01, ***P<0.001. CIRP, cold-inducible RNA-binding protein; ECM, extracellular matrix; iNOS, nitric oxide synthase; COX-2, cyclooxygenase-2; MMPs, matrix metalloproteinases; ADAMTS5, ADAM metallopeptidase with thrombospondin type 1 motif 5.

release inhibitor GW4869 suppressed the secretion of CIRP in human chondrocytes (Fig. 2I). These results indicate that CIRP is overexpressed and released in an exosomal manner in OA chondrocytes, which may contribute to the progression of OA.

CIRP enhances inflammatory cytokine expression and ECM degradation in chondrocytes. From the aforementioned results, it was observed that the expression of CIRP was upregulated in human OA chondrocytes. This upregulation was associated with increased expression of inflammatory cytokines and MMPs, suggesting that CIRP may play a role in progression of OA. OA progression is strongly associated with inflammatory responses and ECM degradation in chondrocytes. To study the role of CIRP in OA, chondrocytes were treated with CIRP concentrations ranging from 0-8 μg/ml. It was found that 4 μg/ml CIRP did not affect cell viability, while 8 μg/ml CIRP did (data not shown). Therefore, the present study chose to use 4 μg/ml CIRP to treat chondrocytes and analyze its effect on inflammatory cytokines expression. The results demonstrated that CIRP treatment increased the expression of IL-6, IL-1β and TNFα (Fig. 3A). Additionally, the expression of iNOS and COX-2, two crucial inflammatory response factors in OA, was also markedly upregulated following CIRP treatment (Fig. 3A and B). The present study then examined the effect of CIRP on the expression of extracellular matrix proteins. As shown in Fig. 3C, treatment with 4 μg/ml CIRP markedly decreased collagen II expression and increased the expression of MMP1, MMP3, MMP13 and ADAMTS5 at mRNA level. The western blotting results in Fig. 3D also showed that CIRP induced the upregulation of MMP1, MMP3, MMP13 and ADAMTS5, along with the downregulation of collagen II, with IL-1β treatment serving as a positive control. Furthermore, Immunofluorescence results indicated that collagen II expression was markedly reduced, while MMP13 expression was induced by CIRP treatment (Fig. 3E).

CIRP-induced inflammatory response and ECM degradation dependent on TLR4/NF-κB/NLRP3 signaling. Next, chondrocytes were treated with 4 μg/ml CIRP and cells were collected for RNA-seq. Fig. 4A shows the genes affected by CIRP treatment. KEGG signaling pathway enrichment analysis indicated that the differentially expressed genes were highly enriched in functions related to inflammatory processes, including TNF-α signaling, cytokine-cytokine receptor interaction, NOD-like receptor signaling and Toll-like receptor signaling pathways (Fig. 4B). The activation of NF-κB signaling

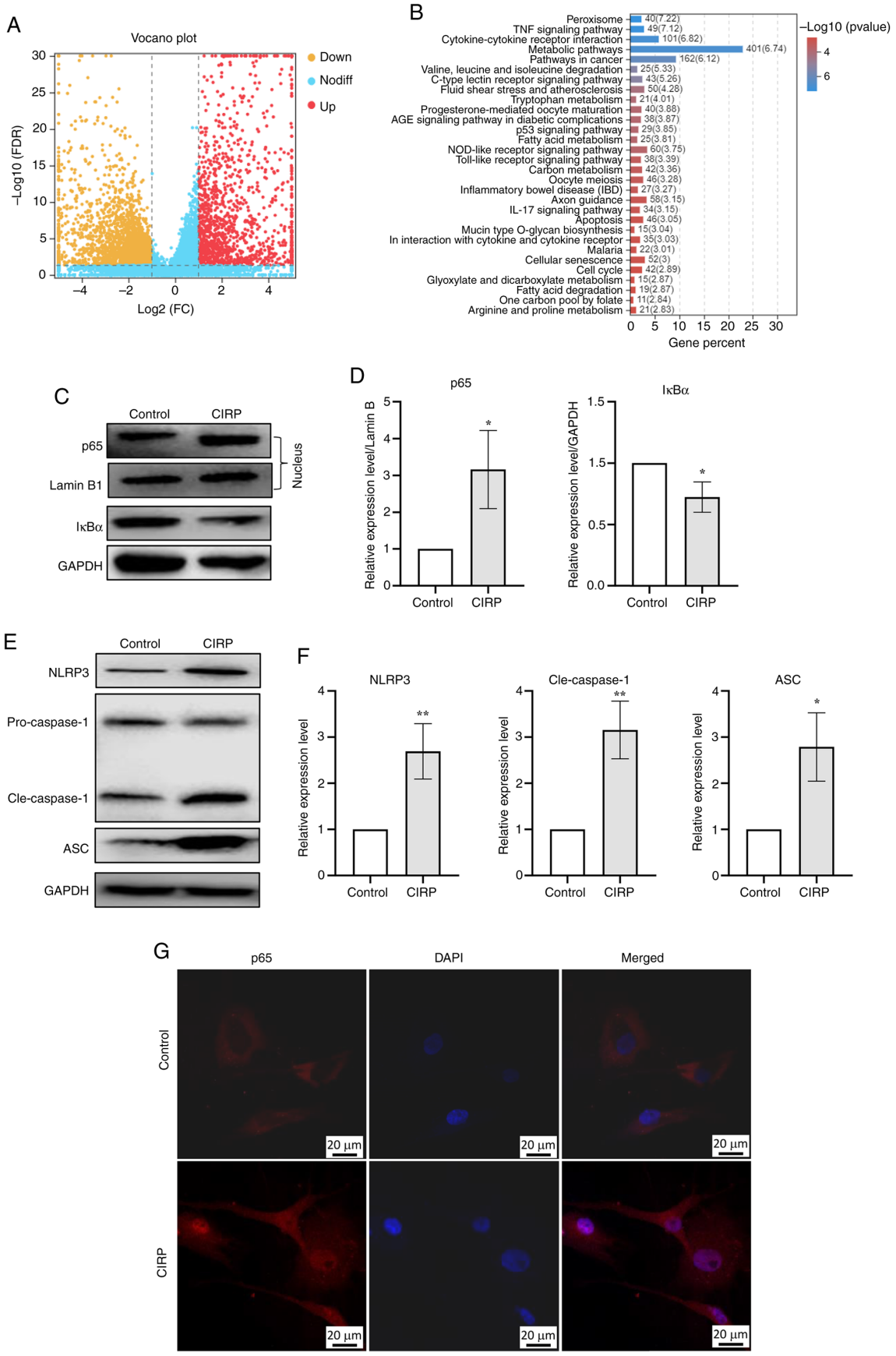

Figure 4. Effect of CIRP on NF-κB/NLRP3 pathway activation. (A) Volcano plot showing differentially expressed genes between control and CIRP-treated chondrocytes. (B) Kyoto Encyclopedia of Genes and Genomes pathway enrichment analysis; a higher P-value (-Log10) indicates greater enrichment and the top 30 enriched pathways are displayed. (C and D) Western blotting showing the protein expression of IκBα in chondrocytes and p65 in the nucleus. (E and F) Western blotting showing the protein expression of NLRP3, cleaved-caspase-1 and ASC. (G) Immunofluorescence analysis of p65 nuclear translocation, with DAPI staining for nuclei (scale bar, 20 μm). n=3, *P<0.05, **P<0.01. CIRP, cold-inducible RNA-binding protein; NLRP3, NLR family pyrin domain containing 3; Cle, cleaved.

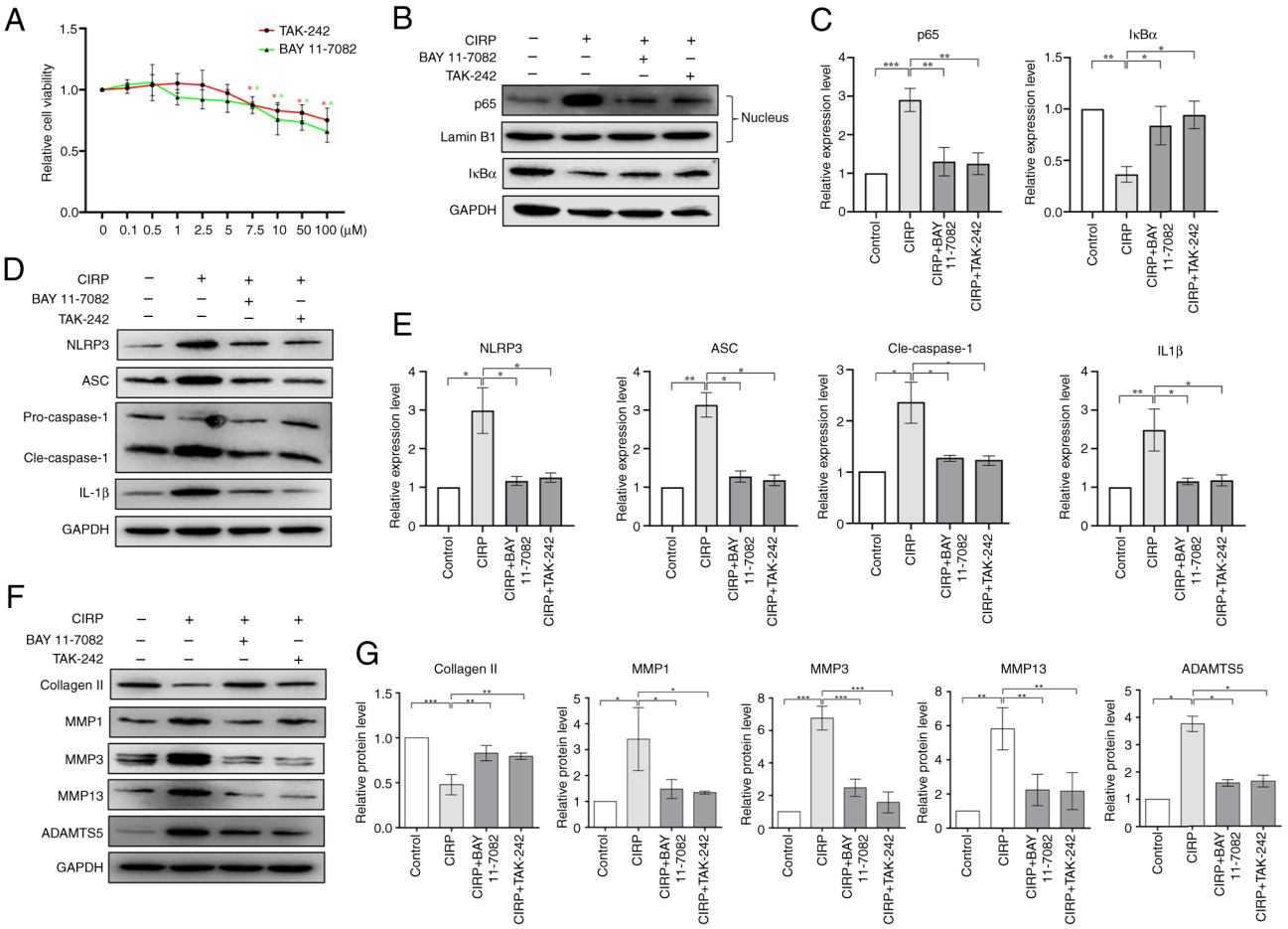

Figure 5. Dependence of CIRP-induced chondrocyte damage on TLR4/NF-κB signaling. (A) Cytotoxicity of BAY 11-7082 and TAK-242 on chondrocytes at various concentration for 48 h, assessed using a CCK-8 assay. (B and C) Western blotting showing the protein expression of IκBα in chondrocytes and p65 in the nucleus of chondrocytes following treatment with the two inhibitors. (D and E) Western blotting showing the protein expression NLRP3, cleaved-caspase-1, ASC and IL-1β following treatment with the two inhibitors. (F and G) Western blotting showing the expression of extracellular matrix proteins in chondrocytes following treatment with the two inhibitors. n=3, *P<0.05, **P<0.01, ***P<0.001. CIRP, cold-inducible RNA-binding protein; TLR4, Toll-like receptor 4; NLRP3, NLR family pyrin domain containing 3; Cle, cleaved.

pathway plays crucial roles in the inflammatory response and cartilage degradation associated with OA. Studies have reported that CIRP can activate the NF-κB signaling pathway via TLR4/MD2. The present study found that 4 μg/ml CIRP increased the expression of p65 in the nucleus and promoted the degradation of IκBα (Fig. 4C and D). It was observed that CIRP markedly induced the nuclear translocation of p65 in chondrocytes (Fig. 4G). These results demonstrate that CIRP induces NF-κB activation in human chondrocytes. The present study also analyzed the activation of NF-κB downstream NLRP3 inflammasome signaling pathway and the results showed that CIRP induced the upregulation of NLRP3, ASC and cleaved-caspase1 (Fig. 4E and F).

It has been reported that extracellular CIRP stimulates inflammatory responses by activating its receptors, including TLR4, IL-6R and TREM-1 (41,44,45). Based on the RNA-seq analysis, the present study sought to determine whether inhibition of TLR4/NF-κB signaling pathway would eliminate CIRP's function. The inhibitors BAY 11-7082 and TAK-242 were used to block the TLR4/NF-κB signaling pathway. Fig. 5A shows their toxicity on human chondrocytes. Next, chondrocytes were pretreated with 5 μM BAY 11-7082 or TAK-242 and then treated with 4 μg/ml CIRP to analyze the effects of NF-κB signaling pathway inhibition on CIRP. The results in Fig. 5B and C indicate that treatment with 5 μM BAY 11-7082 and TAK-242 inhibited the activation of the NF-κB signaling pathway. Furthermore, these two inhibitors suppressed CIRP-induced activation of the NLRP3 signaling pathway (Fig. 5D and E). Additionally, these two inhibitors suppressed CIRP-induced downregulation of collagen II and upregulation of MMP1, MMP3, MMP13 and ADAMTS5 (Fig. 5F and G). These results indicate that CIRP-induced inflammatory responses and ECM degradation in human chondrocytes are dependent on the TLR4/NF-κB/NLRP3 signaling pathway.

CIRP as a putative target of miR-145-5p. Subsequently, miRWalk, TargetScan, StarBase, miRDIP and a chondrogenesis-related microRNA databases were applied to predict the miRNAs that could potentially regulate CIRP expression. A Venn diagram was created based on comparisons of these miRNAs (Fig. 6A), which revealed six candidate miRNAs of interest. Among them, has-miR-145-5p has been reported to play an important role in chondrogenesis (34-36). Therefore, it was hypothesized that miR-145-5p could influence OA progression by targeting CIRP. To validate this hypothesis, online tools indicated the presence of specific binding sites between CIRP mRNA and miR-145-5p

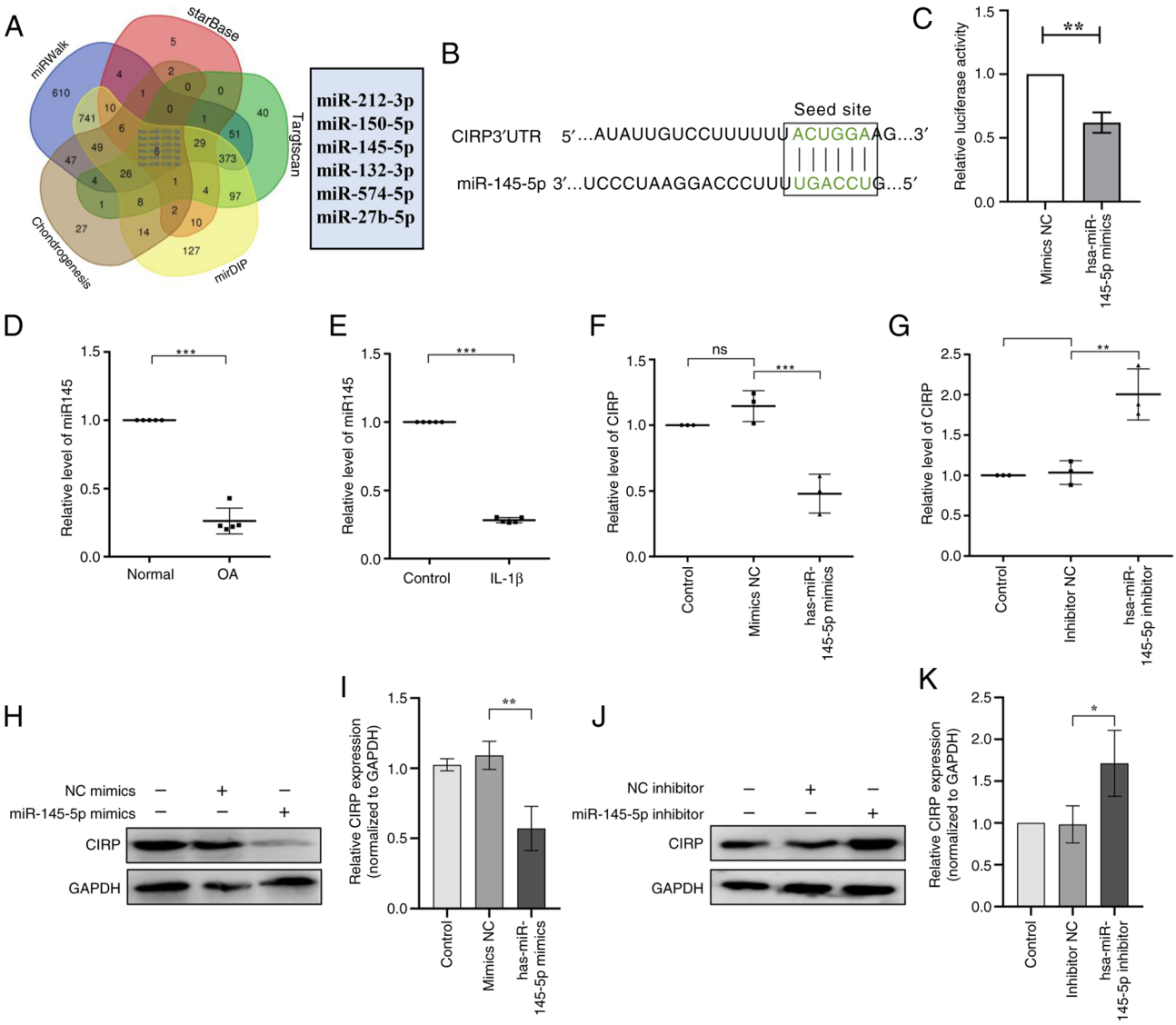

Figure 6. CIRP is a putative target of miR-145. (A) Comparisons of predicted miRNAs that regulate CIRP in a Venn map. (B) Binding sites between has-miR-145-5p and CIRP predicted by online websites. (C) Luciferase activity of CIRP-WT in 293T cells treated with miR-145-5p mimic or NC-mimic assessed by dual luciferase reporter gene assay. (D) Expression of miR-145-5p in OA chondrocytes and normal chondrocytes examined by qPCR. (E) Expression of miR-145-5p in chondrocytes and IL-1β treated chondrocytes examined by qPCR. (F-K) Expression of CIRP in chondrocytes after alteration of miR-145-5p determined by qPCR or western blotting. n=3, *P<0.05, **P<0.01, ***P<0.001. CIRP, cold-inducible RNA-binding protein; miR, microRNA; WT, wild-type; NC, negative control; OA, osteoarthritis; qPCR, quantitative PCR.

(Fig. 6B), suggesting that CIRP might be a downstream target gene of miR-145-5p. Moreover, a dual luciferase reporter gene assay showed that, compared with the NC-mimic control, luciferase activity decreased after the introduction of miR-145-5p mimic and the CIRP-WT reporter plasmid (Fig. 6C). Next, the expression of miR-145-5p in OA human chondrocytes and IL-1β treated human chondrocytes was analyzed by qPCR. The results revealed that the miR-145-5p expression was reduced in OA chondrocytes compared with normal chondrocytes. Moreover, chondrocytes treated with IL-1β exhibited lower miR-145-5p expression compared with untreated chondrocytes (Fig. 6D and E). Subsequently, the expression of CIRP in human chondrocytes treated with miR-145-5p mimic or miR-145-5p inhibitor was assessed. The results revealed that miR-145-5p mimic led to increased miR-145-5p expression (Fig. S1A) and decreased CIRP expression (Fig. 6F, H and I), whereas the miR-145-5p inhibitor decreased miR-145-5p (Fig. S1B) induced the opposite effects (Fig. 6G, J and K). Taken together, these results indicated that miR-145-5p is downregulated in OA and specifically binds to and suppresses CIRP expression.

miR-145-5p maintains chondrocyte function in vitro. Subsequently, to investigate the effect of miR-145-5p on human chondrocytes, the cells were transfected with miR-145-5p mimic, followed by overexpression of CIRP to induce the inflammation and ECM degradation in chondrocytes. qPCR and western blotting analysis were conducted to evaluate the mRNA and protein expression of ECM-related proteins (collagen II, ADAMTS5 and MMPs) as well as pro-inflammatory factors (iNOS, COX-2, IL-6, TNF-α and IL-1β). As shown in Fig. 7A, B and E, the expression of pro-inflammatory factors was markedly increased after CIRP overexpression but was reduced by miR-145-5p mimic treatment. In Fig. 7C-E, the expression of collagen II was markedly decreased following

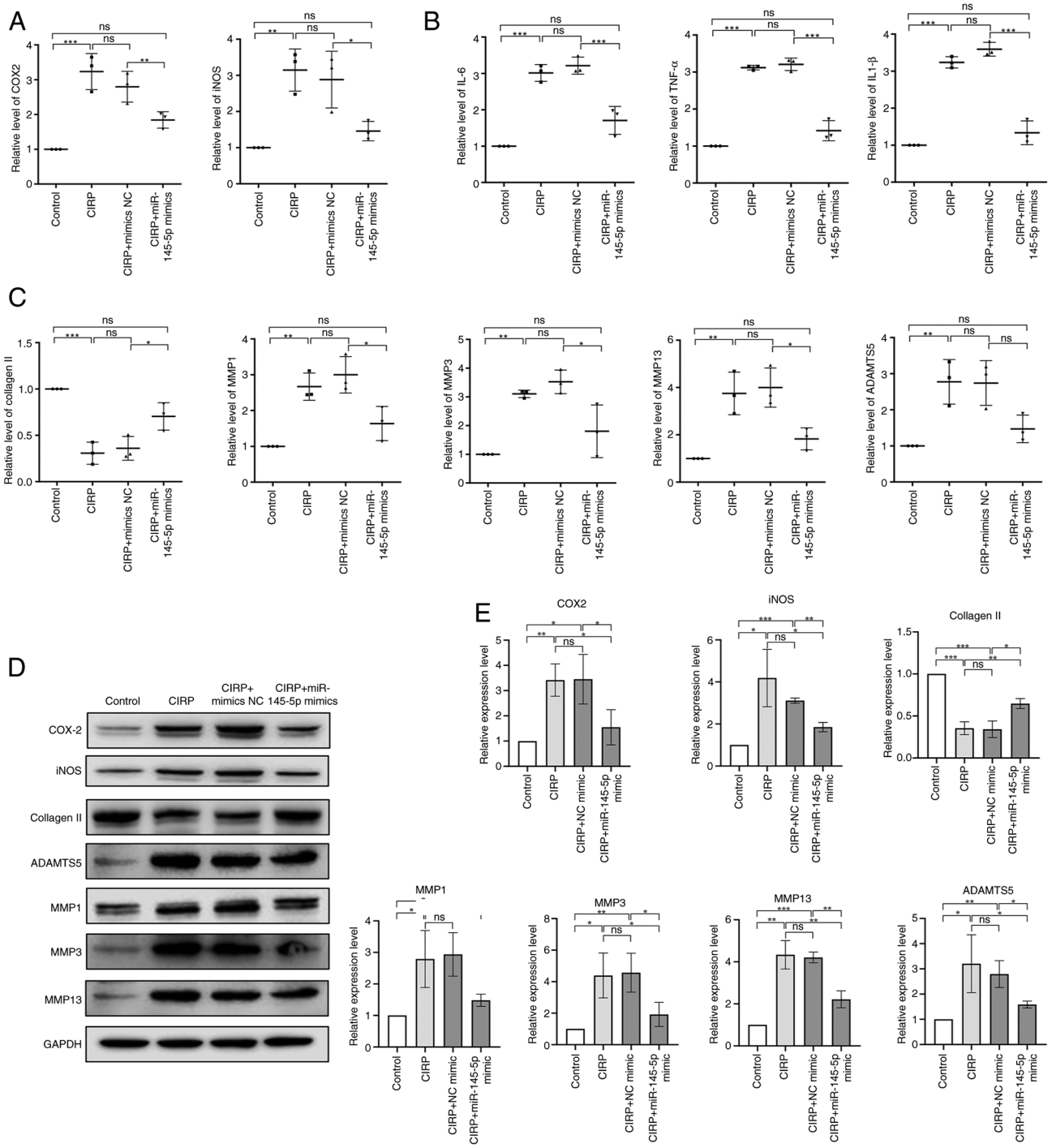

Figure 7. miR-145-5p elevation suppresses CIRP induced damage of chondrocytes. (A and B) Chondrocytes were transfected with miR-145-5p mimics or CIRP, IL-6, IL-1β, TNF-α, iNOS and COX-2 expression were analyzed by qPCR. (C) Chondrocytes were transfected with miR-145-5p mimics or CIRP, the mRNA expression of extracellular matrix proteins of chondrocytes were analyzed by qPCR. (D and E) Chondrocytes were transfected with miR-145-5p mimics or CIRP, the protein expression of iNOS, COX-2 and extracellular matrix proteins of chondrocytes were analyzed by western blotting. n=3, *P<0.05, **P<0.01, ***P<0.001. miR, microRNA; CIRP, cold-inducible RNA-binding protein; iNOS, inducible nitric oxide synthase; COX-2, cyclooxygenase-2; qPCR, quantitative PCR.

CIRP overexpression but was increased by miR-145-5p mimic treatment, with the expression of other ECM-related proteins showing inverse results. These findings indicated that miR-145-5p overexpression markedly increased collagen II expression, decreased the expression of MMP1, MMP3, MMP13 and ADAMTS5 and reduced inflammatory response in human chondrocytes. Moreover, the damage induced by CIRP overexpression in human chondrocytes was alleviated by overexpressed miR-145-5p overexpression. These results indicate that miR-145-5p plays a key role in maintaining the function of chondrocytes *in vitro*.

miR-145-5p suppresses IL-1β-induced CIRP/NF-κB/NLRP3 signaling pathway activation. The present study investigated the role of miR-145-5p in IL-1β-induced activation of the CIRP/NF-kB/NLRP3 signaling pathway. It was found that

treatment with 10 ng/ml IL-1β increased the expression of p65 in the nucleus and promoted the degradation of IκBα in human chondrocytes (Fig. 8A and B). Additionally, IL-1β induced the nuclear translocation of p65 (Fig. 8C). However, the activation of the NF-κB signaling pathway was suppressed by treatment with miR-145-5p mimics (Fig. 8A-C). Moreover, IL-1β-induced expression of CIRP and activated the downstream NLRP3 inflammasome signaling pathway, both of which were suppressed by treatment with miR-145-5p mimics (Fig. 8D and E). These results indicated that miR-145-5p exerts a protective function in chondrocytes by inhibiting the CIRP/NF-κB/NLRP3 signaling pathway.

miR-145-5p inhibits articular cartilage degradation in vivo. Finally, the effect of miR-145-5p on mice with OA was evaluated *in vivo*. The mice were divided into four groups: One sham group and three DMM groups (DMM group, DMM injected with miR-NC exosomes group and DMM injected with miR-145-5p mimic exosomes group). hematoxylin and eosin staining was conducted to observe the pathological changes in the articular cartilage of the knee joint and S/O staining was used to assess articular cartilage degeneration. The S/O staining revealed that the articular cartilage in the sham control group exhibited a normal, red-dyed area and a smooth surface. By contrast, the thickness of the articular cartilage was markedly reduced in both the DMM group and the DMM injected with miR-NC exosomes group compared with the sham control group. Notably, the DMM injected with miR-145-5p mimic exosomes group showed a thicker red-dyed area and a smoother surface of articular cartilage compared with the DMM injected with miR-NC exosomes group (Fig. 9A), suggesting that miR-145-5p plays a role in attenuating cartilage matrix degradation. Additionally, immunohistochemical assays for MMP3 and collagen II were also performed in the OA model. In the DMM group and the DMM injected with miR-NC exosomes group, the expression of MMP3 was markedly increased compared with the sham group, while the DMM injected with miR-145-5p mimic exosomes group exhibited decreased expression of MMP3 (Fig. 9B). Similarly, the expression of collagen II was markedly reduced in the DMM group and the DMM injected with miR-NC group compared with the sham group, while the DMM injected with miR-145-5p mimic exosomes group exhibited elevated expression levels (Fig. 9B). Therefore, these results indicate that, as demonstrated by the *in vivo* experiments, overexpressing of miR-145-5p can hinder OA-related damage.

Discussion

OA is a chronic, age-associated degenerative joint disease featured by cartilage degeneration and inflammatory response, which imposes a significant social and economic burden (1,46). Currently, pain management and end-stage joint replacement surgery are the most used strategies for OA treatment. However, these approaches do not stop or reverse cartilage degeneration and destruction (3,47). Therefore, research has shifted its focus toward understanding the pathogenesis of OA and identifying new strategies to prevent its early onset (4). An increasing number of studies have highlighted that the diagnostic and therapeutic potential of miRNAs in treatment of OA (28,48). Additionally, exosomes have emerged as potential natural vehicles for miRNA delivery in disease therapy. The current study aimed to investigate the effects of the novel pro-inflammatory factor CIRP on OA progression through the activation of the TLR4/NF-κB/NLRP3 inflammasome signaling pathway. Furthermore, its key findings provided evidence that exosomes overexpressing miR-145-5p can attenuate OA damage by repressing CIRP.

CIRP is an RNA-binding protein known for its tumor-promoting function and our research group has focused on it for an extended period (15,49). CIRP has been identified as a significant pro-inflammatory factor, playing important roles in various inflammation diseases, including sepsis, neuroinflammation, colitis-associated cancer and endothelial cell inflammation (17,18,24,50). Two studies have been reported that CIRP levels were evaluated in the synovial fluid of patients with OA and rheumatoid arthritis, indicating a positive correlation between CIRP and OA (26,27). However, the specific roles of CIRP in OA progression and the underlying mechanisms remain unclear. The present study aimed to investigate the function of CIRP in OA progression. It first assessed its expression and found that CIRP was markedly upregulated in OA chondrocytes. Additionally, a previous study has detected CIRP in the synovial fluid of OA patients and it has been reported that CIRP can be released by macrophages during sepsis (51). The present study then examined the expression of CIRP in the exosomes derived from OA chondrocytes and discovered that these chondrocytes released higher levels of CIRP in an exosomal manner. Furthermore, treatment with IL-1β increased the expression of CIRP in both whole chondrocyte cells and their exosomes. These findings may help to explain the elevated levels of CIRP observed in the synovial fluid of OA patients.

Next, the present study investigated the influence of CIRP on the expression of pro-inflammatory cytokines and MMP. During OA progression, the balance of metabolic process is disrupted. In response to inflammatory cytokines, such as iNOS, chondrocytes secret many metalloproteinases that contribute to extracellular matrix degradation (52,53). The results demonstrated that CIRP markedly upregulated the expression of pro-inflammatory cytokines, including iNOS and COX-2. Additionally, the expression of matrix metalloproteinase, such as MMP1, MMP3, MMP13 and ADAMTS5, was markedly elevated following CIRP treatment. Importantly, CIRP also decreased the expression of collagen II. These findings suggested that CIRP is positively associated with inflammation and extracellular matrix degradation in OA and the underlying molecular mechanisms require further investigation.

Furthermore, the present study treated human chondrocytes with CIRP and performed RNA-seq to explore the downstream effects. The RNA-seq analysis revealed that CIRP treatment induced differential gene expression enriched in pathways related to TNF-α signaling, cytokine-cytokine receptor interaction, IL-17 signaling, NOD-like receptor signaling and TLR signaling pathway. These pathways are well-established contributors to OA progression. For example, elevated circulating TNF-α levels are associated with severe OA and cartilage loss and small molecules that targeting

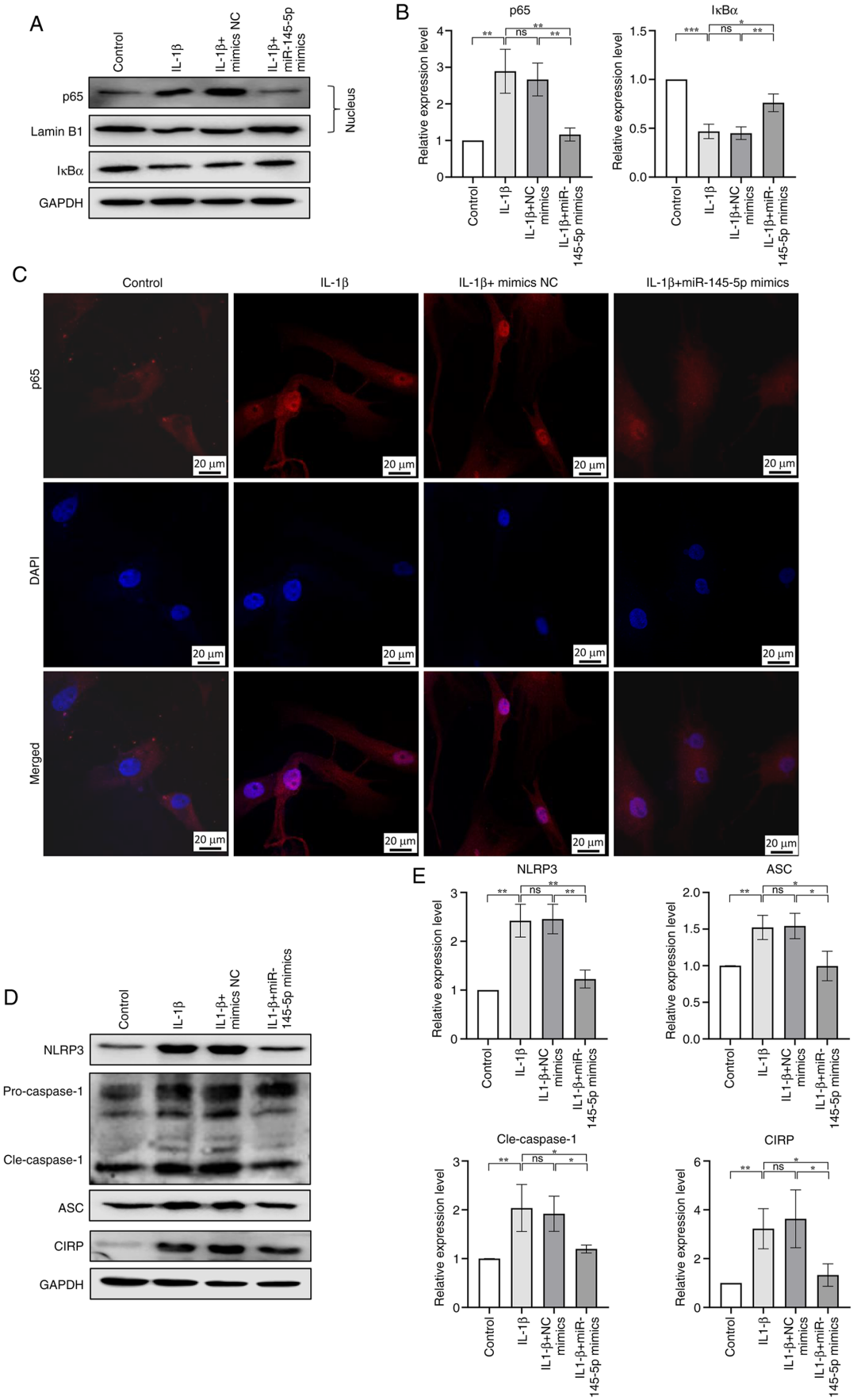

Figure 8. miR-145-5p elevation suppressed CIRP induced NF-κB/NLRP3 activation. (A and B) Chondrocytes were transfected with miR-145-5p mimics or IL-1β treatment, western blotting results showed the protein expression of IκBα in the cytoplasm and p65 in the nucleus of chondrocytes. (C) The nuclei translocation of p65 was detected by the immunofluorescence combined with DAPI staining for the nuclei. (D and E) western blotting results showed the protein expression NLRP3, cleaved-caspase-1, ASC and CIRP in chondrocytes transfected with miR-145-5p mimics or IL-1β treatment. n=3, *P<0.05, **P<0.01, ***P<0.001. miR, microRNA; CIRP, cold-inducible RNA-binding protein; NLRP3, NLR family pyrin domain containing 3; Cle, cleaved.

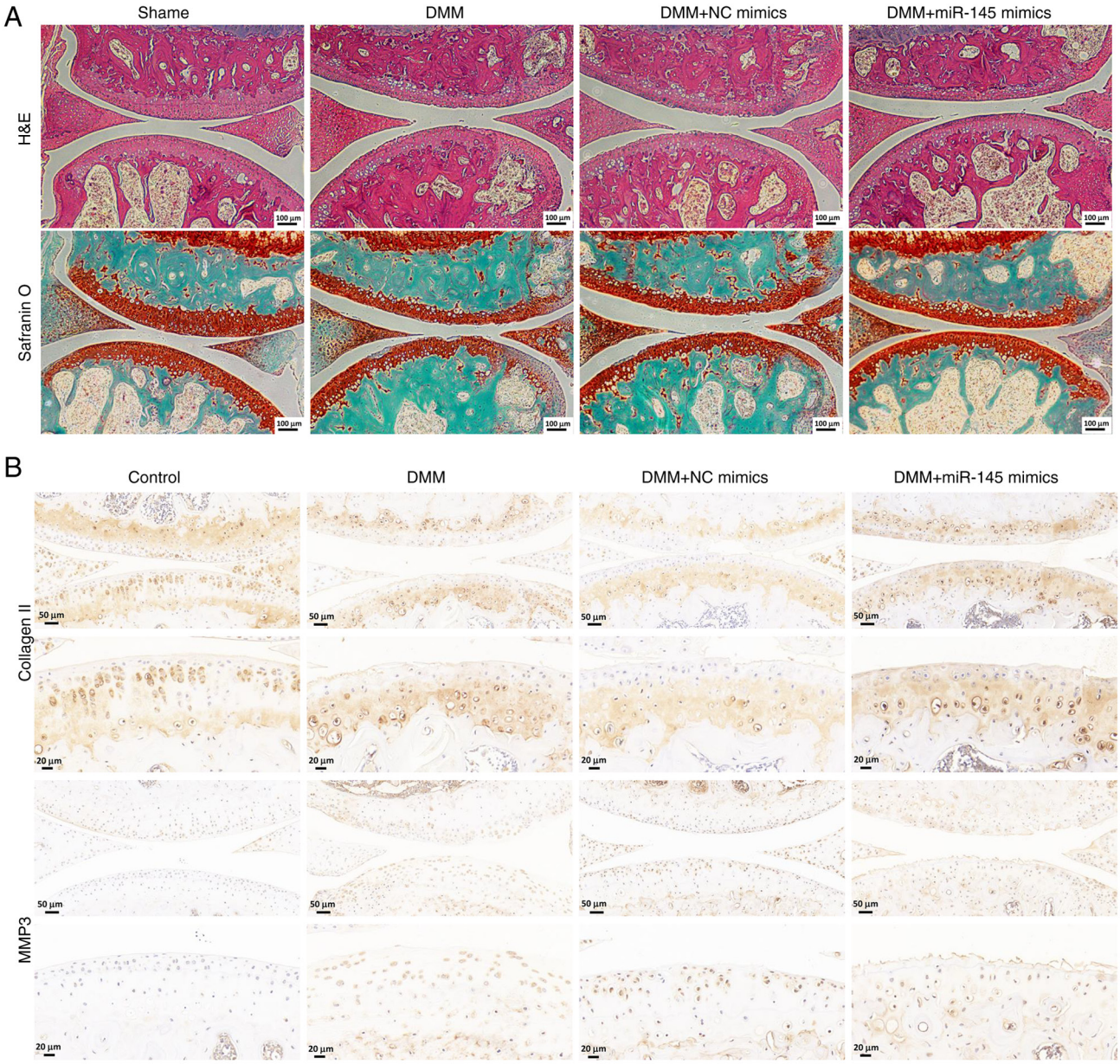

Figure 9. miR-145-5p elevation suppresses OA progression in animal model. (A) Pathological changes of cartilage tissues of the knee joint using hematoxylin and eosin staining and Safranin O staining. (B) Immunohistochemical staining of collagen II and MMP-3 in cartilage tissues of OA mice. miR, microRNA; OA, osteoarthritis; MMPs, matrix metalloproteinases.

TNF-α receptors have demonstrated protective effects against OA (54,55). Previous studies have shown that extracellular CIRP can stimulate inflammatory responses by interacting with its receptors such as TLR4, IL-6R and triggering receptor expressed on myeloid cells 1 (24,56,57). The RNA-seq data also suggested that CIRP affects the TLR signaling pathway. Notably, TLR4 is overexpressed in human OA chondrocytes (58,59) and is a well-studied upstream activator of the NF-κB signaling pathway (60), implying that CIRP may activate NF-κB through TLR4 signaling. To investigate this hypothesis, the present study assessed the activation of NF-κB and found that CIRP increased the nuclear translocation of p65, a hallmark of NF-κB pathway activation. Additionally, CIRP upregulated the expression of IL-1β, a key pro-inflammatory cytokine. The TLR4/NF-κB axis and NLRP3/ASC/procaspase-1 inflammasome complex are critical for the maturation and secretion of IL-1β (61,62). It was further confirmed that CIRP activates the NLRP3 inflammasome. These findings suggested that CIRP promotes inflammation and ECM degradation in OA by activating NF-κB/NLRP3 inflammasome signaling pathway via TLR4. To validate this mechanism, two inhibitors, BAY 11-7082 (an NF-κB inhibitor) and TAK-242 (a TLR4 inhibitor), were used to inhibit the TLR4/NF-κB signaling pathway. The two inhibitors effectively suppressed CIRP-induced nuclear translocation of p65. Furthermore, they inhibited the activation of downstream NLRP3 inflammasome and the expression of IL-1β. Importantly, treatment with these inhibitors also reversed CIRP-induced ECM degradation.

miRNAs, a type of conserved endogenous non-coding RNAs, regulate target mRNA stability or translation by directly binding to their targets. Growing evidence demonstrates that a number of miRNAs exert protective functions

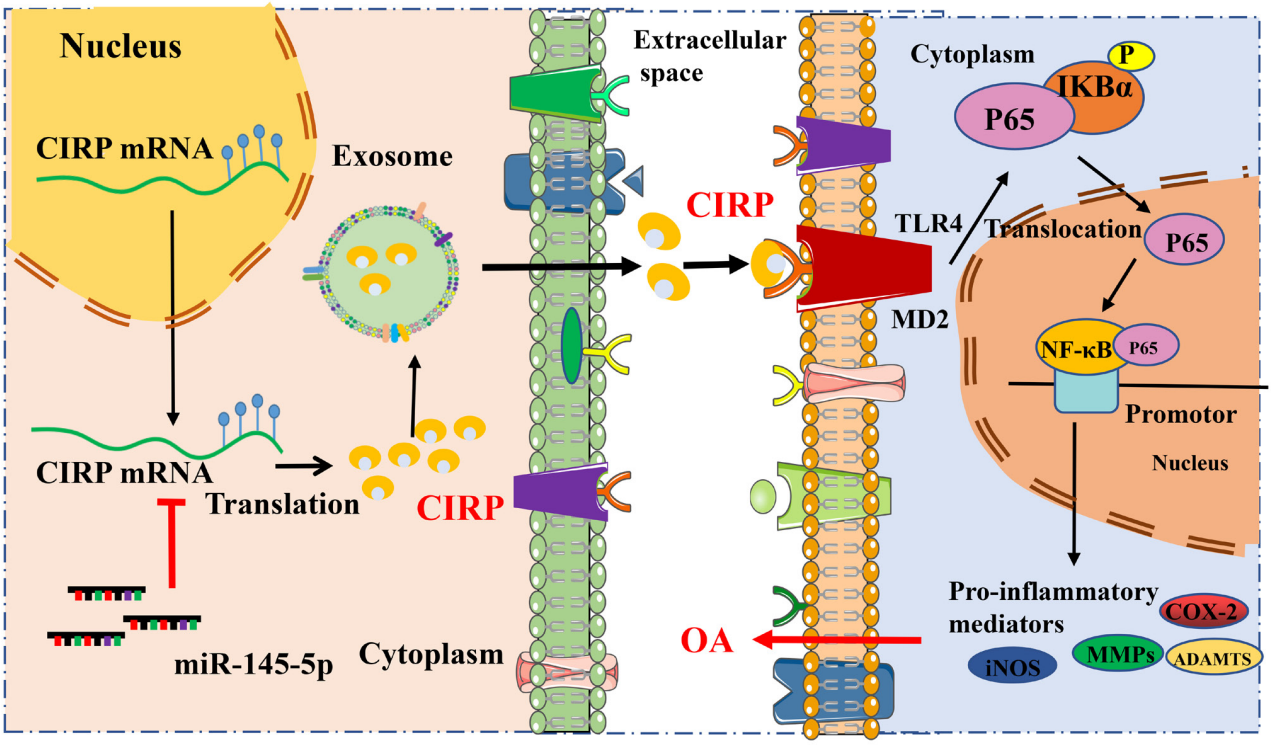

Figure 10. Schematic illustration of targeting of CIRP attenuates osteoarthritis progression and the underlying mechanism. CIRP can be secreted in the form of exosomes and acts as a pro-inflammatory factor that activates the TLR4/NF-κB/NLRP3 signaling pathway, promoting the inflammatory response, ECM degradation and the progression of OA. Additionally, CIRP is identified as a target of miR-145, which inhibits its expression in OA. CIRP, cold-inducible RNA-binding protein; TLR4, Toll-like receptor 4; NLRP3, NLR family pyrin domain containing 3; OA, osteoarthritis; ECM, extracellular matrix; miR, microRNA; iNOS, inducible nitric oxide synthase; COX-2, cyclooxygenase-2; MMPs, matrix metalloproteinases.

in chondrocytes by directly targeting multiple mRNAs involved in the development of OA. miR-145 is a well-known non-coding RNA involved in the pathogenesis of various inflammation-related diseases, such as colitis and inflammation-associated colon cancer (63), rheumatoid arthritis (64) and myocardial ischemia/reperfusion injury (65). The current study found that the expression of miR-145 was decreased in the cartilage of OA patients and in chondrocytes treated with IL-1β, highlighting its possible role in the pathogenesis of OA. More importantly, CIRP was identified as a target of miR-145-5p in chondrocytes. The chondrocyte-protective function of miR-145-5p was assessed both *in vivo* and *in vitro*. It was found that miR-145-5p could suppress the inflammatory response, ECM degradation and activation of NF-κB/NLRP3 signaling pathway induced by overexpression of CIRP or IL-1β treatment *in vitro*. The results were consistent with a previous study showing that miR-145 plays a protective role in chondrocytes during OA progression (34). *In vivo* experiments further supported the idea that exosomal delivery of miR-145 could suppress OA progression. The present study found that miR-145 suppresses the expression of CIRP in OA, suggesting that miR-145 may play an important role in modulating the inflammatory response and pathological progression of OA. While miR-145 is a significant microRNA that suppresses CIRP expression, it is not the only one identified in the literature. Previous study has reported that miR-383-5p can target CIRP mRNA and inhibit its expression in ovarian granulosa cells (66). Additionally, Lin *et al* (67) recently demonstrated that CIRP is a direct target of miR-377-3p in nasopharyngeal carcinoma. Aside from these two miRNAs, no other microRNAs have been documented to inhibit CIRP expression in the literature. Analysis using databases such as miRWalk, TargetScan, StarBase, miRDIP and chondrogenesis-related microRNA resources revealed six candidate microRNAs that may also regulate CIRP expression. Further investigation demonstrated specific binding sites between CIRP mRNA and miR-145-5p, with decreased expression of miR-145-5p observed in OA chondrocytes. Consequently, the present study focused on miR-145 for more detailed study. The dual luciferase reporter gene assay confirmed that miR-145-5p suppresses CIRP expression in OA chondrocytes. Further research is needed to explore the potential existence of other microRNAs that may regulate CIRP expression in the context of OA.

In conclusion, the present study confirmed that CIRP can be secreted in the form of exosomes, functions as a pro-inflammatory factor and activates the TLR4/NF-κB/NLRP3 signaling pathway, thereby promoting the inflammatory response, ECM degradation and the progression of OA (Fig. 10). It also established that CIRP is a target of miR-145-5p, which inhibits CIRP expression in OA. *In vitro*, miR-145-5p was shown to suppress the inflammatory response, ECM degradation and the activation of the NF-κB/NLRP3 signaling pathway induced by either CIRP overexpression or IL-1β treatment. Furthermore, *in vivo* delivery of miR-145-5p via exosomes was effective in inhibiting OA progression, highlighting the potential of exosome-based miR-145-5p delivery as a therapeutic strategy. Overall, the findings suggested a novel strategy for OA

treatment by targeting pro-inflammatory factors such as CIRP, with miR-145-5p acting as a key regulator of CIRP expression. However, the clinical relevance of CIRP in alleviating OA is still in its early stages. While there is promising potential for targeting CIRP in OA treatment, this area remains an emerging field of research. The therapeutic implications of CIRP inhibition warrant further exploration through clinical trials to determine whether CIRP suppression can indeed provide significant relief or disease modification in OA patients. It is important to note the limitations of the present study. It would be an oversimplification to assert that suppressing CIRP expression will directly lead to the suppression of OA. Although CIRP has been implicated in the inflammatory response and cartilage degradation associated with OA, the relationship between CIRP and OA is probably complex and multifactorial. Therefore, while targeting CIRP may present therapeutic potential, it is unlikely to be a sufficient strategy for fully mitigating OA. A comprehensive understanding of additional factors and pathways is essential when considering CIRP as a therapeutic target for OA. Future investigations should focus on identifying the underlying mechanisms at play in this relationship to improve the informing of therapeutic strategies.

Acknowledgements

Not applicable.

Funding

The present study was supported by the joint foundation of Luzhou Government and Southwest Medical University (grant no. 2024LZXNYDJ104); the Shenzhen Medical Research Fund (grant no. A2403030); Natural Science Foundation of China (grant no. 82172831); Shenzhen Science and Technology Projects (grant no. JSGG20220831110400001, KCXFZ20230731093059012) and Shenzhen High-level Hospital Construction Fund (grant no. 1801024).

Availability of data and materials

The data generated in the present study may be requested from the corresponding author.

Authors' contributions

WCS was responsible for data curation, formal analysis, funding acquisition and original draft. YL was responsible for data curation, formal analysis, the original draft manuscript and methodology. JGF was responsible for data curation, formal analysis and validation. JHL was responsible for formal analysis and validation. QFH was responsible for formal analysis and investigation. DXH and YXC were responsible for formal analysis and investigation. HYS and WY were responsible for investigation and validation. WS was responsible for conceptualization, funding acquisition, formal analysis and validation. QY was responsible for conceptualization, funding acquisition, formal analysis and original draft. WCS and QY confirm the authenticity of all the raw data. All authors read and approved the final manuscript.

Ethics approval and consent to participate

The collection of cartilage tissues from patients was approved by the Ethics Committee of Shenzhen Second People's Hospital (approval no. 2023-157-01YJ). Samples used in this study were collected after all patients received detailed explanation of the research and submitted written informed consents. All *in vivo* experiments procedures were approved by the Animal Research Committee of Southwest Medical University (approval no. 20220307-004).

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

References

1. Martel-Pelletier J, Barr AJ, Cicuttini FM, Conaghan PG, Cooper C, Goldring MB, Goldring SR, Jones G, Teichtahl AJ and Pelletier JP: Osteoarthritis. Nat Rev Dis Primers 2: 16072, 2016.
2. Loeser RF, Goldring SR, Scanzello CR and Goldring MB: Osteoarthritis: A disease of the joint as an organ. Arthritis Rheum 64: 1697-1707, 2012.
3. Bijlsma JW, Berenbaum F and Lafeber FP: Osteoarthritis: An update with relevance for clinical practice. Lancet 377: 2115-2126, 2011.
4. Glyn-Jones S, Palmer AJ, Agricola R, Price AJ, Vincent TL, Weinans H and Carr AJ: Osteoarthritis. Lancet 386: 376-387, 2015.
5. Yang B, Kang X, Xing Y, Dou C, Kang F, Li J, Quan Y and Dong S: Effect of microRNA-145 on IL-1beta-induced cartilage degradation in human chondrocytes. FEBS Lett 588: 2344-2352, 2014.
6. Tu C, Huang X, Xiao Y, Song M, Ma Y, Yan J, You H and Wu H: Schisandrin A inhibits the IL-1β-Induced inflammation and cartilage degradation via suppression of MAPK and NF-κB signal pathways in rat chondrocytes. Front Pharmacol 10: 41, 2019.
7. Chabane N, Zayed N, Afif H, Mfuna-Endam L, Benderdour M, Boileau C, Martel-Pelletier J, Pelletier JP, Duval N and Fahmi H: Histone deacetylase inhibitors suppress interleukin-1beta-induced nitric oxide and prostaglandin E2 production in human chondrocytes. Osteoarthritis Cartilage 16: 1267-1274, 2008.
8. Zhou S, Liu G, Si Z, Yu L and Hou L: Glycyrrhizin, an HMGB1 inhibitor, suppresses interleukin-1β-induced inflammatory responses in chondrocytes from patients with osteoarthritis. Cartilage 13 (2_Suppl): 947S-955S, 2021.
9. Frommer KW, Schaffler A, Rehart S, Lehr A, Muller-Ladner U and Neumann E: Free fatty acids: Potential proinflammatory mediators in rheumatic diseases. Ann Rheum Dis 74: 303-310, 2015.
10. Xu HC, Wu B, Ma YM, Xu H, Shen ZH and Chen S: Hederacoside-C protects against AGEs-induced ECM degradation in mice chondrocytes. Int Immunopharmacol 84: 106579, 2020.
11. Gao F and Zhang S: Loratadine alleviates advanced glycation end Product-induced activation of NLRP3 inflammasome in human chondrocytes. Drug Des Devel Ther 14: 2899-2908, 2020.
12. Liao Y, Tong L, Tang L and Wu S: The role of cold-inducible RNA binding protein in cell stress response. Int J Cancer 141: 2164-2173, 2017.
13. Zhong P and Huang H: Recent progress in the research of cold-inducible RNA-binding protein. Future Sci OA 3: FSO246, 2017.
14. Wellmann S, Buhrer C, Moderegger E, Zelmer A, Kirschner R, Koehne P, Fujita J and Seeger K: Oxygen-regulated expression of the RNA-binding proteins RBM3 and CIRP by a HIF-1-independent mechanism. J Cell Sci 117: 1785-1794, 2004.

15. Sun W, Liao Y, Yi Q, Wu S, Tang L and Tong L: The mechanism of CIRP in regulation of STAT3 phosphorylation and Bag-1/S expression Upon UVB radiation. Photochem Photobiol 94: 1234-1239, 2018.
16. Aziz M, Brenner M and Wang P: Extracellular CIRP (eCIRP) and inflammation. J Leukoc Biol 106: 133-146, 2019.
17. Qiang X, Yang WL, Wu R, Zhou M, Jacob A, Dong W, Kuncewitch M, Ji Y, Yang H, Wang H, *et al*: Cold-inducible RNA-binding protein (CIRP) triggers inflammatory responses in hemorrhagic shock and sepsis. Nat Med 19: 1489-1495, 2013.
18. Sakurai T, Kashida H, Watanabe T, Hagiwara S, Mizushima T, Iijima H, Nishida N, Higashitsuji H, Fujita J and Kudo M: Stress response protein cirp links inflammation and tumorigenesis in colitis-associated cancer. Cancer Res 74: 6119-6128, 2014.
19. Zhou M, Yang WL, Ji Y, Qiang X and Wang P: Cold-inducible RNA-binding protein mediates neuroinflammation in cerebral ischemia. Biochim Biophys Acta 1840: 2253-2261, 2014.
20. Vande Walle L, Van Opdenbosch N, Jacques P, Fossoul A, Verheugen E, Vogel P, Beyaert R, Elewaut D, Kanneganti TD, van Loo G and Lamkanfi M: Negative regulation of the NLRP3 inflammasome by A20 protects against arthritis. Nature 512: 69-73, 2014.
21. McAllister MJ, Chemaly M, Eakin AJ, Gibson DS and McGilligan VE: NLRP3 as a potentially novel biomarker for the management of osteoarthritis. Osteoarthritis Cartilage 26: 612-619, 2018.
22. Chen Z, Zhong H, Wei J, Lin S, Zong Z, Gong F, Huang X, Sun J, Li P, Lin H, *et al*: Inhibition of Nrf2/HO-1 signaling leads to increased activation of the NLRP3 inflammasome in osteoarthritis. Arthritis Res Ther 21: 300, 2019.
23. Liu Q, Zhang D, Hu D, Zhou X and Zhou Y: The role of mitochondria in NLRP3 inflammasome activation. Mol Immunol 103: 115-124, 2018.
24. Zhou K, Cui S, Duan W, Zhang J, Huang J, Wang L, Gong Z and Zhou Y: Cold-inducible RNA-binding protein contributes to intracerebral hemorrhage-induced brain injury via TLR4 signaling. Brain Behav 10: e01618, 2020.
25. Elliott EI and Sutterwala FS: Initiation and perpetuation of NLRP3 inflammasome activation and assembly. Immunol Rev 265: 35-52, 2015.
26. Yu L, Li QH, Deng F, Yu ZW, Luo XZ and Sun JL: Synovial fluid concentrations of cold-inducible RNA-binding protein are associated with severity in knee osteoarthritis. Clin Chim Acta 464: 44-49, 2017.
27. Yoo IS, Lee SY, Park CK, Lee JC, Kim Y, Yoo SJ, Shim SC, Choi YS, Lee Y and Kang SW: Serum and synovial fluid concentrations of cold-inducible RNA-binding protein in patients with rheumatoid arthritis. Int J Rheum Dis 21: 148-154, 2018.
28. Felekkis K, Pieri M and Papaneophytou C: Exploring the feasibility of circulating miRNAs as diagnostic and prognostic biomarkers in osteoarthritis: Challenges and opportunities. Int J Mol Sci 24: 13144, 2023.
29. Lin Z, Jiang T, Zheng W, Zhang J, Li A, Lu C and Liu W: N6-methyladenosine (m6A) methyltransferase WTAP-mediated miR-92b-5p accelerates osteoarthritis progression. Cell Commun Signal 21: 199, 2023.
30. Mao G, Zhang Z, Hu S, Zhang Z, Chang Z, Huang Z, Liao W and Kang Y: Exosomes derived from miR-92a-3p-overexpressing human mesenchymal stem cells enhance chondrogenesis and suppress cartilage degradation via targeting WNT5A. Stem Cell Res Ther 9: 247, 2018.
31. Chen H, Yao H, Chi J, Li C, Liu Y, Yang J, Yu J, Wang J, Ruan Y, Pi J and Xu JF: Engineered exosomes as drug and RNA co-delivery system: New hope for enhanced therapeutics? Front Bioeng Biotechnol 11: 1254356, 2023.
32. Jiang M, Jike Y, Liu K, Gan F, Zhang K, Xie M, Zhang J, Chen C, Zou X, Jiang X, *et al*: Exosome-mediated miR-144-3p promotes ferroptosis to inhibit osteosarcoma proliferation, migration, and invasion through regulating ZEB1. Mol Cancer 22: 113, 2023.
33. Zhang X, Wang J, Liu N, Wu W, Li H, Lu W and Guo X: Umbilical cord Blood-derived M1 macrophage exosomes loaded with cisplatin target ovarian cancer in vivo and reverse cisplatin resistance. Mol Pharm 20: 5440-5453, 2023.
34. Hu G, Zhao X, Wang C, Geng Y, Zhao J, Xu J, Zuo B, Zhao C, Wang C and Zhang X: MicroRNA-145 attenuates TNF-α-driven cartilage matrix degradation in osteoarthritis via direct suppression of MKK4. Cell Death Dis 8: e3140, 2017.
35. Zhou J, Sun J, Markova DZ, Li S, Kepler CK, Hong J, Huang Y, Chen W, Xu K, Wei F and Ye W: MicroRNA-145 overexpression attenuates apoptosis and increases matrix synthesis in nucleus pulposus cells. Life Sci 221: 274-283, 2019.
36. Tian K, Deng B, Han X, Zheng H, Lin T, Wang Z, Zhang Y and Wang G: Over-expression of microRNA-145 elevating autophagy activities via downregulating FRS2 expression. Comb Chem High Throughput Screen 27: 127-135, 2024.
37. Wang X, Lu W, Xia X, Zhu Y, Ge C, Guo X, Zhang N, Chen H and Xu S: Selenomethionine mitigate PM2.5-induced cellular senescence in the lung via attenuating inflammatory response mediated by cGAS/STING/NF-κB pathway. Ecotoxicol Environ Saf 247: 114266, 2022.
38. Chen Z, Zhang M, Zhao Y, Xu W, Xiang F, Li X, Zhang T, Wu R and Kang X: Hydrogen sulfide contributes to uterine quiescence through inhibition of NLRP3 inflammasome activation by suppressing the TLR4/NF-κB signalling pathway. J Inflamm Res 14: 2753-2768, 2021.
39. Livak KJ and Schmittgen TD: Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) method. Methods 25: 402-408, 2001.
40. Wang S, Li W, Zhang P, Wang Z, Ma X, Liu C, Vasilev K, Zhang L, Zhou X, Liu L, *et al*: Mechanical overloading induces GPX4-regulated chondrocyte ferroptosis in osteoarthritis via Piezo1 channel facilitated calcium influx. J Adv Res 41: 63-75, 2022.
41. Gong T, Wang QD, Loughran PA, Li YH, Scott MJ, Billiar TR, Liu YT and Fan J: Mechanism of lactic acidemia-promoted pulmonary endothelial cells death in sepsis: Role for CIRP-ZBP1-PANoptosis pathway. Mil Med Res 11: 71, 2024.
42. Zhou M, Aziz M, Yen HT, Ma G, Murao A and Wang P: Extracellular CIRP dysregulates macrophage bacterial phagocytosis in sepsis. Cell Mol Immunol 20: 80-93, 2023.
43. Zhang P, Bai L, Tong Y, Guo S, Lu W, Yuan Y, Wang W, Jin Y, Gao P, Liu J, *et al*: CIRP attenuates acute kidney injury after hypothermic cardiovascular surgery by inhibiting PHD3/HIF-1α-mediated ROS-TGF-β1/p38 MAPK activation and mitochondrial apoptotic pathways. Mol Med 29: 61, 2023.
44. Ye L, Tang X, Liu F, Wei T, Xu T, Jiang Z, Xu L, Xiang C, Yuan X, Shen L, *et al*: Targeting CIRP and IL-6R-mediated microglial inflammation to improve outcomes in intracerebral hemorrhage. J Adv Res: Sep 9, 2025 doi: 10.1016/j.jare.2025.09.012 (Epub ahead of print).
45. Han J, Zhang Y, Ge P, Dakal TC, Wen H, Tang S, Luo Y, Yang Q, Hua B, Zhang G, *et al*: Exosome-derived CIRP: An amplifier of inflammatory diseases. Front Immunol 14: 1066721, 2023.
46. Palazzo C, Nguyen C, Lefevre-Colau MM, Rannou F and Poiraudeau S: Risk factors and burden of osteoarthritis. Ann Phys Rehabil Med 59: 134-138, 2016.
47. Carr AJ, Robertsson O, Graves S, Price AJ, Arden NK, Judge A and Beard DJ: Knee replacement. Lancet 379: 1331-1340, 2012.
48. Liu J, Wu X, Lu J, Huang G, Dang L, Zhang H, Zhong C, Zhang Z, Li D, Li F, *et al*: Exosomal transfer of osteoclast-derived miRNAs to chondrocytes contributes to osteoarthritis progression. Nat Aging 1: 368-384, 2021.
49. Sun W, Bergmeier AP, Liao Y, Wu S and Tong L: CIRP sensitizes cancer cell responses to ionizing radiation. Radiat Res 195: 93-100, 2021.
50. Zhang F, Yang WL, Brenner M and Wang P: Attenuation of hemorrhage-associated lung injury by adjuvant treatment with C23, an oligopeptide derived from Cold-inducible RNA-binding protein. J Trauma Acute Care Surg 83: 690-697, 2017.
51. Murao A, Tan C, Jha A, Wang P and Aziz M: Exosome-mediated eCIRP release from macrophages to induce inflammation in sepsis. Front Pharmacol 12: 791648, 2021.
52. Ying X, Peng L, Chen H, Shen Y, Yu K and Cheng S: Cordycepin prevented IL-β-induced expression of inflammatory mediators in human osteoarthritis chondrocytes. Int Orthop 38: 1519-1526, 2014.
53. Schmidt N, Pautz A, Art J, Rauschkolb P, Jung M, Erkel G, Goldring MB and Kleinert H: Transcriptional and post-transcriptional regulation of iNOS expression in human chondrocytes. Biochem Pharmacol 79: 722-732, 2010.
54. Stannus O, Jones G, Cicuttini F, Parameswaran V, Quinn S, Burgess J and Ding C: Circulating levels of IL-6 and TNF-α are associated with knee radiographic osteoarthritis and knee cartilage loss in older adults. Osteoarthritis Cartilage 18: 1441-1447, 2010.
55. Zhao Y, Li Y, Qu R, Chen X, Wang W, Qiu C, Liu B, Pan X, Liu L, Vasilev K, *et al*: Cortistatin binds to TNF-α receptors and protects against osteoarthritis. EBioMedicine 41: 556-570, 2019.

56. Zhou M, Aziz M, Denning NL, Yen HT, Ma G and Wang P: Extracellular CIRP induces macrophage endotoxin tolerance through IL-6R-mediated STAT3 activation. JCI Insight 5: e133715, 2020.
57. Denning NL, Aziz M, Murao A, Gurien SD, Ochani M, Prince JM and Wang P: Extracellular CIRP as an endogenous TREM-1 ligand to fuel inflammation in sepsis. JCI Insight 5: e134172, 2020.
58. Gomez R, Villalvilla A, Largo R, Gualillo O and Herrero-Beaumont G: TLR4 signalling in osteoarthritis-finding targets for candidate DMOADs. Nat Rev Rheumatol 11: 159-170, 2015.
59. Barreto G, Senturk B, Colombo L, Brück O, Neidenbach P, Salzmann G, Zenobi-Wong M and Rottmar M: Lumican is upregulated in osteoarthritis and contributes to TLR4-induced pro-inflammatory activation of cartilage degradation and macrophage polarization. Osteoarthritis Cartilage 28: 92-101, 2020.
60. Liu L, Gu H, Liu H, Jiao Y, Li K, Zhao Y, An L and Yang J: Protective effect of resveratrol against IL-1β-induced inflammatory response on human osteoarthritic chondrocytes partly via the TLR4/MyD88/NF-κB signaling pathway: An 'in vitro study'. Int J Mol Sci 15: 6925-6940, 2014.
61. Zhang A, Wang P, Ma X, Yin X, Li J, Wang H, Jiang W, Jia Q and Ni L: Mechanisms that lead to the regulation of NLRP3 inflammasome expression and activation in human dental pulp fibroblasts. Mol Immunol 66: 253-262, 2015.
62. Wang C, Gao Y, Zhang Z, Chen C, Chi Q, Xu K and Yang L: Ursolic acid protects chondrocytes, exhibits anti-inflammatory properties via regulation of the NF-κB/NLRP3 inflammasome pathway and ameliorates osteoarthritis. Biomed Pharmacother 130: 110568, 2020.
63. Dougherty U, Mustafi R, Zhu H, Zhu X, Deb D, Meredith SC, Ayaloglu-Butun F, Fletcher M, Sanchez A, Pekow J, *et al*: Upregulation of polycistronic microRNA-143 and microRNA-145 in colonocytes suppresses colitis and Inflammation-associated colon cancer. Epigenetics 16: 1317-1334, 2021.
64. Tang J, Yi S and Liu Y: Long non-coding RNA PVT1 can regulate the proliferation and inflammatory responses of rheumatoid arthritis fibroblast-like synoviocytes by targeting microRNA-145-5p. Hum Cell 33: 1081-1090, 2020.
65. Liu Z, Tao B, Fan S, Pu Y, Xia H and Xu L: MicroRNA-145 protects against myocardial ischemia reperfusion injury via CaMKII-Mediated antiapoptotic and Anti-inflammatory pathways. Oxid Med Cell Longev 2019: 8948657, 2019.
66. Li Y, Wu X, Miao S and Cao Q: MiR-383-5p promotes apoptosis of ovarian granulosa cells by targeting CIRP through the PI3K/AKT signaling pathway. Arch Gynecol Obstet 306: 501-512, 2022.
67. Lin TY, Jia JS, Luo WR, Lin XL, Xiao SJ, Yang J, Xia JW, Zhou C, Zhou ZH, Lin SJ, *et al*: ThermomiR-377-3p-induced suppression of Cirbp expression is required for effective elimination of cancer cells and cancer stem-like cells by hyperthermia. J Exp Clin Cancer Res 43: 62, 2024.