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EXPERIMENTAL / BASIC SCIENCE RESEARCH

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CTHRC1 inhibits the proliferation and promotes apoptosis of osteoarthritis chondrocytes by activating Wnt / ß-Catenin pathway

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ABSTRACT

This study aimed to elucidate the function of CTHRC1 and its linkage to the Wnt/β-catenin signaling pathway in the pathogenesis of osteoarthritis (OA), and to preliminarily explore whether a similar molecular interplay exists in rheumatoid arthritis (RA). We employed an integrated strategy combining bioinformatics, in vitro, and in vivo approaches. Bioinformatic screening of GEO-derived RNA-seq data identified CTHRC1 as a key differentially expressed gene in osteoarthritis. Its functional role was subsequently investigated in OA chondrocyte models, where we measured proliferation (via CCK-8 and EdU assays) and apoptosis (by Western blot analysis of Bax, Bcl-2, and Cleaved Caspase-3), along with key proteins in the Wnt/β-catenin pathway. Furthermore, to assess its relevance to inflammatory arthritis in vivo, we utilized a collagen-induced arthritis (CIA) rat model, evaluating clinical arthritis indices, inflammatory cytokine levels, and joint histopathology by HE staining. We found that CTHRC1 expression was significantly upregulated in OA tissues. Functional enrichment analysis indicated its close association with the Wnt/β-catenin signaling pathway. In vitro experiments confirmed increased CTHRC1 expression in IL-1β–induced OA chondrocytes, while knockdown of CTHRC1 effectively promoted cell proliferation and inhibited apoptosis. Mechanistic studies revealed that the protective effects of CTHRC1 knockdown were reversed by the Wnt/β-catenin pathway agonist BML-284, confirming that CTHRC1 mediates chondrocyte degeneration through activation of this pathway. Furthermore, intra-articular knockdown of CTHRC1 in a CIA rat model significantly alleviated joint swelling, reduced levels of inflammatory factors (IL-1β, IL-6, and TNF-α), and effectively mitigated synovial inflammation and collagen deposition. This study identifies CTHRC1 as an upregulated gene in OA and validates its role in promoting chondrocyte dysfunction. CTHRC1 knockdown reverses these effects by attenuating Wnt/β-catenin signaling, a mechanism confirmed by pharmacological rescue. Preliminary in vivo evidence further suggests a similar pathogenic role in RA. Collectively, CTHRC1 emerges as a potential therapeutic target in arthritis through modulation of the Wnt/β-catenin axis.

Keywords: Osteoarthritis, CTHRC1, Wnt/β-catenin, proliferation, apoptosis, Rheumatoid Arthritis.

INTRODUCTION

Osteoarthritis (OA) and Rheumatoid Arthritis (RA) are two common forms of disabling joint disease, characterized by distinct pathological features and treatment strategies. OA is primarily a degenerative disorder marked by progressive articular cartilage degradation and chondrocyte loss. Clinical management focuses on symptom relief, and there is a notable lack of effective therapies that halt disease progression[1,2]. In contrast, RA is an autoimmune-mediated inflammatory disease, with core pathological manifestations including synovitis, inflammatory cell infiltration, and joint bone erosion. Its treatment relies heavily on immunosuppressants and biologics to control inflammation[3].

Despite their differing etiologies, research has revealed shared pathological links between OA and RA at the molecular level. For instance, PPARγ in RA promotes autophagy to accelerate reactive oxygen species (ROS) clearance, thereby inhibiting ROS-mediated macrophage polarization and NLRP3 inflammasome activation[4]. Correspondingly, in OA, PPARγ agonists can similarly mitigate cartilage degeneration by reducing ROS generation and suppressing the mitochondrial apoptosis pathway[5]. Furthermore, the NF-κB signaling pathway plays a critical role in both diseases: Sappanone A exerts anti-inflammatory effects in RA models by inhibiting this pathway[6], while in OA chondrocytes, miR-485-3p promotes proliferation and reduces apoptosis by suppressing the NF-κB pathway[7]. These findings suggest significant convergence in molecular pathways between OA and RA, despite their differing clinical presentations.

Collagen triple helix repeat containing 1 (CTHRC1), a secreted glycoprotein involved in tissue repair and remodeling, is increasingly recognized for its role in arthritis pathogenesis[8,9]. Studies demonstrate that CTHRC1 is highly expressed in the cartilage and peripheral blood of OA patients and participates in IL-1β-induced chondrocyte apoptosis via regulation of the JNK1/2 signaling pathway[9]. In RA, CTHRC1 is abnormally expressed in serum and is closely associated with pannus formation and levels of inflammatory cytokines[10,11], suggesting it may be an important molecular link between the two arthritides. However, whether CTHRC1 shares a common signaling regulatory mechanism in both OA and RA remains to be elucidated.

The Wnt/β-catenin signaling pathway plays a crucial role in the pathogenesis of both OA and RA[12,13]. In OA, aberrant activation of this pathway directly inhibits chondrocyte proliferation, promotes apoptosis, and enhances matrix degradation[14,15]. In RA, its activation is closely linked to synovial cell hyperplasia, release of pro-inflammatory factors, and bone erosion[16]. The downstream effector molecules of this pathway, p-Smad1/5/8, have also been shown to play complex roles in cartilage and bone metabolism in arthritis[17,18]. Although some studies suggest CTHRC1 may regulate Wnt signaling in other systems[19], it remains unknown whether CTHRC1, Wnt/β-catenin, and p-Smad1/5/8 form a unified signaling axis in OA chondrocytes, and what role this axis plays in the inflammatory environment of RA.

Based on this background, this study aims to systematically clarify the common mechanism of action of CTHRC1 in OA and RA. We hypothesize that CTHRC1 drives chondrocyte dysfunction in OA and exacerbates inflammatory joint destruction in RA by activating the Wnt/β-catenin/p-Smad1/5/8 signaling axis. By integrating bioinformatic analysis, in vitro OA chondrocyte models, and an in vivo CIA rat arthritis model, this research not only reveals the core role of this novel CTHRC1-Wnt/β-catenin-p-Smad1/5/8 regulatory axis in arthritis but also provides experimental evidence for developing targeted therapeutic strategies across arthritis types.

METHODS

RNA-Seq Datasets

The Gene Expression Omnibus (GEO) database is a public repository for high-throughput genomic data, including microarrays and next-generation sequencing (NGS) datasets. In this study, we downloaded and analyzed the RNA-seq dataset GSE114007(20) from GEO, which comprises 20 human knee cartilage samples from osteoarthritis (OA) patients and 18 samples from normal cartilage tissues.

Analysis of differentially expressed genes (DEGs)

To identify differentially expressed genes (DEGs) between normal and osteoarthritis (OA) groups, we performed analysis using the DESeq2 R package. Genes with an adjusted p-value ≤ 0.05 and an absolute log2 fold change ($|\log2FC|$) ≥ 1 were considered statistically significant[21].

Enrichment analysis of DEGs

Functional enrichment analysis of DEGs was performed using the Database for Annotation, Visualization, and Integrated Discovery (DAVID). DAVID provides comprehensive functional annotation tools for the systematic analysis of large gene lists. Gene Ontology (GO) biological process terms with a p-value < 0.05 were considered statistically significant.

Cell cultures and osteoarthritis model

The mouse chondrocyte line ATDC5 was obtained from the American Type Culture Collection (ATCC, USA) and maintained in DMEM/F12 medium supplemented with 10% fetal bovine serum. Cells were cultured at 37°C in a humidified incubator with 5% CO_2. To establish an in vitro OA model, cells were treated with 10 ng/ml interleukin-1β (IL-1β; PeproTech, USA) for 24 hours. A control group (NC) was established by adding an equivalent volume of phosphate-buffered saline (PBS) to the culture medium.

Cell transfection and treatment

Chondrocytes in the logarithmic growth phase were transfected with sh-NC (negative control) or sh-CTHRC1 using Lipofectamine 3000. Following successful transfection, cells were treated with the Wnt/β-catenin agonist BML-284 (1 μM, MedChem Express, HY-19987) for 24 hours.

RT-qPCR assay

Total RNA was extracted from cells using TRIzol reagent according to the manufacturer's protocol. Complementary DNA (cDNA) was synthesized from the extracted RNA using a reverse transcription kit. Quantitative real-time PCR (qPCR) was performed with SYBR Green Master Mix following the manufacturer's instructions. All primers were designed and synthesized by Shanghai Sangon Bioengineering Co., Ltd. GAPDH served as the internal control, and relative gene expression levels were calculated using the $2^{(-\Delta\Delta Ct)}$ method.

CCK-8 assay for Cell Proliferation

Cells from each group in the logarithmic growth phase were seeded into 96-well plates at a density of 2×10^4 cells per well. After 24 hours of culture, 10 μl of CCK-8 solution was added to each well and incubated for 1 hour. The absorbance at 450 nm was then measured using a microplate reader. All experiments were performed with three biological replicates.

Western Blot assay

Total protein was extracted from chondrocytes using RIPA lysis buffer, and protein concentration was determined with a BCA assay kit. Subsequently, 40 μg of protein per sample was separated by SDS-PAGE and transferred onto a PVDF membrane. The membrane was blocked with 5% skim milk for 2 hours at room temperature and then incubated with the following primary antibodies at 4°C for 24 hours: Bcl-2 (SAB, 40639), Bax (Proteintech, 50599-2-Ig), cleaved caspase-3 (Cell Signaling Technology, #9664), p-Smad1/5/8 (CST, 13820), Smad1/5/8 (Abcam, ab13723), β-catenin (Abcam, ab32572), and GAPDH (Abcam, ab8245). After incubation, the membrane was washed three times with TBST and incubated with an HRP-conjugated secondary antibody for 1 hour at room temperature. Following additional TBST washes, protein bands were visualized using an ECL substrate, and band intensities were quantified with ImageJ software.

EdU proliferation assay

Chondrocytes from different experimental groups were seeded into 6-well plates at a density of 2×10^5 cells per well and cultured for 24 hours. The culture medium was then replaced with medium containing 10 μmol/L 5-ethynyl-2'-deoxyuridine (EdU; Beyotime, China) and incubated for an additional 24 hours. Following incubation, cells were fixed with 4% paraformaldehyde for 15 minutes at room temperature. Cell nuclei were counterstained with Hoechst stain (Invitrogen), and EdU incorporation was visualized using fluorescence microscopy (IX73, Olympus).

Establishment of the CIA (Collagen-Induced Arthritis) rat model

All animal experimental protocols were reviewed and approved by the Animal Ethics and Welfare Committee (AEWC) of Yan'an Hospital Affiliated to Kunming Medical University (Approval No.:2025049), and all procedures were strictly performed in accordance with relevant animal welfare guidelines. Male Sprague-Dawley (SD) rats aged 6-8 weeks and weighing 180-220 g were randomly assigned using a computer-generated random number table into three groups (n=3 per group): (1) blank control group, (2) CIA model group, and (3) CIA + Ad-shRNA-CTHRC1 treatment group. All animals were individually housed under standard conditions (temperature 22°C, humidity 50%, 12-hour light/dark cycle) with free access to food and water. Bovine type II collagen was dissolved in 0.05 mol/L acetic acid and emulsified with an equal volume of Freund's incomplete adjuvant (IFA). On day 1, rats received a subcutaneous injection at the tail base with 2 mg/kg of the collagen-IFA emulsion, except for the positive control and treatment group 3, which received 1.5 mg/kg. A booster injection was administered on day 7 using 1 mg/kg of the emulsion for most groups, with adjustments for specific groups: 0.5 mg/kg for the positive control and treatment group 3, and 0.7 mg/kg for treatment groups 1–2. Control animals received an equivalent volume of sterile saline. Prior to the initial immunization, baseline ankle joint volume was recorded for all animals. Following induction, ankle joint circumference was measured and documented photographically on days 7, 14, 21, and 28. Joint swelling was quantified by calculating the swelling index relative to baseline measurements. To minimize potential bias, the researchers responsible for disease induction were blinded to group assignments until the animals developed clinical signs of disease. Furthermore, all subsequent histological assessments and molecular analyses were conducted by investigators

who remained blinded to the experimental groups throughout the evaluation process.

Treatment with Ad-shRNA-CTHRC1 viral vector in CIA rats

On days 14 and 21 post-immunization, CIA rats were randomly allocated to the following treatment groups: Ad-shRNA-CTHRC1 group: rats received intra-articular injections of 2×10^8 PFU Ad-shRNA-CTHRC1 viral vector in both ankle joints; Control shRNA group: rats were injected with the same volume of control shRNA vector in both ankle joints; PBS control group: rats received an equal volume of PBS in both ankle joints. All animals were euthanized on day 28 for subsequent analysis.

Arthritis index assessment

Arthritis severity in the hind paws and ankle joints of rats was evaluated every three days by two independent observers using a standardized scoring system. The assessment was based on the following criteria: 0: No swelling or erythema; 1: Mild swelling confined to the ankle joint; 2: Edema extending to the dorsal paw surface; 3: Severe swelling involving both the ankle and paw; 4: Joint stiffness, deformity, or ankylosis.

Enzyme-linked immunosorbent assay (ELISA) for serum cytokines

On day 28, rats were anesthetized and blood samples were collected from the abdominal aorta. After clotting at room temperature, samples were centrifuged at 3,000 rpm for 20 min to obtain serum. Serum concentrations of IL-1β, IL-6, and TNF-α were quantified using commercial ELISA kits (Mlbio, China; IL-1β: Cat# ml037361, IL-6: Cat# ml064292, TNF-α: Cat# ml002859) according to the manufacturer's protocols. All reagents were prepared as instructed, and the assay procedure included sample incubation and color development steps. Absorbance was measured at 450 nm using a microplate reader, and cytokine concentrations were determined based on standard curves prepared with reference standards.

Hematoxylin and eosin (H&E) staining

On day 28, rats were euthanized and ankle joints were collected and fixed in 4% paraformaldehyde at room temperature for 48 hours. Tissues were then decalcified in 10% EDTA (pH 7.4) for four weeks, with the decalcification solution replaced every three days. Following decalcification, samples were dehydrated through a graded ethanol series, embedded in paraffin, and sectioned at 5 μm thickness. Tissue sections were stained with hematoxylin and eosin (H&E) for histological evaluation of synovial pathology. Stained sections were imaged and analyzed using light microscopy.

Masson's trichrome staining

Paraffin-embedded synovial tissue sections (5 μm thickness) were deparaffinized and rehydrated through a graded ethanol series. The staining procedure was performed as follows: sections were first stained with Weigert's iron hematoxylin for 5-10 minutes, briefly differentiated in acidic ethanol for 5-15 seconds, and rinsed in distilled water. Subsequently, sections were treated with Masson blueing solution for 3-5 minutes and rinsed again. Cytoplasmic staining was performed using Ponceau-Fuchsin solution for 5-10 minutes, followed by distilled water rinsing. Sections were then washed with weak acid solution for 1 minute, treated with phosphomolybdic acid solution for 1-2 minutes, and rinsed again with weak acid solution. Collagen was specifically stained by immersion in aniline blue solution for 1-2 minutes, followed by weak acid solution rinsing. Dehydration was achieved through sequential immersion in 95% ethanol (2-3 seconds) and absolute ethanol (two changes of 5-10 seconds each), followed by clearing in xylene (two changes of 1 minute each). Finally, sections were mounted with neutral balsam and air-dried. Stained sections were examined under a light microscope to evaluate synovial hyperplasia, bone and cartilage erosion, and inflammatory cell infiltration.

Statistical analysis

Statistical analyses were performed using GraphPad Prism software (version 8.0). Data are presented as the mean ± standard deviation (SD). For comparisons between two groups, an unpaired two-tailed Student's t-test was used. For comparisons among more than two groups, one-way analysis of variance (ANOVA) was applied, followed by an appropriate post-hoc test (e.g., Tukey's test) for multiple comparisons. A p-value of less than 0.05 was considered statistically significant.

RESULTS

Identification and functional enrichment of differentially expressed genes in OA

Analysis of RNA-seq data identified hundreds of significantly differentially expressed genes (DEGs) between osteoarthritis (OA) and normal cartilage samples, as shown in the heatmap (Figure 1A). Gene Ontology (GO) enrichment analysis indicated

that upregulated DEGs in OA were significantly associated with biological processes including skeletal system development, regulation of cell adhesion, extracellular matrix organization, negative regulation of cell differentiation, vasculature development, positive regulation of cell death, and mitotic cell cycle progression (Figure 1B). In contrast, downregulated DEGs were primarily enriched in fundamental biological processes such as development, biological adhesion, cellular processes, and multicellular organismal processes (Figure 1B).

To identify key genes involved in these processes, we analyzed the DEGs and found that CTHRC1 expression was significantly upregulated in OA tissues compared to normal controls (Figure 2A). Further investigation into the functional role of CTHRC1 through canonical pathway, GO biological process, and WikiPathways analyses revealed its significant enrichment in pathways including malignant pleural mesothelioma and Wnt signaling pathway (Figure 2B).

The upregulation of CTHRC1 in an in vitro OA model is accompanied by suppressed proliferation and enhanced apoptosis

To investigate the role of CTHRC1 in OA, an in vitro OA model was established by stimulating chondrocytes

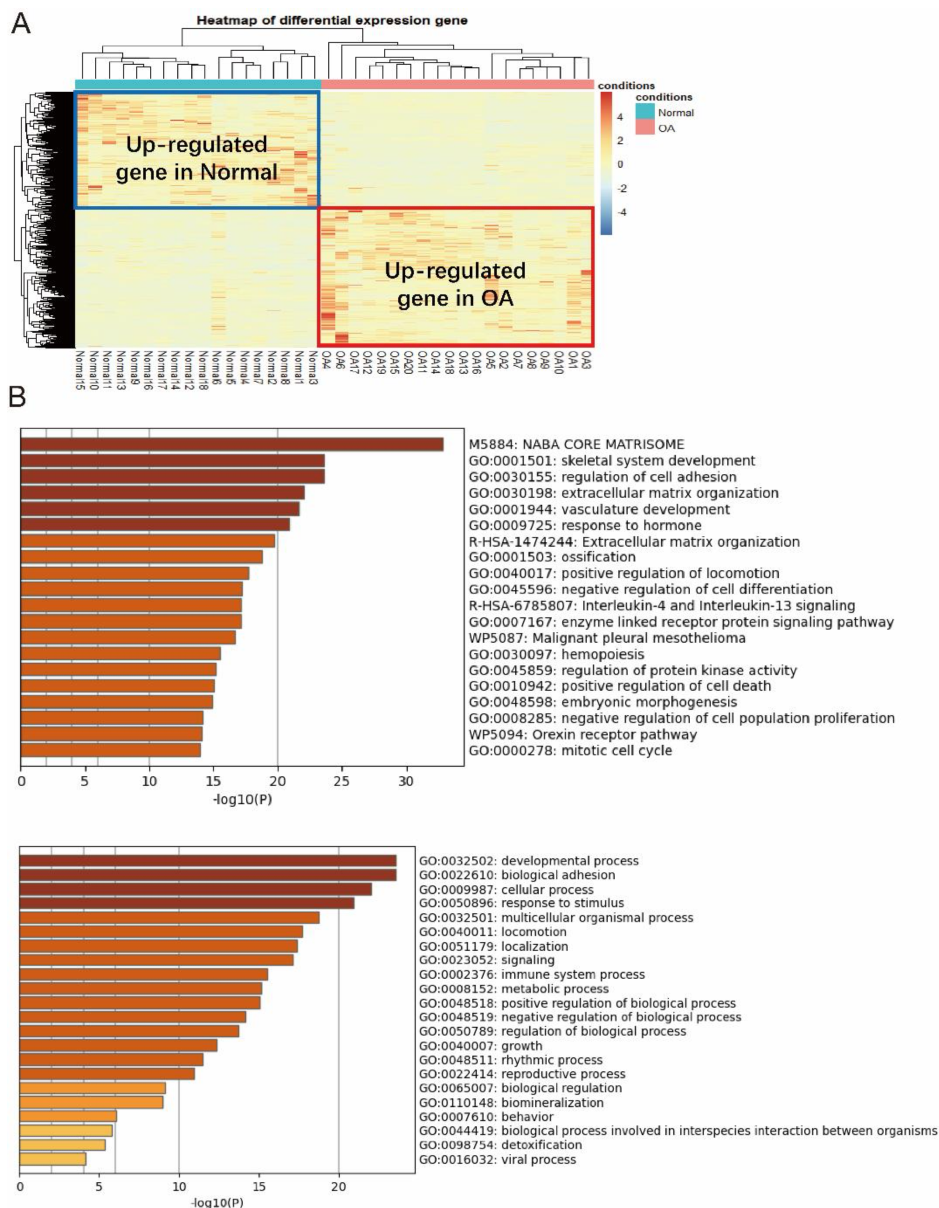

Fig. 1 — Identification and functional enrichment of DEGs. (A) Heatmap of DEGs in OA group and normal group. (B) A bar plot depicting the prominent GO terms associated with upregulated and downregulated genes in individuals with OA.

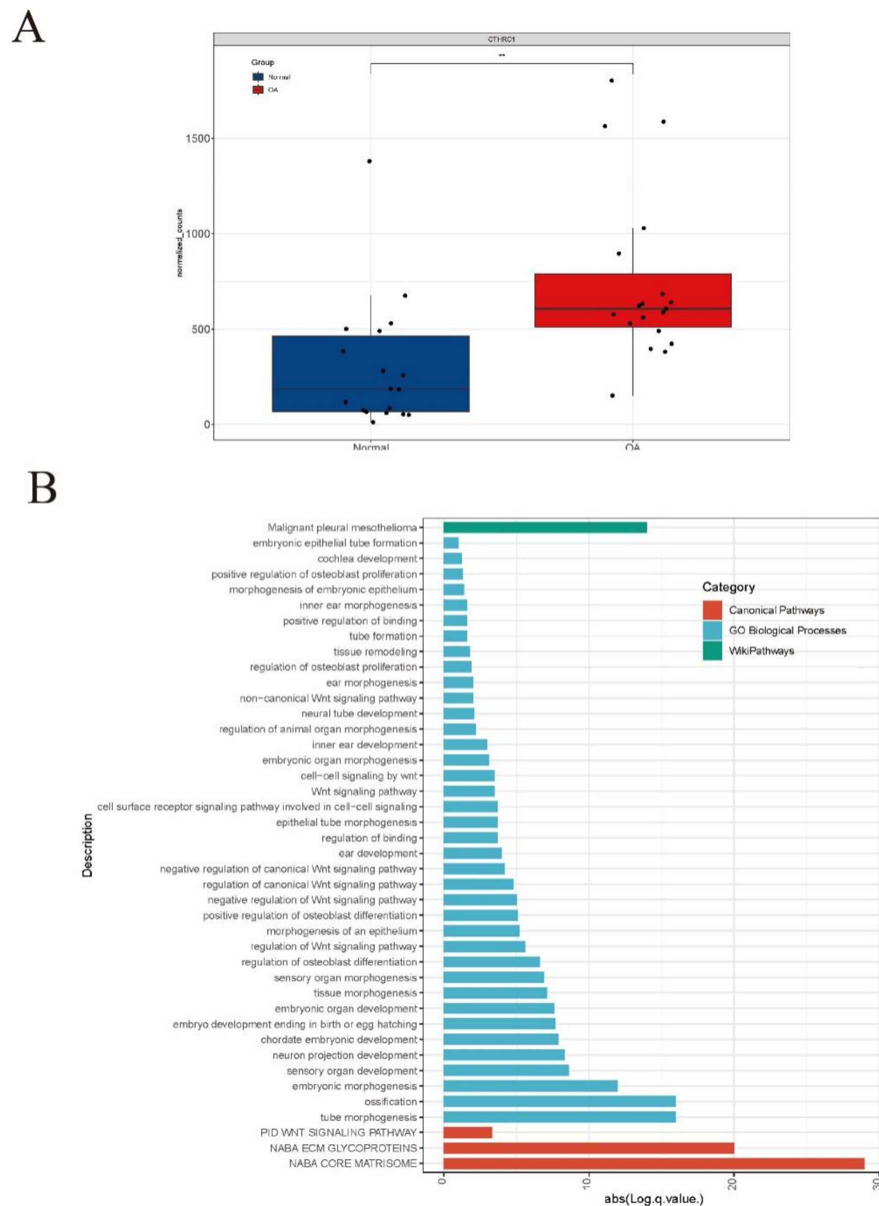

*Fig. 2 — CTHRC1 was up-regulated in OA tissues. (A) Expression levels of CTHRC1 in human OA cartilage tissues compared to normal controls (n=18 OA, n=18 normal, **p < 0.01). (B) The single gene functional enrichment analysis of CTHRC1.*

with IL-1β. In this model, IL-1β treatment led to a significant reduction in cell viability (Figure 3A) and decreased proliferation (Figure 3B). Concurrently, the apoptosis rate was markedly increased compared to the normal control (NC) group (Figure 3C). Western blot analysis of apoptosis-related proteins showed a substantial upregulation of Bax and cleaved caspase-3 expression, along with a significant downregulation of Bcl-2 in IL-1β-treated cells (Figure 3D). We next examined whether CTHRC1 expression was altered under these OA-mimicking conditions. Both protein and mRNA levels of CTHRC1 were found to be significantly elevated following IL-1β stimulation, as demonstrated by western blot and RT-qPCR analyses (Figure 3E, 3F). These results indicate that CTHRC1 expression is upregulated in IL-1β-induced chondrocyte injury, suggesting a potential association of CTHRC1 with the dysregulated cell viability and apoptosis observed in this in vitro OA model.

Suppression of CTHRC1 enhances chondrocyte viability and mitigates apoptosis in an OA model

To investigate the role of CTHRC1 in regulating chondrocyte proliferation and apoptosis in OA, we suppressed CTHRC1 expression by transfecting cells

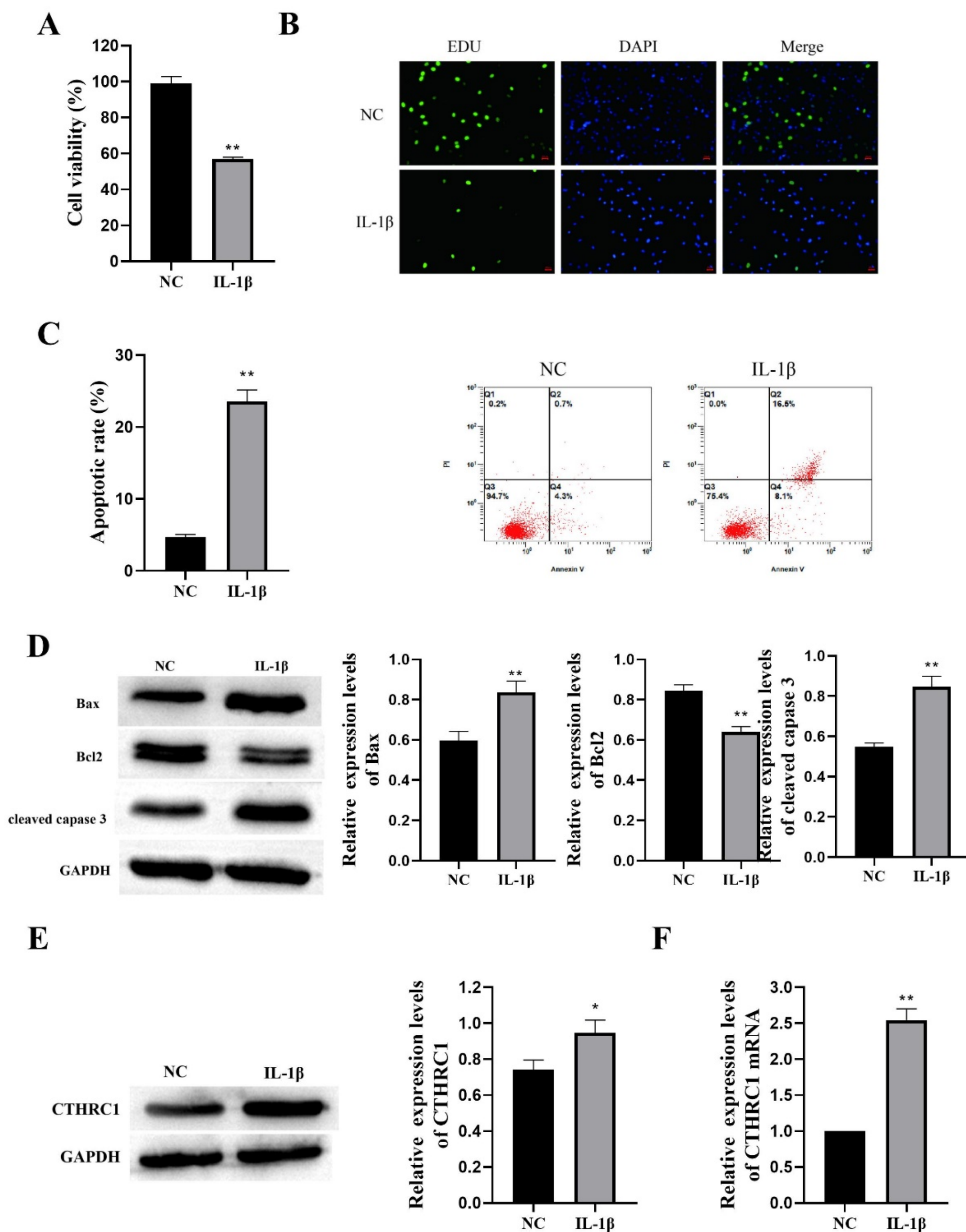

Fig. 3 — The upregulation of CTHRC1 in an in vitro OA model is accompanied by suppressed proliferation and enhanced apoptosis. (A) Cell viability measured by CCK-8 assay in control and IL-1β-treated chondrocytes ($p < 0.01$). (B) Cell proliferation assessed by EdU assay (scale bar: 20 μm). (C) Apoptosis rate quantified by flow cytometry ($p < 0.01$). (D) Western blot analysis of apoptosis-related proteins Bax, Bcl-2 and cleaved caspase-3 ($p < 0.01$). (E) Western blot analysis of CTHRC1 protein expression ($p < 0.05$). (F) CTHRC1 mRNA expression detected by RT-qPCR ($p < 0.01$).*
All data are presented as mean ± SEM from three independent experiments and were analyzed using unpaired Student's t-test. GAPDH was used as a loading control for western blot analyses.

with sh-CTHRC1. Transfection effectively reduced both CTHRC1 protein and mRNA levels (Figure 4A, 4B). Subsequent functional assays demonstrated that CTHRC1 knockdown increased cell viability, as measured by CCK-8 assay (Figure 4C), and enhanced cell proliferation, as evidenced by EdU staining (Figure 4D). Moreover, flow cytometric analysis revealed that sh-CTHRC1 significantly reduced chondrocyte apoptosis compared with control groups (Figure 4E). Consistent with these phenotypic changes, western blot analysis of apoptosis-related proteins showed that CTHRC1 knockdown substantially downregulated

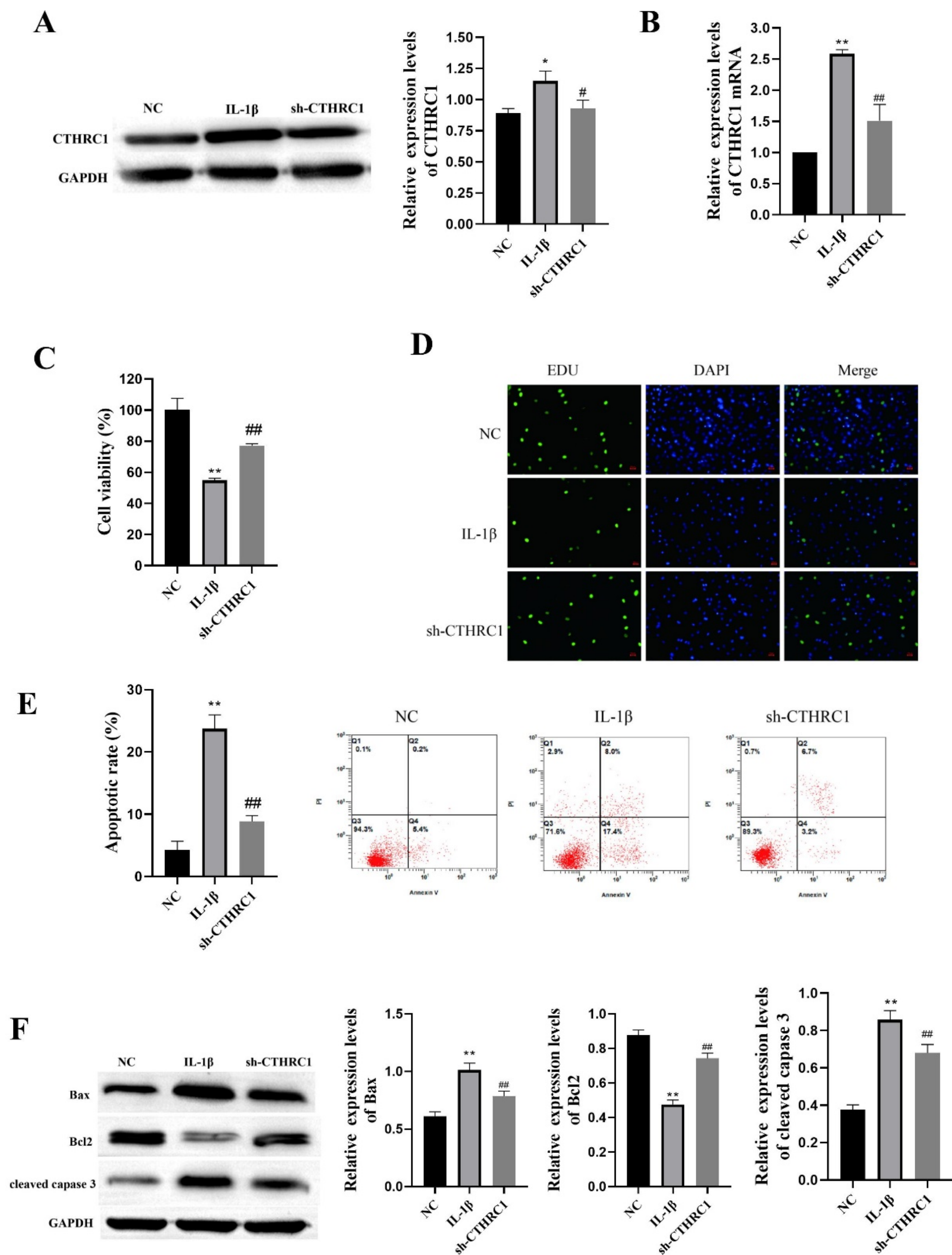

*Fig. 4 — Suppression of CTHRC1 enhances chondrocyte viability and mitigates apoptosis in an OA model. (A) Western blot analysis of CTHRC1 protein expression (*P < 0.05 for NC vs. IL-1β; #P < 0.05 for IL-1β vs. sh-CTHRC1). (B) CTHRC1 mRNA expression measured by RT-qPCR (**P < 0.01 for NC vs. IL-1β; ##P < 0.01 for IL-1β vs. sh-CTHRC1). (C) Cell viability assessed by CCK-8 assay (**P < 0.01 for NC vs. IL-1β; ##P < 0.01 for IL-1β vs. sh-CTHRC1n). (D) Cell proliferation evaluated by EdU assay (scale bar: 20 μm). (E) Apoptosis rate determined by flow cytometry (**P < 0.01 for NC vs. IL-1β; ##P < 0.01 for IL-1β vs. sh-CTHRC1). (F) Western blot analysis of apoptosis-related proteins Bax, Bcl-2 and cleaved caspase-3 (**P < 0.01 for NC vs. IL-1β; ##P < 0.01 for IL-1β vs. sh-CTHRC1).*

All data are expressed as mean ± SEM from three independent experiments and were analyzed by ordinary one-way ANOVA. GAPDH was used as a loading control for western blot analyses.

the expression of the pro-apoptotic proteins Bax and cleaved caspase-3, while upregulating the anti-apoptotic protein Bcl-2 relative to the IL-1β-treated group (Figure 4F). Taken together, these data indicate that suppression of CTHRC1 promotes proliferation and attenuates apoptosis in OA chondrocytes, supporting a functional role for CTHRC1 in these processes.

CTHRC1 knockdown attenuates Wnt/ ß-catenin-associated signaling in an OA model

Previous studies have indicated that CTHRC1 can modulate the Wnt/β-catenin signaling pathway(22). To further investigate whether CTHRC1 regulates the Wnt/β-catenin pathway in our OA model, we examined key downstream signaling molecules. Western blot analysis showed that knockdown of CTHRC1 significantly decreased protein levels of p-Smad1/5/8 and β-catenin in OA chondrocytes (Figure 5A).

Activation of Wnt/ß-catenin signaling reverses the protective effects of CTHRC1 knockdown in OA chondrocytes

To investigate whether the observed effects of CTHRC1 are mediated through mechanisms involving the Wnt/β-catenin signaling pathway, we performed a rescue experiment using the pathway-specific agonist BML-284 in CTHRC1-knockdown OA chondrocytes (sh-CTHRC1 + BML-284). First, we confirmed that BML-284 treatment did not significantly alter CTHRC1 expression itself (Figure 6A, 6B), suggesting its action is likely downstream of CTHRC1. Specifically, BML-284 significantly attenuated the promotive effect of sh-CTHRC1 on cell proliferation, as measured by both CCK-8 (Figure 6C) and EdU assays (Figure 6D). In parallel, BML-284 reversed the reduction in apoptosis caused by sh-CTHRC1, leading to a marked increase in the apoptosis rate as assessed by flow cytometry (Figure 6E). Consistent with these phenotypic observations, Western blot analysis of apoptosis-related proteins showed that BML-284 treatment reversed the changes induced by sh-CTHRC1, increasing the expression of pro-apoptotic proteins Bax and cleaved caspase-3 while decreasing the level of the anti-apoptotic protein Bcl-2 (Figure 6F). To assess the underlying signaling activity at the protein level, Western blot analysis showed that the decreases in β-catenin and p-Smad1/5/8 levels resulting from CTHRC1 knockdown were also restored upon BML-284 treatment (Figure 6G). Taken together, the rescue of both functional phenotypes and associated signaling molecule levels by BML-284 supports a model in which CTHRC1 contributes to the dysregulation of chondrocyte proliferation and apoptosis in OA, at least in part, through mechanisms associated with the Wnt/β-catenin signaling axis.

Preliminary assessment of the potential ameliorative role of CTHRC1 knockdown in a CIA rat model

To explore the potential role of CTHRC1 in rheumatoid arthritis (RA) pathogenesis, we performed a preliminary in vivo study using a collagen-induced arthritis (CIA) rat model. As this was an exploratory study with a sample size of n = 3 per group, the following conclusions are preliminary in nature. Western blot analysis indicated elevated CTHRC1 protein expression in the joints of CIA rats compared to control animals (Figure 7G). This expression was effectively reduced by intra-articular injection of Ad-

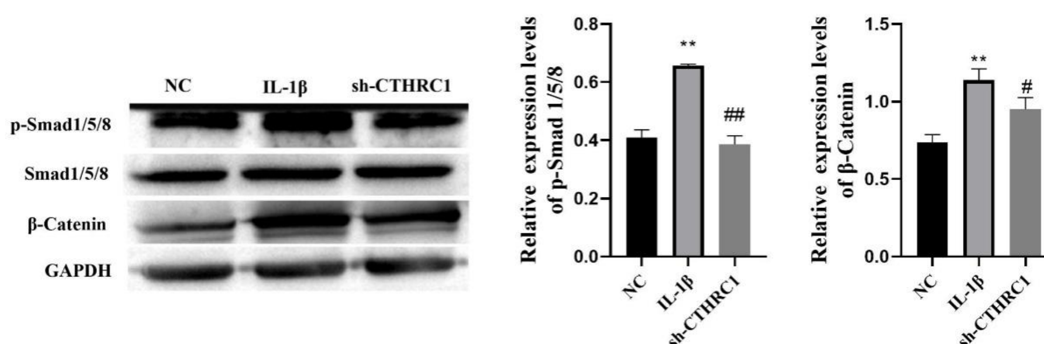

*Fig. 5 — CTHRC1 knockdown attenuates Wnt/β-catenin-associated signaling in an OA model. (A) Western blot analysis of p-Smad1/5/8 and β-catenin protein expression in OA chondrocytes (**P < 0.01 for NC vs. IL-1β; ##P < 0.01 for IL-1β vs. sh-CTHRC1). All data are expressed as mean ± SEM from three independent experiments and were analyzed by ordinary one-way ANOVA. GAPDH was used as a loading control for western blot analyses.*

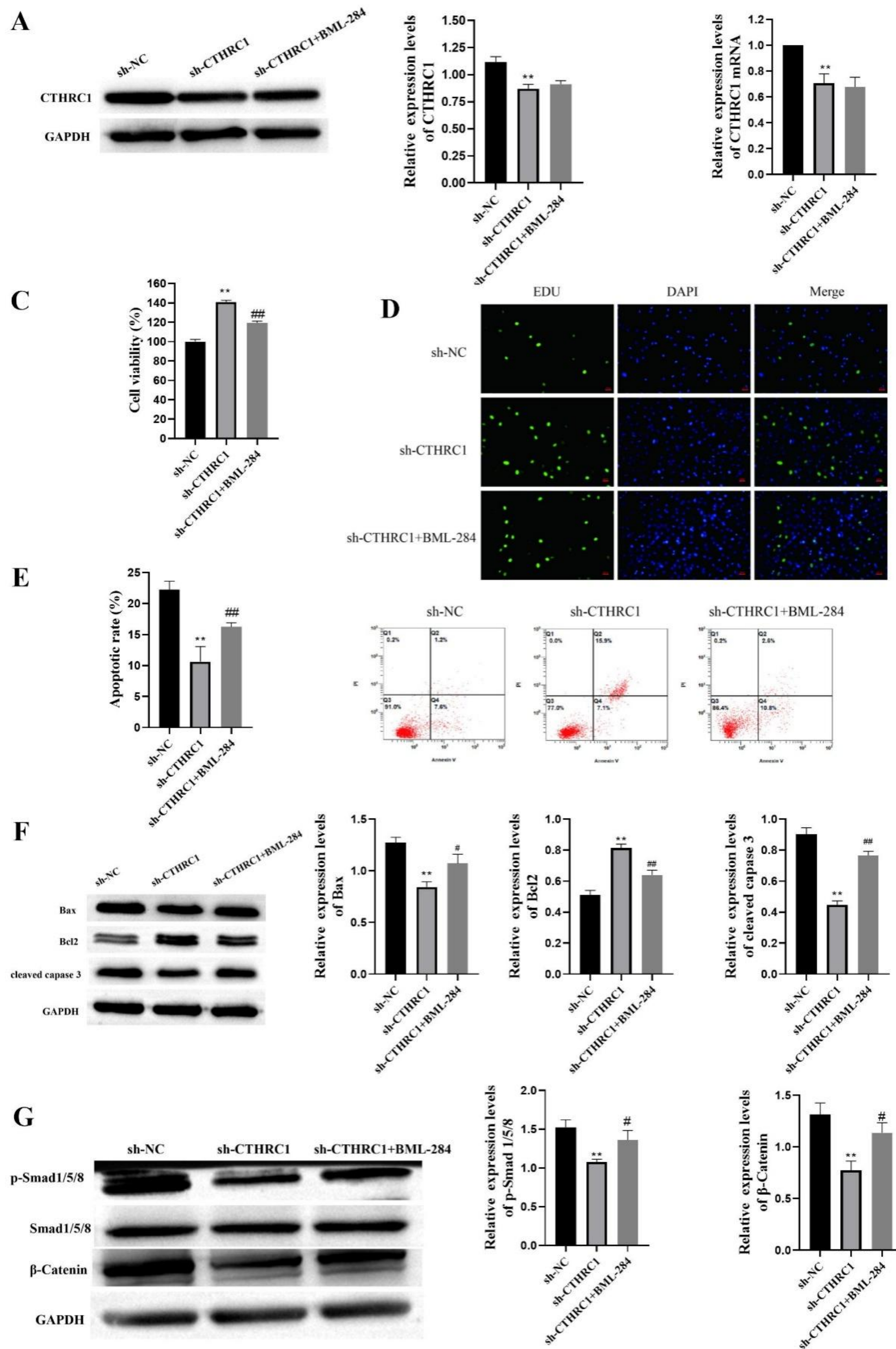

*Fig. 6 — Activation of Wnt/β-catenin signaling reverses the protective effects of CTHRC1 knockdown in OA chondrocytes. (A)Western blot analysis of CTHRC1 protein expression in OA chondrocytes (**P < 0.01 for sh-NC vs. sh-CTHRC1; NS for sh-CTHRC1 vs. sh-CTHRC1+BML-284). (B) CTHRC1 mRNA expression measured by RT-qPCR (**P < 0.01 for sh-NC vs. sh-CTHRC1; NS for sh-CTHRC1 vs. sh-CTHRC1+BML-284). (C) Cell viability assessed by CCK-8 assay (**P < 0.01 for sh-NC vs. sh-CTHRC1; ##p < 0.01 for sh-CTHRC1 vs. sh-CTHRC1+BML-284). (D) Cell proliferation evaluated by EdU assay (scale bar: 25 μm). (E) Apoptosis rate determined by flow cytometry (**P < 0.01 for sh-NC vs. sh-CTHRC1; ##p < 0.01 for sh-CTHRC1 vs.sh-CTHRC1+BML-284). (F) Western blot analysis of apoptosis-related proteins Bax, Bcl-2 and cleaved caspase-3 (**P < 0.01 for sh-NC vs. sh-CTHRC1; ##p < 0.01 or #p < 0.05 for sh-CTHRC1 vs. sh-CTHRC1+BML-284). (G) Western blot analysis of p-Smad1/5/8 and β-catenin protein expression (**P < 0.01 for sh-NC vs. sh-CTHRC1; #p < 0.05 for sh-CTHRC1 vs.sh-CTHRC1+BML-284).*
All data are expressed as mean ± SEM from three independent experiments and were analyzed by ordinary one-way ANOVA. GAPDH was used as a loading control for western blot analyses.

shRNA-CTHRC1 (Figure 7G). In this preliminary assessment, CTHRC1 knockdown was associated with several positive outcomes: reduced arthritis index scores (Figure 7A), attenuated joint swelling (Figure 7B), and decreased serum levels of the pro-inflammatory cytokines IL-1β, IL-6, and TNF-α (Figure 7C). Histological examination via H&E staining suggested that CTHRC1 silencing may alleviate joint structural disruption and inflammatory cell infiltration (Figure 7D). Masson's trichrome staining further indicated a reduction in collagen fiber deposition and joint fibrosis following CTHRC1 knockdown (Figure 7E). Additionally, western blot analysis showed that CTHRC1 downregulation was associated with inhibited activity of the Wnt/β-catenin signaling pathway in joint tissues (Figure 7F).

DISCUSSION

Osteoarthritis is an age-related disease that mainly affects individuals over the age of 65, and significantly impacts their quality of life[23]. This

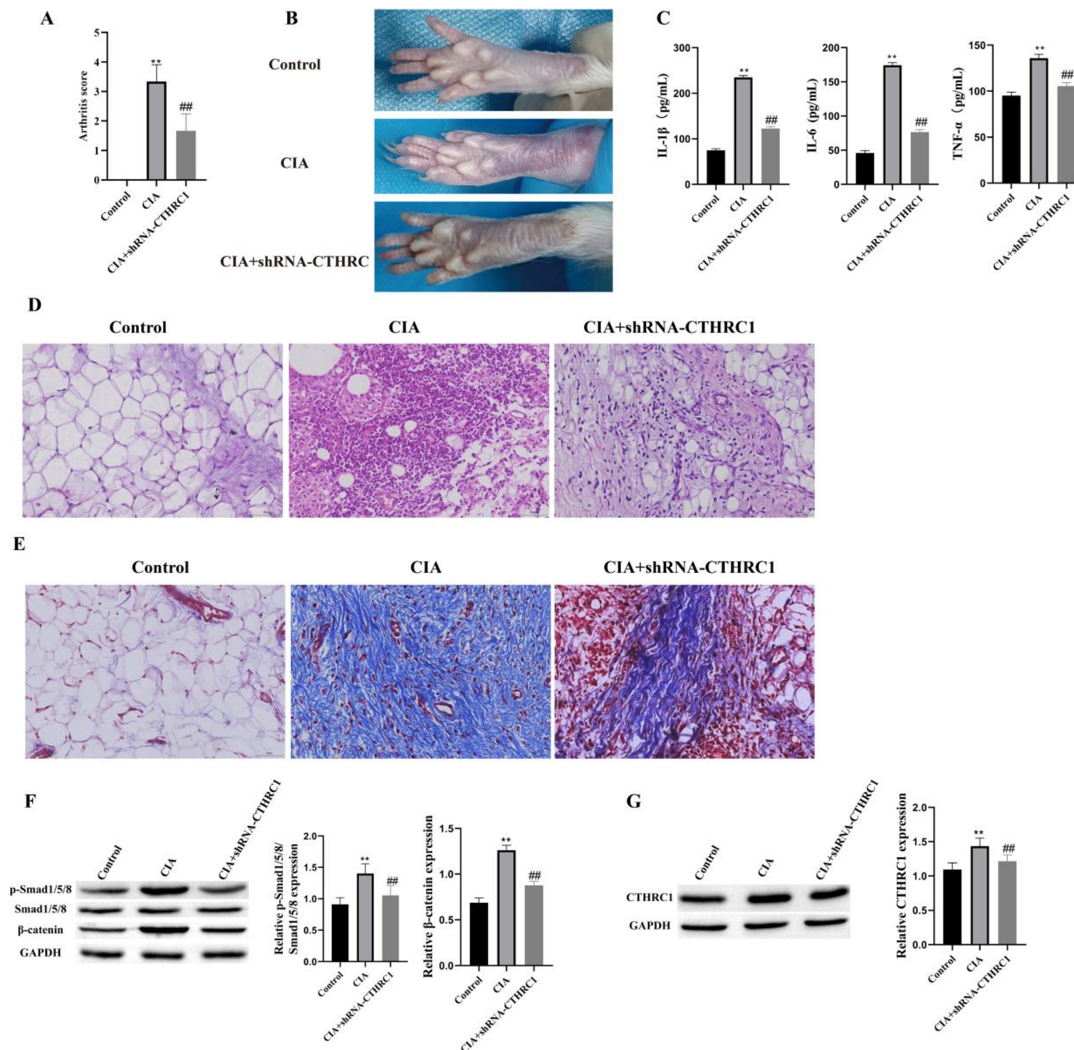

*Fig. 7 — Preliminary assessment of the potential ameliorative role of CTHRC1 knockdown in a CIA rat model. (A) Arthritis index scores recorded every three days throughout the study (**p < 0.01 for Control vs. CIA; ##p < 0.01 for CIA vs. CIA+shRNA-CTHRC1). (B) Representative photographs of hind paw morphology from each experimental group. (C) Serum levels of IL-1β, IL-6, and TNF-α measured by ELISA (**p < 0.01 for Control vs. CIA; ##p < 0.01 for CIA vs. CIA+shRNA-CTHRC1). (D) Histopathological evaluation of synovial tissue by H&E staining (scale bar: 50 μm). (E) Collagen deposition assessed by Masson's trichrome staining (scale bar: 50 μm). (F) Western blot analysis of p-Smad1/5/8 and β-catenin protein expression in joint tissues (**p < 0.01 for Control vs. CIA; ##p < 0.01 for CIA vs. CIA+shRNA-CTHRC1). (G) Western blot analysis of CTHRC1 protein expression in joint tissues (**p < 0.01 for Control vs. CIA; ##p < 0.01 for CIA vs. CIA+shRNA-CTHRC1).*
All data are expressed as mean ± SEM from three independent experiments and were analyzed by ordinary one-way ANOVA. GAPDH was used as a loading control for western blot analyses.

disease is characterized by cartilage destruction, osteophyte formation and synovial hyperplasia, and the main characteristics of soft bone degradation are up-regulation of stromal degrading enzymes and extracellular matrix[24]. Current treatments aim to alleviate patient symptoms, but effective therapeutic options are limited. Therefore, elucidating the pathogenesis of knee osteoarthritis has an important role in the treatment of knee osteoarthritis. As one of the cellular components of articular cartilage, chondrocytes play an important role in maintaining the integrity of the articular cartilage matrix[25]. In the pathological process of knee osteoarthritis, chondrocytes suffer from apoptosis, and then show up-regulation of stroma-degrading enzymes and loss of extracellular matrix, thus promoting cartilage thinning. Cell survival plays a key role in the remission of knee osteoarthritis[26]. In this study, we found the that DEGs between OA and normal were significantly enriched in the skeletal system development, regulation of cell adhesion, positive regulation of cell proliferation and multicellular organismal process. Additionally, we observed higher expression of CTHRC1 in OA group. Our in vitro assays demonstrated that CTHRC1 inhibits proliferation and promoted apoptosis of OA chondrocytes by activating the Wnt/β-catenin. Therefore, CTHRC1 represents a promising preclinical therapeutic target for osteoarthritis, though its clinical translation will require further validation in disease-specific models and subsequent clinical studies.

CTHRC1 is a secreted protein, which can repair vascular injury and participate in vascular remodeling. Recent studies have shown that CTHRC1 is abnormally expressed in various solid tumors, including gastric cancer, breast cancer and colorectal cancer[27,28]. Overexpression of CTHRC1 promotes tumor development through a variety of signaling pathways, including angiogenic tumor-associated macrophage invasion and epithelial-mesenchymal transformation[29]. CTHRC1 overexpression promotes ectopic endometrial stromal cell proliferation, migration, and invasion via activation of the Wnt/β-catenin pathway[30]. We found that the expression of CTHRC1 was significantly up-regulated in OA tissues and chondrocytes. In this study, we found that knockdown of CTHRC1 significantly promoted the proliferation of OA chondrocytes and inhibited apoptosis.

The Wnt/β-catenin signaling pathway is a critical regulator of chondrocyte homeostasis, influencing a wide range of cellular processes including proliferation, differentiation, and apoptosis[31] . Its dysregulation is increasingly recognized as a key factor in osteoarthritis (OA) pathogenesis. While previous studies, such as that by Lu et al.[32]), have highlighted the role of certain compounds in promoting chondrocyte proliferation via this pathway, the upstream regulators that drive its aberrant activation in OA remain incompletely understood. Our findings identify CTHRC1 as a novel upstream activator of the Wnt/β-catenin pathway in OA chondrocytes. We demonstrate that CTHRC1 knockdown suppresses the activity of the Wnt/β-catenin pathway and its downstream effector p-Smad1/5/8, leading to promoted chondrocyte proliferation and inhibited apoptosis. Most importantly, the functional significance of this axis was definitively confirmed through rescue experiments using the specific Wnt/β-catenin pathway agonist BML-284. The application of BML-284 effectively reversed the suppressive effects of CTHRC1 knockdown on the Wnt/β-catenin/p-Smad1/5/8 pathway. Consequently, the pro-proliferative and anti-apoptotic phenotypes resulting from CTHRC1 knockdown were significantly attenuated. This successful pharmacological rescue provides direct evidence that the functional impact of CTHRC1 on chondrocyte fate is mechanistically dependent on its activation of the Wnt/β-catenin/p-Smad1/5/8 signaling cascade. It should be noted, however, that our study lacks direct measurements of canonical Wnt pathway activity, such as TOPFlash reporter assays. Therefore, while our findings strongly suggest the involvement of Wnt/β-catenin signaling in CTHRC1-mediated chondrocyte regulation, the conclusions remain supportive rather than definitive, and further validation using direct pathway activity readouts is warranted in future studies.

This study originated from an exploration into the mechanisms of osteoarthritis (OA). In an OA chondrocyte model, we found that inhibiting CTHRC1, by regulating the Wnt/β-catenin pathway, suppressed chondrocyte proliferation and promoted apoptosis. Given that OA and rheumatoid arthritis (RA) share key pathological processes, such as inflammatory pathways and matrix degradation[33-35], we hypothesized that the CTHRC1/Wnt/β-catenin axis might be a common pathogenic signaling pathway. To explore this hypothesis, we conducted an in vivo investigation using a collagen-induced arthritis (CIA) rat model. Notably, in this preliminary, hypothesis-generating study with a limited sample size (n=3 per group), the experimental data suggested that silencing CTHRC1 in the CIA model, likewise via suppression of the Wnt/β-catenin pathway, may exert anti-inflammatory and joint-protective effects. Therefore, our collective data, derived from both OA

and RA disease models, provide preliminary evidence supporting the CTHRC1/Wnt/β-catenin pathway as a potential common therapeutic target for mitigating arthritic pathologies. While this study offers initial insights into the role of the CTHRC1/Wnt/β-catenin pathway in inflammatory arthritis using the CIA model, it is important to acknowledge the etiological differences between this model and primary OA, and the preliminary nature of the in vivo findings underscores the need for future validation in larger-scale studies.

CONCLUSIONS

This study reveals the regulatory role of CTHRC1 in chondrocyte function within an in vitro OA cell model. The findings demonstrate that CTHRC1 exacerbates chondrocyte damage by modulating signaling associated with the Wnt/β-catenin pathway. A similar mechanism was observed in a RA animal model, where CTHRC1 influenced this pathway and exhibited potential anti-inflammatory and tissue-protective effects in joint tissues. These results provide experimental evidence for further elucidating the role of the CTHRC1/Wnt/β-catenin axis in the pathogenesis of arthritis and suggest its potential as a therapeutic target for intervening in arthritis progression.

Ethics approval and consent to participate: The analysis of human osteoarthritis data was based on the publicly available dataset GSE114007, which was downloaded from the Gene Expression Omnibus (GEO) database. As this study involved analysis of pre-existing, de-identified data, no additional ethical approval was required.
All animal experimental protocols were reviewed and approved by the Animal Ethics and Welfare Committee (AEWC) of Yan'an Hospital Affiliated to Kunming Medical University (Approval No.:2025049). All procedures were conducted in strict accordance with the relevant guidelines outlined in the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

Human Ethics: Not Applicable.

Consent for publication: Not Applicable.

Availability of supporting data: The simulation experiment data used to support the findings of this study are available from the corresponding author upon request.

Competing interests: The authors declare no competing interests.

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Authors' contributions: Haifeng Li and Jiayu Xiao designed and supervised the study. Haibo Li and Yunlou Jiang performed experiments. Rabinsh Raj Dahal and Qian Lu contributed to data analysis and manuscript drafting. Shenghai Wang and Ganglian Li provided technical support and data curation. Jiayu Xiao finalized the manuscript.

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