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Original Article

YAP maintains cartilage stem/progenitor cell homeostasis in osteoarthritis

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ABSTRACT

Background: The cartilage stem/progenitor cells (CSPC) play a critical role in maintaining cartilage homeostasis. However, the effects of phenotypic fluctuations of CSPC on cartilage degeneration and the role of CSPC in the pathogenesis of OA is largely unknown.

Methods: The cartilage samples of 3 non-OA and 10 OA patients were collected. Human CSPC (hCSPC) derived from these patients were isolated, identified, and evaluated for cellular functions. Additionally, chondrocytes derived from OA patients were isolated. The effect of Yes-associated protein (YAP) expression on hCSPC was investigated *in vitro*. The OA rat model was established by Hulth's method. Lentivirus-mediated YAP (Lv-YAP) or lentivirus-mediated YAP RNAi (Lv-YAP-RNAi) was injected intra-articularly to modulate YAP expression in rat joints. In addition, allogeneic rat CSPC (rCSPC) overexpressing or silencing YAP were transplanted by intra-articularly injection. We also evaluated the functions of rCSPC and the OA-related cartilage phenotype in the rat model. Finally, the transcriptome of OA rCSPC overexpressing YAP was examined to explore the potential downstream targets of YAP in rCSPC.

Results: hCSPC derived from OA patients exhibited differential chondrogenesis capacity. Among them, a subset of hCSPC showed pronounced dysfunction, including impaired chondrogenic differentiation, inhibition of proliferation and migration, and downregulation of lubricin. Additionally, YAP was lowly expressed in quiescent non-OA hCSPC, upregulated in activated OA hCSPC, but significantly downregulated in dysfunctional OA hCSPC. Notably, the overexpression of YAP in OA hCSPC improved the proliferation, lubricin production, cell migration, and senescence, while silencing YAP had the opposite effect. In vivo, upregulation of YAP in the joint delayed OA progression and improved the cartilage regeneration capacity of rCSPC. Using transcriptomic analysis, we found that YAP may regulate rCSPC function by upregulating Baculoviral IAP repeat-containing 2 (BIRC2). Importantly, the knockdown of BIRC2 partly blocked the regulation of YAP on the CSPC function.

Conclusion: Dysfunction of CSPC compromises the intrinsic repair capacity of cartilage and impairs cartilage homeostasis in OA. Notably, the transcriptional co-activator YAP plays a critical role in maintaining CSPC function through potential target gene BIRC2.

The Translational Potential of this Article: In this study, we observed targeting the YAP-BIRC2 axis improved the CSPC function and restored the cartilage homeostasis in OA. This study provides a potential stem cell-modifying OA therapy.

1. Introduction

Osteoarthritis (OA) is an age-related disease characterized by progressive cartilage loss. Unfortunately, no well-established strategy has proven effective in delaying or blocking cartilage damage in OA [1]. Notably, it is widely accepted that, despite the lack of effective self-regeneration capacity, articular cartilage possesses endogenous repair mechanisms to maintain tissue homeostasis and counteract microdamage to some extent. In the healthy state, these repair mechanisms can reach a dynamic balance with the deleterious factors that

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drive cartilage degeneration [2,3]. But in OA, this balance is disrupted due to the enhancement of deleterious factors and attenuation of reparative capacity, resulting in progressive cartilage destruction. Importantly, a population of stem cells or progenitor cells within articular cartilage has been well-recognized for several decades [4,5]. These cartilage stem/progenitor cells (CSPC) exhibit properties of adult stem cells, such as self-renewal capacity and multi-lineage differentiation potential, and are assumed to play a critical role in endogenous repair mechanisms [6]. Several studies have demonstrated that CSPC isolated from osteoarthritic cartilage were activated, with high migratory and proliferative activity [4,6,7]. Presumably, this may favor CSPC aggregation at sites of cartilage injury and promote tissue regeneration. In contrast, it has also been found that CSPC derived from a subset of OA patients exhibited a premature senescence phenotype [8]. Similar to the failure of adult stem cells observed in other age-related diseases, the senescence of CSPC has the potential to impair the intrinsic repair mechanisms of cartilage and contribute to the progression of OA [9,10]. Consequently, this phenotypic fluctuation of CSPC may be closely related to the heterogeneity of OA. However, no study has investigated the effect of this phenotypic fluctuation on cartilage degeneration, and the role of CSPC in the pathogenesis of OA is largely unknown. In this study, we observed the phenotypic fluctuation of CSPC in OA and its effect on cartilage homeostasis. We found that the dysfunction of CSPC, partially due to dysregulation of the transcriptional co-activator YAP, contributes to accelerated cartilage destruction. Thus, these data imply that YAP signaling is a potential target to improve the homeostasis of CSPC and cartilage in OA.

2. Material and methods

2.1. Cartilage sample collection and CSPC isolation and identification

Non-OA and OA cartilage samples were collected from patients with traumatic amputation (n = 3) and total knee arthroplasty (n = 10), respectively. OA cartilage samples (n = 10) were obtained from patients who underwent total knee arthroplasty (TKA) with a clinical diagnosis meeting the criteria for knee osteoarthritis [11] and an X-ray image diagnosis meeting the Kellgren–Lawrence classification grade IV [12]. While rat cartilage samples were collected from normal and OA modeling rats. The hCSPC uses the same extraction method as the rCSPC, which is briefly mentioned as follows. Cartilage tissue was washed three times with phosphate buffer saline (PBS), cut into 1–2 mm^2 fragments, and digested in trypsin–EDTA (0.25%, wt/vol; Gibco, USA) for 10 min and 0.2% collagenase type II (Sigma, USA) in Dulbecco's Modified Eagle Medium/Nutrient Mixture F-12 (DMEM/F-12, Gibco, USA) for 4 h. The isolated cells (3×10^5/well) were seeded into six-well plates (Corning, NY) coated with 10 μg/ml of fibronectin (Solarbio, China) in DMEM/F-12 medium with penicillin (100 U/ml), streptomycin (100 μg/ml, Gibco, USA) and 10% fetal bovine serum (FBS, Gibco, USA) for 20 min. The non-adherent cells were removed. Adherent cells were collected for further identification. The non-adherent cells are chondrocytes. Multipotential differentiation assays and flow cytometry were used to identify CSPC. Cells in Passage 3 were plated in 6 well plates (2×10^4/well) and cultured for 14 days in OriCell osteogenic or adipogenic culture medium (Cyagen, USA). Then cells were stained with alizarin red S or an oil red solution. 3×10^5 cells were put into a 15 ml conical tube, centrifugated into pellets, and cultured for 21 days in OriCell chondrogenic culture medium, which contained TGFβ-3 (Cyagen, USA) and stained with Alcian blue. Antibodies against CD44, CD90, CD31, and CD45 (Thermofisher, USA) were used for flow cytometry (BD FACSCalibur, USA). 1×10^5 cells in Passage 3 were suspended in 500 μl PBS containing 20 μg/ml antibody before analyses.

2.2. OA chondrocytes isolation and culture

All the OA chondrocytes were derived from the OA cartilage tissue mentioned above. OA chondrocytes were obtained using methods described in our previous study [13]. Rat-derived OA cartilage cells and human-derived cartilage cells were extracted using the same method. In brief, the articular cartilage of the OA patients was harvested and digested with 0.2% collagenase type II (Sigma, USA) at 37 °C for 4 h. Cells were cultured in DMEM/F-12 (Gibco, USA) supplemented with 10% FBS (Gibco, USA) at 37 °C. Cells in passage 3 were used for co-culture. Furthermore, we characterized OA chondrocyte morphology and proliferation under the microscope. The cells from each OA patient were not pooled at the time of the co-culture experiment.

2.3. 5-Ethynyl-2′-deoxyuridine (EdU) incorporation assay

Proliferating cells were determined by the EdU Imaging Kits (APExBIO, USA). Cells were incubated with 10 μM EdU for 2 h before EdU staining, and cell nuclei were stained with Hoechst 33,342 at 5 μg/ml for 30 min. The cells were counted in five random fields per well. The percentage of EdU-positive cells was calculated using Image-Pro Plus version 6.0 for Windows (Media Cybernetics, USA). For detecting proliferating cells in the rat OA model, 100 μl 10 μM EdU was injected intra-articularly on day 38. Four days later, rats were sacrificed, and the joints were harvested. Paraffin sections were evaluated using the EdU Kit and stained with Hoechst 33,342. Each paraffin section was counted in five random fields.

2.4. Transwell migration assay

Cell migration assays were performed using 24-well transwell plates with polycarbonate membranes with an 8 μm pore size (Corning, USA). CSPC in DMEM/F12 was added to the upper chamber at a density of 2.5×10^5 (200 μl/well), and the lower chamber was filled with 10% FBS in the culture medium. Cells that did not penetrate the membrane were removed. Cells were stained with 0.1% crystal violet and counted in six random fields per well.

2.5. Lentivirus infection and small interfering RNA (siRNA) transfection

Lv-YAP, Lv-YAP-RNAi, and Lv-Vector were synthesized and packaged by Genechem (Shanghai, China). The lentivirus-infected OA CSPC following the manufacturer's protocol. Si-YAP, si-Birc2, and nonspecific control siRNA (si-Ctrl) were synthesized by JTSBio (Wuhan, China). The siRNAs were transfected into rats OA-CSPC using jetPRIME transfection reagent (Polyplus, France) following the manufacturer's protocol. The

Table 1
The siRNAs sequences

Name	RNA oligo sequences (5′ to 3′)
si-YAP1-1	GGUCAGAGAUACUUCUAATT
	UUAAGAAGUAUCUCUGACCTT
si-YAP1-2	CCGGGAUGACUCAGGAAUUTT
	AAUUCCUGAGUCAUCCCGGTT
si-YAP1-3	GCUGCCACCAAGUUAGAUATT
	UAUCUAACUUGGUGGCAGCTT
si-Yap1-1	UUGUGAUUUAAGAAGUAUCUC
	GAUACUUCUUAAAUCACAAUG
si-Yap1-2	AUCAUUGUGAUUUAAGAAGUA
	CUUCUUAAAUCACAAUGAUCA
si-Yap1-3	UGUUGUUGUCUGAUCAUUGUG
	CAAUGAUCAGACAACAACAUG
si-Birc2-1	UGGAUUUGUACCAUUCUUCUG
	GAAGAAUGGUACAAAUCCAAG
si-Birc2-2	ACUCAUUGGUUCCUUUAAGGG
	CUUAAAGGAACCAAUGAGUCC
si-Birc2-3	UCAUAUUCUGAAUCUCAUCCU
	GAUGAGAUUCAGAAUAUGAAG

siRNAs sequences are shown in Table 1.

2.6. The co-culture of OA-hCSPC with OA-chondrocyte

A co-culture system used six-well transwell plates (Corning, USA). 2.5×10^5 hCSPC, hCSPC(YAP^{high}), or hCSPC(YAP^{low}) in Passage 3 were seeded in the upper compartments, and 2.5×10^5 OA-chondrocyte in Passage 3 were cultured in the lower compartments in DMEM/F12 with 10% FBS. Co-cultures were maintained for 7 days before evaluation.

2.7. Animal experiments

The *in vivo* study was conducted with the approval of the Ethics Committee for Animal Experiments in Central South University (Resolution no:2019sydw209). The Department of Laboratory Animals of Central South University provided 7-week-old Sprague–Dawley (SD) rats (male). All SD rats were maintained under standard light conditions (12-h light/dark cycle) at room temperature. Rats were performed Hulth modeling of the knee joint, and PBS, lentivirus and rCSPC were injected into the joint cavity according to the experiments, and euthanized as well as taken at different time periods. All surgeries and evaluations of these groups were done on the same day to minimize potential confounders. Furthermore, all cartilage samples were collected from each group for cell culture, RT-qPCR, western blot, and histological evaluation.

2.8. Histological evaluation

The joint is fixed with 4% Paraformaldehyde and decalcified for 1 month before being dehydrated and embedded with paraffin. Paraffin sections were stained for histological evaluation using safranin O-fast green, anti-CD44 (15675-1-AP, Proteintech, China), anti-CD90 (66,766-1-lg, Proteintech, China), anti-YAP (AF6328, Affinity, China), anti-Collagen II (Col II, GB11021, Servebio, China), anti-Collagen I (Col I, bs-0578R, Bioss, China) and anti-Collagen X (Col X, DF13214, Affinity, China). Secondary antibodies were detected using a fluorescent secondary antibody (Proteintech, China) or Rabbit streptavidin-biotin detection system kit (ZSGB-Bio, China) according to the manufacturer's protocol. Joint pathology was quantified using the Osteoarthritis Research Society International (OARSI) scoring system.

2.9. RNA-sequencing and functional annotation

5×10^6 rCSPC in Passage 3 were infected with or without Lv-YAP for 72 h and then subjected to further RNA preparation, library construction, sequencing, and analysis. The rCSPC from each rat knee joint cartilage sample were not pooled at the time of each experiment. Genes with an adjusted $P < 0.05$ and an absolute value of $|\log2 \text{ (fold change)}| > 1$ found by the "limma" R package were assigned as differentially expressed genes (DEGs). Gene Ontology (GO) term enrichment, Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis, and annotation were conducted with "clusterProfiler" and "org.Hs.eg.db" R packages. Expression heatmaps were visualized with "pheatmap" R package.

2.10. Real-time quantitative polymerase chain reaction (RT-qPCR)

The total RNA of cells was extracted using the RNAfast200 Total RNA Kit (220,011, Fastagen, China). Reverse transcription reactions were performed using HiScript II Q RT SuperMix for qPCR (+gDNA wiper) (R223-01, Vazyme, China). RT-qPCR was conducted using ChamQ Universal SYBR qPCR Master Mix (Vazyme, China) according to the manufacturer's protocol. An initial denaturation step was carried out at 95 °C for 15 min, followed by 40 cycles of denaturation at 95 °C for 10 s, annealing at 56 °C for 30 s, and extension at 72 °C for 30 s. Gene transcriptional levels were normalized to those of β-actin and calculated using the log2–△△Ct method. The primer sequences are shown in Table 2.

2.11. Western blotting

Protein extracts were subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), and the proteins were transferred to polyvinylidene fluoride (PVDF) membranes and blocked in blocking buffer (5% skimmed milk) for 1 h. The membranes were incubated overnight at 4 °C with primary antibodies against Lubricin (bs-11175R, Bioss, China), YAP (AF6328, Affinity, China), SRY-box transcription factor 9 (SOX9, ET1611-56, Huabio, China), bone morphogenetic protein 4 (BMP4, EM1706-23, Huabio, China), RUNX family transcription factor 2 (RUNX2, ET1612-47, Huabio, China), matrix metallopeptidase 3 (MMP3, AF0217, Affinity, China), Focal adhesion kinase (FAK, BS6899, Bioworld, China), Col I (bs-0578R, Bioss, China), Col II (GB11021, Servicebio, China), Col X (DF13214, Affinity, China), Birc2 (DF6167, Affinity, China), snail family transcriptional repressor 2 (Snai2, AF4002, Affinity, China), zinc finger E-box binding homeobox 2 (Zeb2, AF5278, Affinity, China), cyclin D2 (Ccnd2, AF5410, Affinity, China), and glyceraldehyde-3-phosphate dehydrogenase (GAPDH). The following day, the membranes were incubated with fluorophore-conjugated secondary antibody or HRP-conjugated secondary antibody at room temperature for 1 h and developed in electrochemiluminescence (ECL) Western blot detection reagents (Biosharp, China). The band was analyzed using UVP Chem studio PLUS 815 (Analytik Jena, Germany).

2.12. Statistical analysis

Values in the text and figures are expressed as the mean ± SD unless otherwise noted. Statistical significance was determined by Student's t-test or analysis of variance (ANOVA) using SPSS 17.0 (SPSS, Inc., Chicago, IL, USA). Spearman correlation analysis was applied to evaluate correlations among continuous variables. R version 4.0.3 was used for omics data analyses. Differentially expressed genes (DEGs) between two subclusters were calculated with the R package "limma" ($|\log2 \text{ FC}| > 1$ and adjusted $P < 0.05$). P values in GO and KEGG enrichment analyses were adjusted using the "Benjamini & Hochberg" method. Differences with $p < 0.05$ were considered statistically significant.

3. Results

3.1. CSPC dysfunction in OA

Non-OA and OA cartilage samples were collected from patients with traumatic amputation (n = 3) and total knee arthroplasty (n = 10), respectively. Patient-specific information is shown in Supplementary Table 1. The cells from each patient were not pooled at the time of each experiment. The isolated cells were successfully induced to undergo chondrogenic, osteogenic, and adipogenic differentiation (Fig. 1A). These cells were negative for endothelial cell markers CD31 and CD45 and positive for mesenchymal stem cell markers CD44 and CD90 (Fig. 1B). Therefore, these cells were identified as hCSPC. By functional evaluation, we found that the hCSPC derived from OA patients exhibited differential chondrogenesis capacity. The chondrosphere diameters of all OA patients formed were from 1.1 mm to 2.2 mm (Fig. 1C). Among them, hCSPC derived from patient 4, 8, and 12 exhibited activated chondrogenesis capacity, including cell pellet diameter, chondrogenic differentiation (Alcian blue and type II collagen immunohistochemistry staining), proliferation (EdU assay), migration (transwell) and functional protein lubricin expression (Fig. 1C–G). These hCSPC were clustered as "Activated OA hCSPC" (Fig. 1C). In contrast, hCSPC derived from patients 7, 9, and 13 exhibited dysfunctional chondrogenesis and hyporesponsiveness to chondrogenic stimuli. Their cell pellets were, on average, 33% smaller than those of non-OA patients (P1, 2, and 3) and

Table 2
Primer sequences used in RT-PCR.

F3	NM_001178096.2	AACACCATGGCACCTTTTGC	TGCTTGGACGACCTGGTTAC
PDPN	NM_001006624.2	AATGTCGGGAAGGTACTCGC	AGGGCACAGAGTCAGAAACG
SCD	NM_005063.5	AGCAGGTAAATTGTCGGGGG	GAGGTTCCTCTTGCTTCGCT
VDR	NM_000376.3	ACCAGAAGCCTTTGGGTCTG	TCCATCCCTGAAGGAGCAGG
BMP4	NM_001202.6	TGGGATTCCCGTCCAAGCTAT	CAGGAATCATGGTGTCTTGACAGA
AXIN2	NM_001363813.1	AGGCCCTGCTGTAAAAGAGAG	AGTTCCTCTCAGCAATCGGC
CXCL12	NM_000609.7	GCCAGCTCTTCCGCTC	CCACGGACGCTCCTGC
TCF4	NM_001083962.2	GATGCTCTGGGGAAAGCACT	GTGCCTGCTGAGAGAGATGG
PHF21B	NM_001135862.3	GCCGAATTAGCATCGTGCC	CGCGGGCAGGGACTATATTT
TERT	NM_001193376.3	CTCCTGCGTTTGGTGGATGA	GGGCATAGCTGAGGAAGGTTT
FANCL	NM_001114636.1	TCTGTGTTTCTCCGGACTTCG	CCCTGAGCCGAGATGAATCC
RYK	NM_001005861.3	CATCCACCCAGACGACTCAG	ACCTAAGGAGCTGGTGATAGGA
FZD7	NM_003507.2	CCTACCGCGCCCTACCTG	AGTACATCAGGCCGTTGGCA
RUNX2	NM_001015051.4	GCGGTGCAAACTTTCTCCAG	GACTCTGTTGGTCTCGGTGG
Yap1	NM_001394328.1	GGCAGGCAATACGGAATATCAA	GGAGAGCTAATTCCCGCTCTG
Ankrd	NM_001107589.1	CATAGACTAACGGCTGCCAAC	CCCGTTACCAGCTCCTCTAC
Ccn1	NM_031327.3	GGATCTGTGAAGTGCGTCCT	CGGACTGGTTCTGGGGATTT
Ccn2	NM_022266.2	TGGCTTGCTCAGGGTAACTG	CTGCCTCCCAAACCAGTCAT
Birc2	XM_017595882.2	GGGACACTCGCGAAGGC	TCTTGAGGGCTATAAATCGCAG

exhibited impaired chondrogenic differentiation (Fig. 1D). Furthermore, these hCSPC also showed inhibition of proliferation and migration and downregulation of lubricin (Fig. 1E–G). Thus, they were clustered as "Dysfunctional OA hCSPC" (Fig. 1C).

3.2. YAP maintains the function of CSPC

To explore the underlying mechanism regulating hCSPC function, we examined the expression of genes associated with stem cell regulation in OA hCSPC. By comparing mRNA expression between non-OA, dysfunctional OA, and activated OA hCSPC, we identified 5 potential genes, including YAP, MMP3, BMP4, SOX9, and RUNX2 (Fig. 2A). Among these identified genes, the protein levels of YAP were lowly expressed in quiescent non-OA hCSPC, upregulated in activated OA hCSPC, and significantly downregulated in dysfunctional OA hCSPC (Fig. 2B).

Next, we explored the role of YAP in OA hCSPC. In the co-culture of OA hCSPC and OA-chondrocytes, CSPC overexpressing YAP (YAP^{high} hCSPC) exhibited increased expression of lubricin and FAK. Meanwhile, OA-chondrocytes co-cultured with hCSPC exhibited an alleviated OA phenotype, including elevated Col II and decreased Col I and Col X. Notably, this anti-degenerative paracrine effect of hCSPC was significantly enhanced by overexpression of YAP. In contrast, YAP silencing impaired this effect of hCSPC (YAP^{low} hCSPC) on OA-chondrocytes (Fig. 3A; Supplementary Fig. 1A). Additionally, YAP activated the proliferative activity (EdU assay) and migration ability (transwell migration assay). YAP also attenuated the cellular senescence (SA-β-Gal staining) of OA hCSPC, while silencing YAP aggravated the dysfunction of OA hCSPC (Fig. 3B–D; Supplementary Fig. 1B).

3.3. YAP contributes to CSPC for cartilage regeneration

To investigate the changes of YAP and CSPC in the superficial layer of cartilage along with the early cartilage damage process, 15 rats were randomly divided into three groups. We chose 2 weeks and 4 weeks post-modeling to represent the early stages of cartilage damage. The 8-week-old male SD rats were anesthetized, then Hulth's surgery and sham surgery were performed on rats (n = 5 for each group). Rats were sacrificed at weeks 2 and 4. The knee joints of the were collected, and tissues were processed to obtain paraffin sections. 2 weeks after injury, YAP expression was upregulated and progressively enhanced in cells in the superficial zone of cartilage. Surface markers revealed that most of these YAP^{+} cells were CSPC ($CD44^{+}CD90^{+}$) (Fig. 4A). By isolating rCSPC from injured cartilage, we detected upregulation of YAP and its representative target genes in rCSPC (Fig. 4C and D). Concomitant with YAP expression, rCSPC began to proliferate and increase in number after cartilage injury (Fig. 4A and B).

To investigate the contribution of YAP to cartilage homeostasis, 25 rats were randomly divided into five groups. The rats were anesthetized and operated on as described above. 20 μl of PBS, lentivirus expressing YAP (Lv-YAP) or YAP-RNAi (Lv-YAP-RNAi) was injected intra-articularly 7 days following surgery (n = 5 for each group). It takes at least 5 weeks to develop stable protein expression in the joint after transduction of the lentivirus vector. Thus, the rats were sacrificed for evaluation at week 6. The knee joints of the were collected, and tissues were processed to obtain paraffin sections. Lv-YAP activated the proliferative activity and increased the number of rCSPC in cartilage. In contrast, Lv-YAP-RNAi had the opposite effect (Fig. 5A; Supplementary Fig. 1C). Lv-YAP significantly alleviated the OA phenotype of cartilage, including increasing Col II production, inhibiting Col I and X expression, and improving OARSI score in Safranin O staining, while inhibition of YAP exacerbated OA progression (Fig. 5A; Supplementary Fig. 1D).

To explore the role of YAP in CSPC for cartilage regeneration, 20 rats were randomly divided into four groups. The rats were anesthetized and operated on as described above. For rCSPC transplantation, 100 μl PBS or PBS containing 5×10^6 allogeneic rCSPC in Passage 3 were injected intra-articularly 7 days after surgery (n = 5 for each group). rCSPC were infected or transfected with Lv-YAP or si-YAP 3 days before transplantation. Euthanasia and evaluation of rats were conducted at week 6. The knee joints of the were collected, and tissues were processed to obtain paraffin sections. Intra-articularly, rCSPC transplantation improved the OA phenotype of cartilage. To a large extent, this effect positively correlated with the expression of YAP in rCSPC. YAP^{high} rCSPC exhibit a significant anti-degenerative effect on cartilage, whereas this effect decreases in YAP^{low} rCSPC (Fig. 5C).

3.4. Transcriptomic analysis

RNA sequencing was used to investigate the transcriptome of OA rCSPC overexpressing YAP. Transcriptomics raw data has been uploaded to GSA database (No:CRA009054). Compared to control rCSPC, a total of 4723 DEGs, including 2534 upregulated and 2189 downregulated, were identified in YAP^{high} rCSPC. KEGG enrichment exhibited that these DEGs were primarily enriched in pathways associated with cell cycle, cellular senescence, autophagy, apoptosis, hippo signaling, and TNF signaling (Fig. 6A). The detailed DEGs enriched in these pathways are shown in Fig. 6B.

3.5. YAP may regulate CSPC function by upregulating Birc2

Next, we examined the direct target genes of YAP -TEAD complexes in the transcriptome [14]. We identified four YAP target genes,

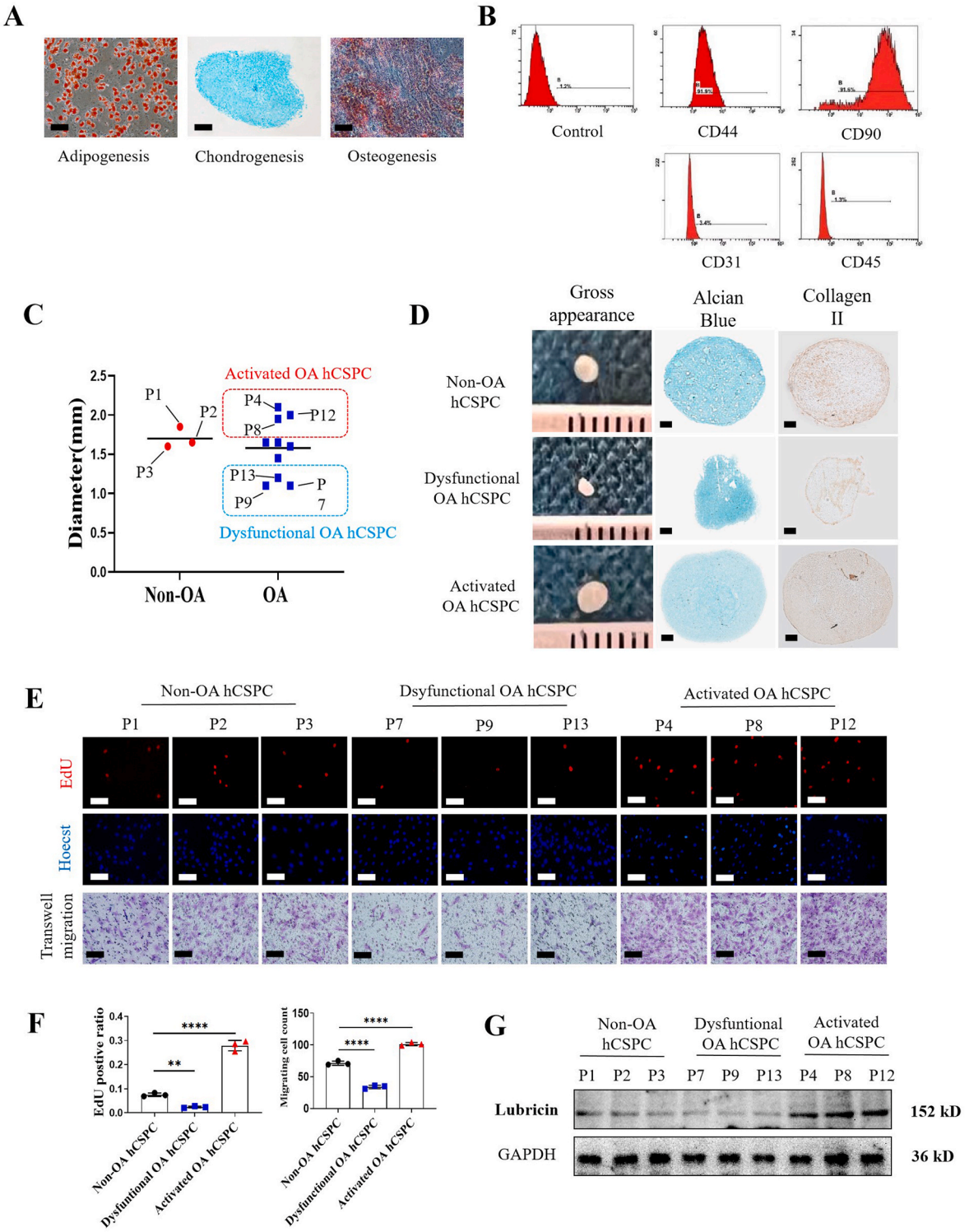

Figure 1. CSPC dysfunction in OA (A) The adipogenesis, chondrogenesis, and osteogenesis of hCSPC. (B) Expression of the surface markers CD44, CD90, CD31, and CD45 as determined by flow cytometry. (C) The diameter of non-OA hCSPC pellets (n = 3) and OA hCSPC pellets (n = 10) after 21 days of chondrogenic induction. (D) The gross appearance (left), Alcian blue (middle) staining, and immunohistochemistry (right) staining for Collagen II of non-OA hCSPC pellet, dysfunctional OA hCSPC pellet, and activated OA hCSPC pellet. Bars = 200 μm. (E,F) The EdU staining (Red) and transwell migration assays of non-OA hCSPC, dysfunctional OA hCSPC, and activated OA hCSPC. Nuclei were stained by Hoechst 33,342 (blue). The cells were counted in five random fields per well. (G) Expression of lubricin in non-OA hCSPC, dysfunctional OA hCSPC, and activated OA hCSPC. Each experiments was repeated three times. Bars = 100 μm. All hCSPC are derived from human cartilage tissue. Cells proliferated to Passage 3 for experiments. Statistical significance was determined by ANOVA. **p < 0.01; ****p < 0.0001.

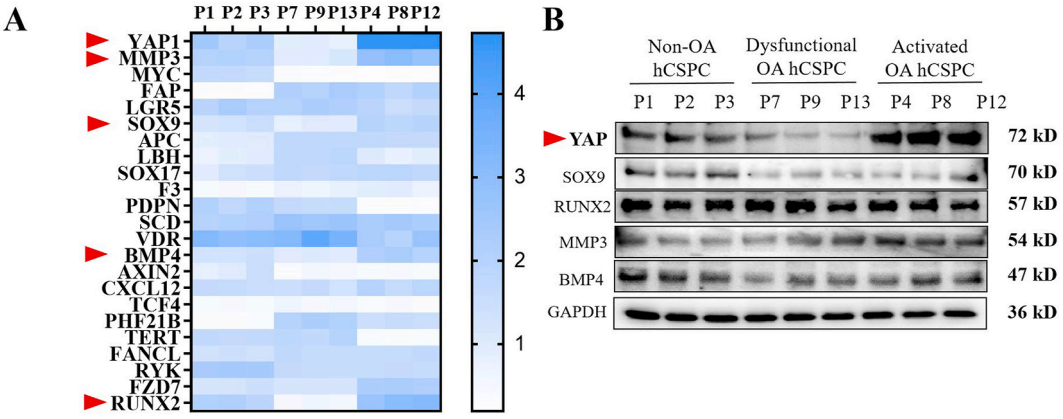

Figure 2. YAP is closely correlated with the functional state of hCSPC. (A) The mRNA expression of the genes associated with stem cell function in non-OA hCSPC, dysfunctional OA hCSPC, and activated OA hCSPC. (B) The protein levels of YAP, SOX9, RUNX2, MMP3, and BMP4 in non-OA hCSPC, activated OA hCSPC, and dysfunctional OA hCSPC. Each experiments was repeated three times. All hCSPC are derived from human cartilage tissue. Cells proliferated to Passage 3 for experiments.

including Birc2, Snai2, Zeb2, and Ccnd2, upregulated in the Lv-YAP-induced DEGs described above (Fig. 6C). Notably, Western blotting showed that the protein level of Birc2 elevated remarkedly after Lv-YAP treatment (Fig. 6D). We further examined the role played by Birc2 in regulating rCSPC function. We identified that the knockdown of Birc2 significantly impaired the cellular functions of rCSPC, including lubricin production, proliferative activity, and migration ability. More importantly, we found that Birc2 knockdown largely blocked the regulation of YAP on the cellular functions of rCSPC (Fig. 7).

4. Discussion

Due to its structural characteristics of lacking blood vessels, articular cartilage has long been thought to lack the ability to repair. However, current studies suggest that cartilage has an intrinsic reparative ability associated with CSPC [3,5,15]. Therefore, the role of CSPC in OA still needs to be further investigated to better understand these physiological properties. One limiting aspect of these kinds of studies is that there are no widely recognized specific surface markers for CSPC. Usually, CSPC are identified by markers of mesenchymal stem cells. In several studies, CD44, CD71, CD90, and CD105 have been used to identify CSPC [16–18]. This study used CD44 and CD90 as surface markers to identify hCSPC (Fig. 1B). Notably, Seol et al. showed that 7–14 days after blunt mechanical impact injury to healthy cartilage explants, a class of migrating cell population appeared at the injury site around the ECM of the explants and showed characteristics of cartilage progenitor cells [19]. This suggests that CSPC are involved in the repair of cartilage injury. A series of studies also confirmed that CSPC play an important role in cartilage repair. In healthy cartilage, CSPC are generally found in the superficial zone of cartilage [20]. When damage occurs in cartilage, CSPC respond to the biological stimuli, migrate to the cartilage damage to proliferate, and secrete collagen II, chitosan, and lubricin for joint resurfacing [19]. Lubricin is the product of the proteoglycan 4 (PRG4) gene, a proteoglycan that serves as a lubricant that protects the integrity of cartilage. It also exerts a protective effect by inhibiting synovial cell hyperproliferation and suppressing chondrocyte senescence [21,22]. In this study, we found that rCSPC activation enhanced gradually in the superficial zone of cartilage along with the progression of cartilage injury (Fig. 4A). Additionally, the percentage of CSPC in normal articular cartilage was 3.49 ± 1.93% and significantly increased in OA cartilage [17]. This change suggests that CSPC are involved in the pathological process of OA. In early OA, aggregated CSPC were found in the fissures of articular cartilage [23,24], implying that CSPC exert a protective effect on articular cartilage by participating in the remodeling of the ECM. In late OA, due to the destruction of the ECM, CSPC migrate between cartilage tissue, subchondral bone, and bone tissue [4], suggesting that it may play a role in information exchange. In this study, we co-cultured OA hCSPC with OA-chondrocytes and observed that after 7 days of co-culture, OA-chondrocytes showed an increase in Col II and a decrease in OA-related phenotypes such as Col I and Col X (Fig. 3A). In the OA rat model, we delayed the osteoarthritis process and OA-related phenotypes in cartilage by intra-articular injection of rCSPC (Fig. 5C). These findings suggest the involvement of CSPC in the maintenance of cartilage homeostasis. Theoretically, CSPC may promote articular cartilage repair through direct differentiation into chondrocytes or paracrine regulation. In a previous study, we used intra-articular transplanted bone marrow-derived MSCs for cartilage repair and observed the homing, adhesion, differentiation, and paracrine effects of MSCs on chondrocytes [13,25,26].

Although CSPC are critical for maintaining cartilage health, little is known about regulating their function. Fellows CR et al. have shown that a premature failure phenotype of CSPC in OA joints [8] is similar to stem cell failure in other age-related diseases [27]. In the present study, compared with non-OA control and activated OA hCSPC, hCSPC in a subset of OA cartilage exhibited dysfunction and hyporesponsiveness to chondrogenic stimuli *in vitro*, including impaired chondrogenic differentiation and regeneration (Fig. 1C and D), poor proliferation, migration, and ability to secrete the functional protein lubricin (Fig. 1E–G). Subsequently, we screened the genes related to stem cell function in non-OA hCSPC and OA hCSPC. We found the strongest correlation of the functional state of CSPC with the transcriptional co-activator YAP, which is lowly expressed in quiescent non-OA hCSPC and upregulated in activated OA hCSPC but appears to be suppressed in dysfunctional OA hCSPC (Fig. 2A and B). YAP is a crucial effector downstream of the Hippo pathway, essential for regenerating multiple organs. It promotes the dedifferentiation of mature cell types and triggers the expansion of stem and progenitor cell pools [14,28,29]. Additionally, YAP promotes stem cell reprogramming [30,31], maintains stem cell stemness, and inhibits stem cell senescence [29]. However, the role of YAP in OA articular cartilage is controversial. Investigators noted that both pharmaceutical and mechanical activation of YAP blocks proinflammatory signaling induced by IL1β and prevents cartilage breakdown and the loss of biomechanical functionality [32]. It has also been suggested that intra-articular injections of Verteporfin, a specific inhibitor of YAP, can delay the OA process [33]. Some investigators have also demonstrated that YAP expression in articular cartilage gradually decreases with the progression of OA [29,34]. Consequently, this dysregulation of YAP may impair the function of stem cells residing in the microenvironment. For example, deletion of YAP in the intestinal epithelium impaired crypt cell proliferation and regeneration in some reports [35,36]. Therefore, YAP

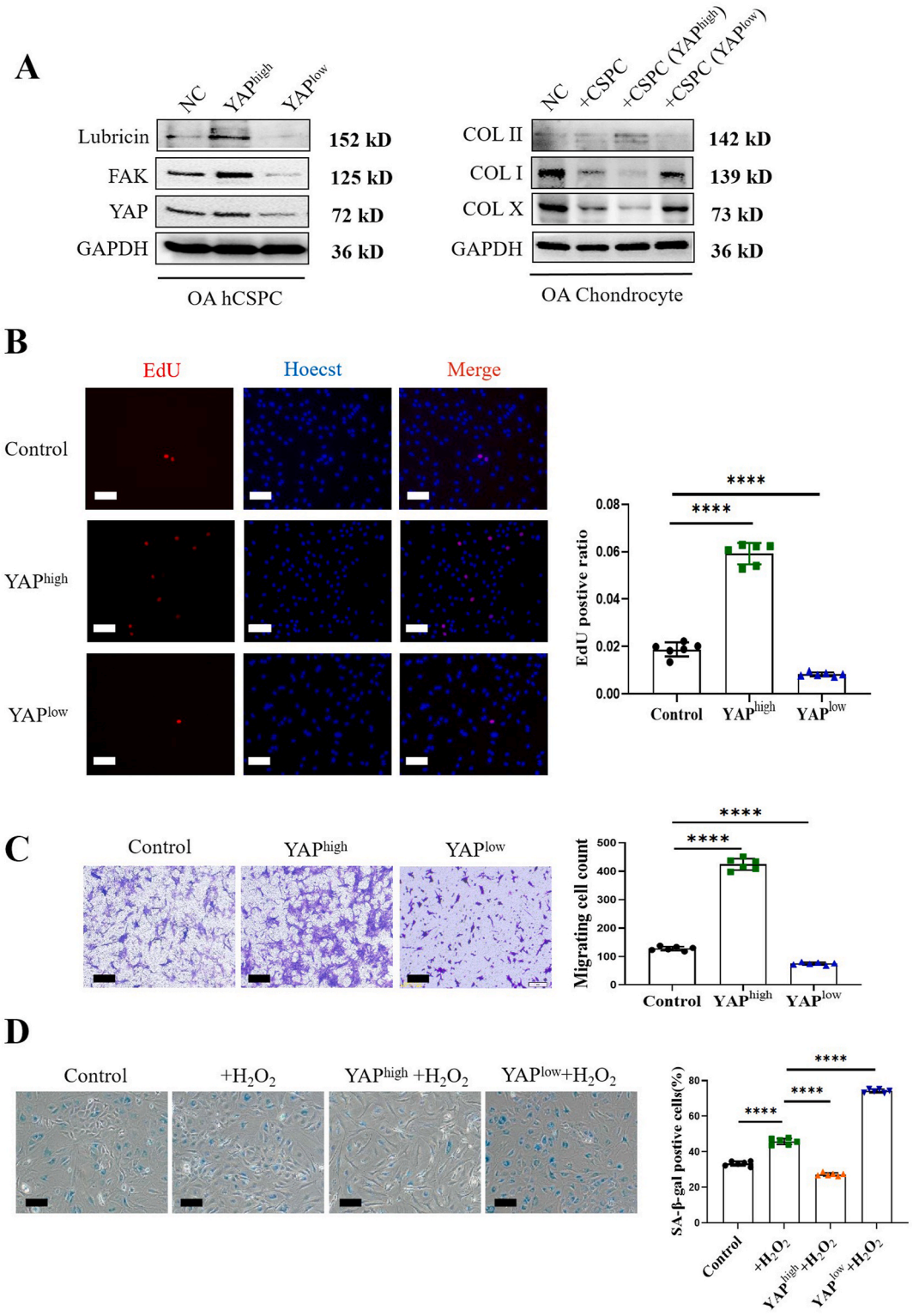

Figure 3. YAP maintains the function of hCSPC. OA-chondrocytes were co-cultured with OA-hCSPC for 7 days. (A) The protein levels of FAK, Lubricin, and YAP in OA-hCSPC (left) and Col II, Col I, and Col X in OA-chondrocytes (right). OA-hCSPC overexpressing or silencing YAP were evaluated by EdU proliferation assay (B), transwell migration assay (C), and SA-β-Gal staining (D). The cells were counted in five random fields per well. Each experiments was repeated three times. All hCSPC and chondrocyte are derived from human cartilage tissue. Cells proliferated to Passage 3 for experiments. The quantitative data are presented as mean ± SD, Statistical significance was determined by ANOVA. Bars = 100 μm ****$p < 0.0001$.

dysregulation can potentially impair the function of CSPC and promote OA progression. However, the effect of YAP on CSPC function is still unclear. In this study, we observed differential YAP expression in the hCSPC of different patients. To further validate the effect of YAP on hCSPC function, we regulated YAP expression within OA hCSPC. We found that the dysfunction was alleviated along with increased YAP expression, and proliferation, migration, and anti-senescence capacity were enhanced. In addition, overexpression of YAP in OA hCSPC

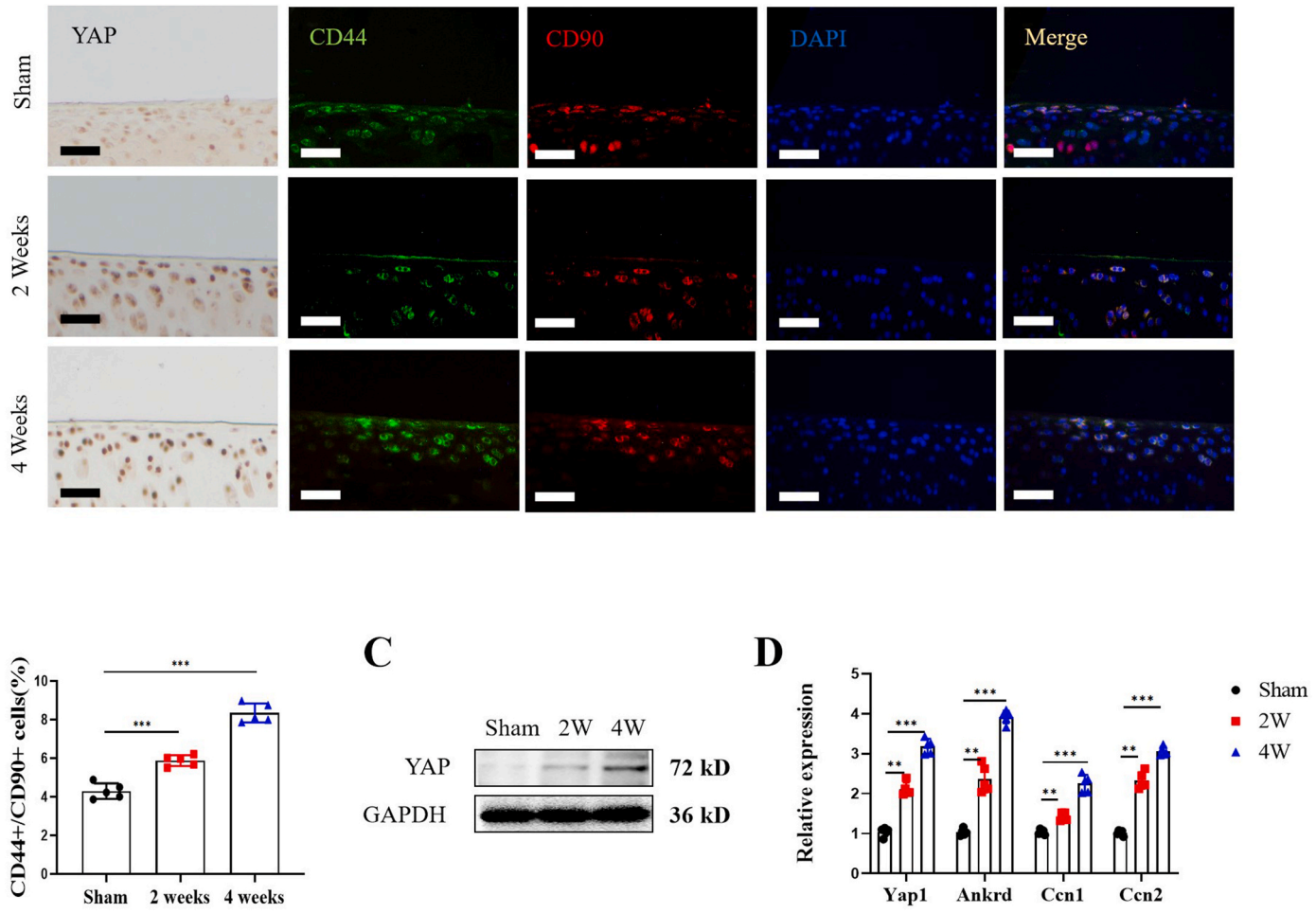

Figure 4. Cartilage injury-induced YAP expression in rCSPC. (A) Immunohistochemistry staining of rat cartilage for CD44 (green), CD90 (red), DAPI (blue), and YAP (brown). Bars = 100 μm. (B) The percentage of CD44+/CD90+ cells in the superficial zone of rat cartilage. C,D Expression of YAP (C) and downstream target genes (D) in rCSPC derived from rat cartilage injury model. Each experiments was repeated three times. All rCSPC are derived from rat cartilage tissue. Cells proliferated to Passage 3 for experiments. Statistical significance was determined by ANOVA. **p < 0.01; ***p < 0.001.

improved its anti-degenerative paracrine effect on OA chondrocytes. In contrast, the knockdown of YAP further impaired the function of OA hCSPC. These findings suggest that YAP is essential for the maintenance of hCSPC (Fig. 3). Furthermore, Fu et al. have shown that the expression of YAP in the superficial zone of cartilage gradually decreases with OA progression, and that upregulation of YAP expression in the OA model alleviates OA progression [29]. Currently, no animal model can completely mimic the naturally occurring OA in humans. However, Hulth's model is one of the most common OA models and is similar to naturally occurring OA to some extent in pathological mechanisms [37]. Notably, we confirmed this finding and demonstrated that the upregulation of YAP in the OA model maintains cartilage homeostasis by improving rCSPC functions. Using transcriptomic analysis, we revealed that BIRC2 might be a critical downstream target of YAP in rCSPC (Fig. 6C and D). BIRC2 is a member of the anti-apoptotic protein family, which inhibits apoptosis and promotes proliferation by binding to TRAF1 and TRAF2. It belongs to the same family as BIRC5, which has recently been recognized as a new biological marker of CSPC [38]. In support of this, Paulina Gil-Kulik et al. showed that MSCs of the Umbilical Cord Wharton's Jelly (WJSC) in younger women giving birth naturally had higher BIRC2 expression and were hypothesized to have better stem cell function [39].

In this study, we propose for the first time that the YAP is critical for the maintenance of CSPC. Additionally, overexpression of YAP in the CSPC improves CSPC function and delays OA progression. This implies that the regulation of YAP is a potential target for OA treatment. However, this study still has some noted limitations. Firstly, due to the limited source of cartilage samples, there is a large age gap between patients in the Non-OA and OA groups. At this stage, we cannot distinguish whether CSPC dysfunction is primarily derived from the effects of aging or the OA microenvironment. Secondly, the CSPC and OA chondrocytes cultured in the monolayer pattern in this study may impair the cell phenotype and affect the outcomes [40]. Thirdly, although results showed that CSPC had regulated the phenotype of chondrocytes, the present results are insufficient to confirm the primary target cells of injected CSPC. CSPC may also affect cells in the rest of the joint, such as synovium. Finally, to make the study more complete, only the downstream targets of YAP were preliminarily explored by RNA-sequence and siRNA silencing techniques (Figs. 6–7). The current results can only suggest that Birc2 may be an essential target downstream of YAP and do not confirm the central role of Birc2 for the function of CSPC. Therefore, all of the above limitations need to be further investigated.

5. Conclusion

Dysfunction of CSPC compromises the intrinsic repair capacity of cartilage and impairs cartilage homeostasis in OA. The transcriptional co-activator YAP plays a critical role in maintaining CSPC function through potential target gene Birc2.

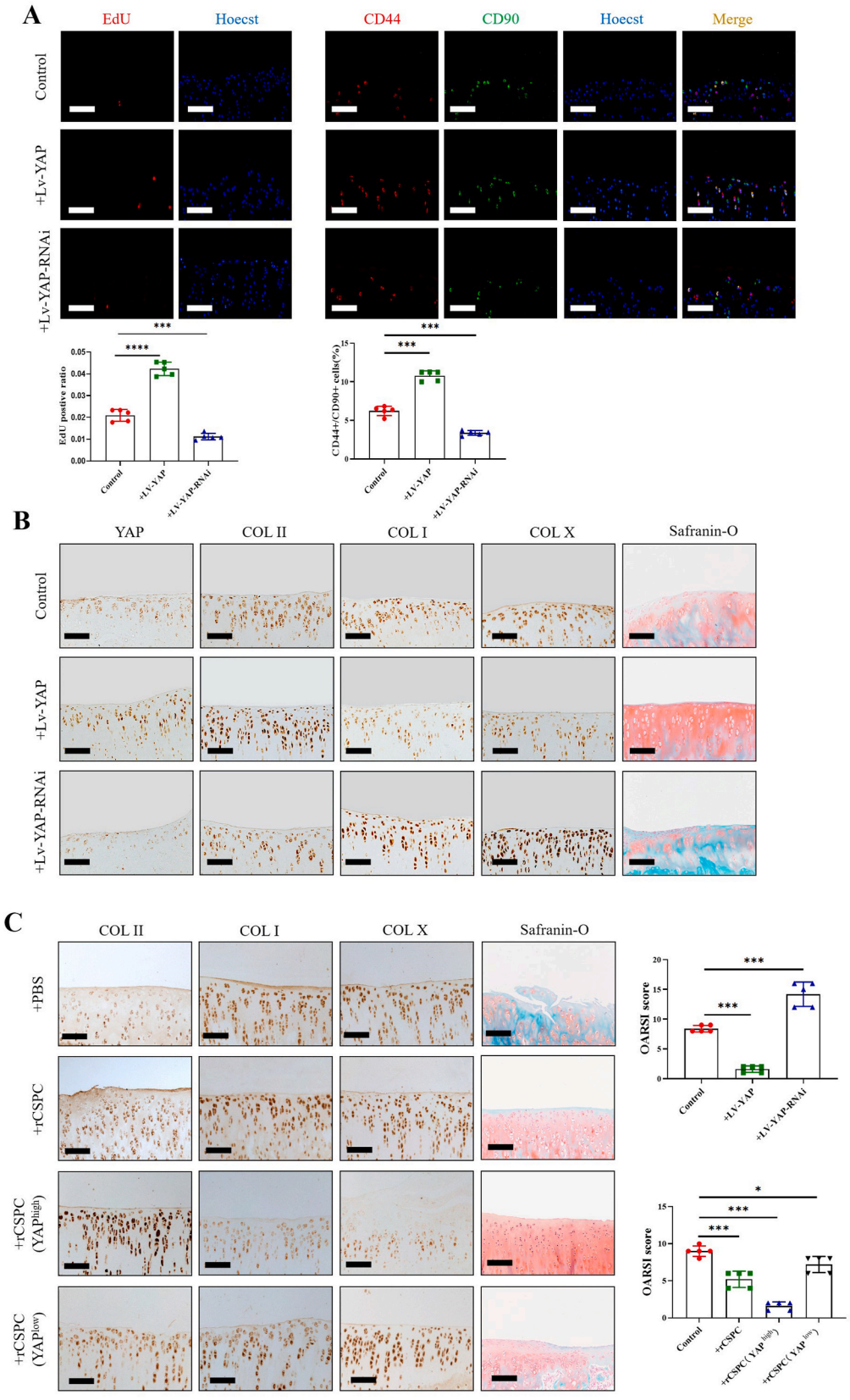

Figure 5. YAP contributes to rCSPC for cartilage regeneration. (A, B) After Lv-YAP-RNAi or Lv-YAP intra-articular injection, cartilage was assessed by EdU proliferation staining (red, left), CD44 (red, right), and CD90 (green) immunofluorescence staining (A), YAP, Col II, Col I, Col X immunohistochemistry staining and safranin O staining (B). (C) After intra-articular injection of rCSPC (5×10^6) overexpressing or silencing YAP, cartilage was evaluated by Col II, Col I, Col X immunohistochemistry, and safranin O staining. The severity of cartilage degeneration was evaluated by OARSI scoring. Each experiments was repeated three times. All rCSPC are derived from rat cartilage tissue. Cells proliferated to Passage 3 for experiments. Statistical significance was determined by ANOVA. Bars = 100 μm *p < 0.05; ***p < 0.001.

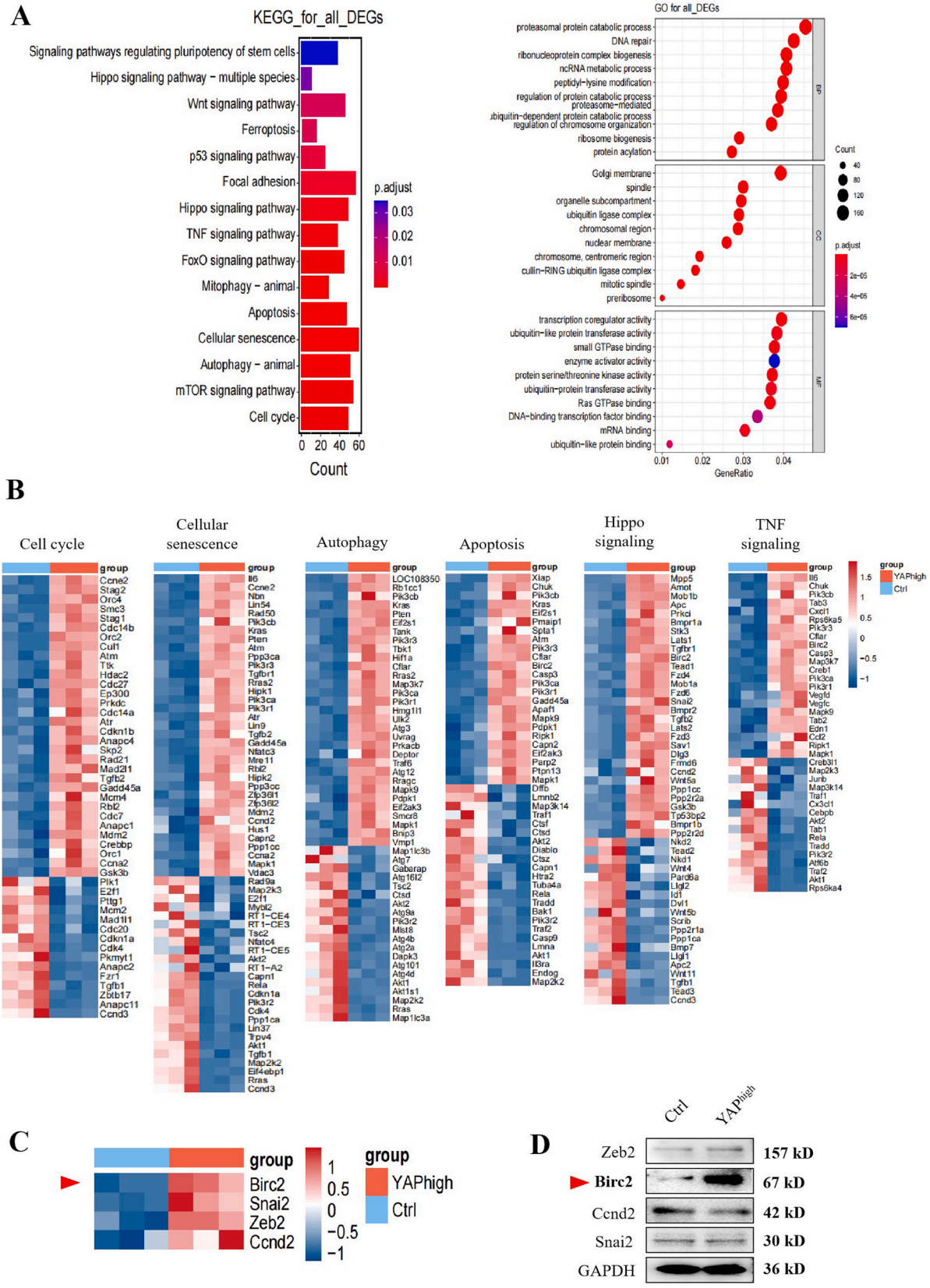

Figure 6. Transcriptomic analysis. (A) KEGG and GO enrichment analysis of DEGs. (B) The detailed DEGs that enriched in cell cycle, cellular senescence, autophagy, apoptosis, Hippo signaling pathway, and TNF signaling pathways. (C) The direct downstream target genes of YAP. (D) The protein level of Birc2, Snai2, Zenb2, and Ccnd2 in rCSPC. Transcriptomic analysis was performed with three biological replicates, and additional experiments were conducted with three technical replicates. All rCSPC are derived from rat cartilage tissue. Cells proliferated to Passage 3 for experiments. The cells from each rat were not pooled at the time of each experiment. Spearman correlation analysis was applied to evaluate correlations among continuous variables.

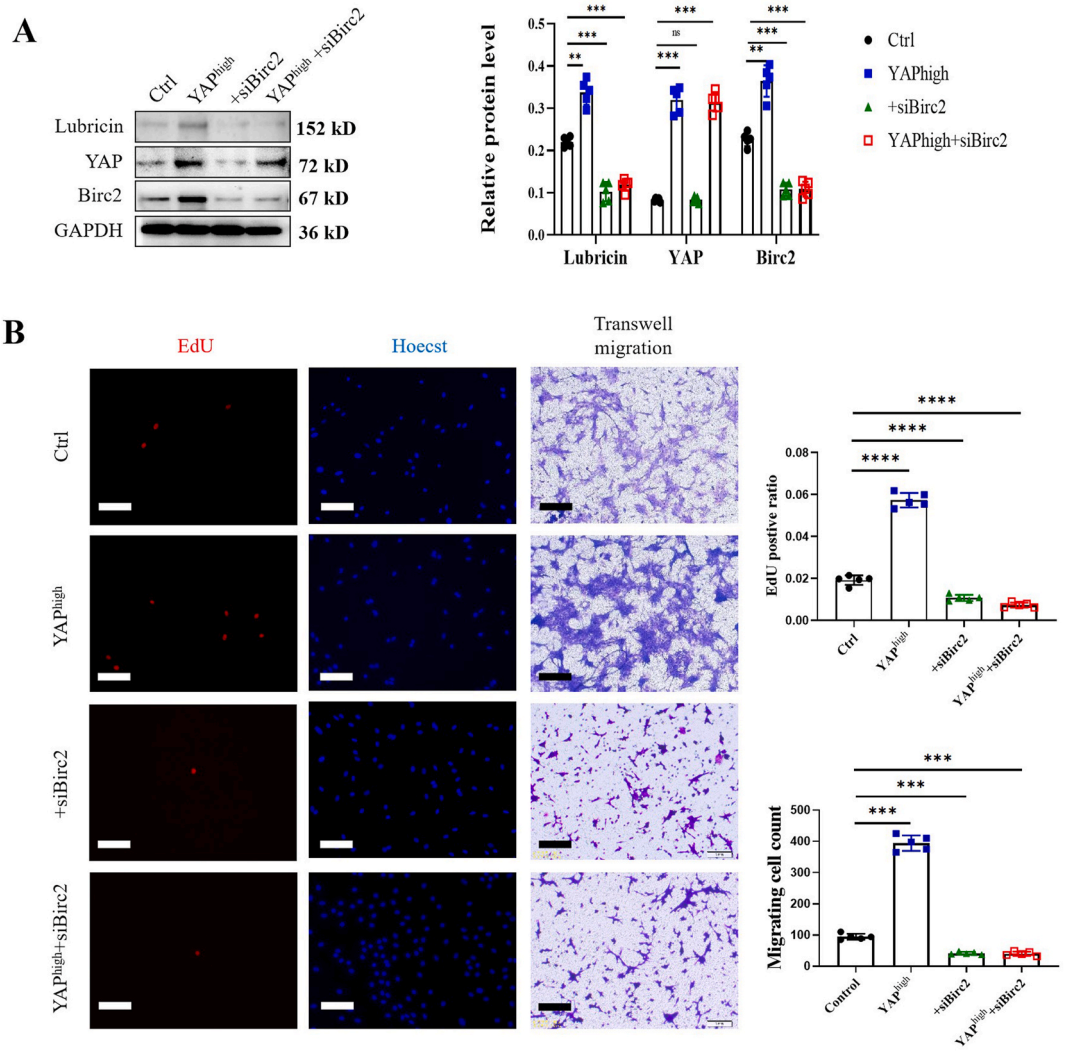

Figure 7. YAP regulates OA-rCSPC function by upregulating Birc2. (A) The expression of Lubricin, YAP, and Birc2 in rCSPC. The protein levels were normalized to GAPDH. (B) rCSPC were evaluated by EdU proliferation assay and transwell migration assay. All rCSPC are derived from rat cartilage tissue. Cells proliferated to Passage 3 for experiments. The cells were counted in five random fields per well. Statistical significance was determined by ANOVA. Bars = 100 μm *p < 0.05; ***p < 0.001, ns: no significance.

Ethics approval and consent to participate

This study protocol was approved by the institutional review board (IRB) of the Third Xiangya Hospital, Central South University (Project title: YAP-BIRC2 axis maintains cartilage stem/progenitor cell homeostasis in osteoarthritis. No: I 22,257. Date: 11/11/2022). The *in vivo* study was conducted with the full approval of the Ethics Committee for Animal Experiments in Central South University (Project title: Effect of YAP on the function of cartilage stem progenitor cells and its mechanism. No: 2019sydw209. Date: 24/10/2019). All experiments involving human tissues and animals were performed per the IRB's guidelines. Each sample was processed only after receiving a signed informed consent form.

Author contribution

LZ conceived and performed most of the experiments. XW wrote the manuscript. GX and JH collected and analyzed the data. ZW and CL raised the animals. XC and YZ provided experimental advice and supervised the study; SW provided funding support. All authors read and approved the final manuscript.

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Declaration of competing interest

The authors declare that they have no competing interests.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jot.2024.03.004.

References

[1] Sharma L. Osteoarthritis of the knee. N Engl J Med 2021;384:51–9.
[2] Hu H, Liu W, Sun C, Wang Q, Yang W, Zhang Z, et al. Endogenous repair and regeneration of injured articular cartilage: a challenging but promising therapeutic strategy. Aging Dis 2021;12:886–901.
[3] McGonagle D, Baboolal TG, Jones E. Native joint-resident mesenchymal stem cells for cartilage repair in osteoarthritis. Nat Rev Rheumatol 2017;13:719–30.
[4] Koelling S, Kruegel J, Irmer M, Path JR, Sadowski B, Miro X, et al. Migratory chondrogenic progenitor cells from repair tissue during the later stages of human osteoarthritis. Cell Stem Cell 2009;4:324–35.
[5] Candela ME, Yasuhara R, Iwamoto M, Enomoto-Iwamoto M. Resident mesenchymal progenitors of articular cartilage. Matrix Biol 2014;39:44–9.
[6] Jiang Y, Tuan RS. Origin and function of cartilage stem/progenitor cells in osteoarthritis. Nat Rev Rheumatol 2015;11:206–12.
[7] Jiang Y, Hu C, Yu S, Yan J, Peng H, Ouyang HW, et al. Cartilage stem/progenitor cells are activated in osteoarthritis via interleukin-1β/nerve growth factor signaling. Arthritis Res Ther 2015;17:327.
[8] Fellows CR, Williams R, Davies IR, Gohil K, Baird DM, Fairclough J, et al. Characterisation of a divergent progenitor cell sub-populations in human osteoarthritic cartilage: the role of telomere erosion and replicative senescence. Sci Rep 2017;7:41421.
[9] Jasper H. Intestinal stem cell aging: origins and interventions. Annu Rev Physiol 2020;82:203–26.
[10] Neumann B, Baror R, Zhao C, Segel M, Dietmann S, Rawji KS, et al. Metformin restores CNS remyelination capacity by rejuvenating aged stem cells. Cell Stem Cell 2019;25:473–485.e8.
[11] Altman R, Asch E, Bloch D, Bole G, Borenstein D, Brandt K, et al. Development of criteria for the classification and reporting of osteoarthritis. Classification of osteoarthritis of the knee. Diagnostic and Therapeutic Criteria Committee of the American Rheumatism Association. Arthritis Rheum 1986;29:1039–49.
[12] Kellgren JH, Lawrence JS. Radiological assessment of osteo-arthrosis. Ann Rheum Dis 1957;16:494–502.
[13] Cao X, Luo P, Huang J, Liang C, He J, Wang Z, et al. Intraarticular senescent chondrocytes impair the cartilage regeneration capacity of mesenchymal stem cells. Stem Cell Res Ther 2019;10:86.
[14] Moya IM, Halder G. Hippo-YAP/TAZ signalling in organ regeneration and regenerative medicine. Nat Rev Mol Cell Biol 2019;20:211–26.
[15] Tong W, Geng Y, Huang Y, Shi Y, Xiang S, Zhang N, et al. In vivo identification and induction of articular cartilage stem cells by inhibiting NF-κB signaling in osteoarthritis. Stem Cell 2015;33:3125–37.
[16] Dowthwaite GP, Bishop JC, Redman SN, Khan IM, Rooney P, Evans DJR, et al. The surface of articular cartilage contains a progenitor cell population. J Cell Sci 2004;117:889–97.
[17] Alsalameh S, Amin R, Gemba T, Lotz M. Identification of mesenchymal progenitor cells in normal and osteoarthritic human articular cartilage. Arthritis Rheum 2004;50:1522–32.
[18] Grogan SP, Barbero A, Diaz-Romero J, Cleton-Jansen A-M, Soeder S, Whiteside R, et al. Identification of markers to characterize and sort human articular chondrocytes with enhanced in vitro chondrogenic capacity. Arthritis Rheum 2007;56:586–95.
[19] Seol D, McCabe DJ, Choe H, Zheng H, Yu Y, Jang K, et al. Chondrogenic progenitor cells respond to cartilage injury. Arthritis Rheum 2012;64:3626–37.
[20] Grogan SP, Duffy SF, Pauli C, Koziol JA, Su AI, D'Lima DD, et al. Zone-specific gene expression patterns in articular cartilage. Arthritis Rheum 2013;65:418–28.
[21] Waller KA, Zhang LX, Elsaid KA, Fleming BC, Warman ML, Jay GD. Role of lubricin and boundary lubrication in the prevention of chondrocyte apoptosis. Proc Natl Acad Sci U S A 2013;110:5852–7.
[22] Elsaid KA, Jay GD, Warman ML, Rhee DK, Chichester CO. Association of articular cartilage degradation and loss of boundary-lubricating ability of synovial fluid following injury and inflammatory arthritis. Arthritis Rheum 2005;52:1746–55.
[23] Brack AS, Rando TA. Tissue-specific stem cells: lessons from the skeletal muscle satellite cell. Cell Stem Cell 2012;10:504–14.
[24] Jang KW, Ding L, Seol D, Lim T-H, Buckwalter JA, Martin JA. Low-intensity pulsed ultrasound promotes chondrogenic progenitor cell migration via focal adhesion kinase pathway. Ultrasound Med Biol 2014;40:1177–86.
[25] Xia H, Liang C, Luo P, Huang J, He J, Wang Z, et al. Pericellular collagen I coating for enhanced homing and chondrogenic differentiation of mesenchymal stem cells in direct intra-articular injection. Stem Cell Res Ther 2018;9:174.
[26] Liang C, Huang J, Luo P, Wang Z, He J, Wu S, et al. Platelet-derived microparticles mediate the intra-articular homing of mesenchymal stem cells in early-stage cartilage lesions. Stem Cell Dev 2020;29:414–24.
[27] McKay BR, Ogborn DI, Bellamy LM, Tarnopolsky MA, Parise G. Myostatin is associated with age-related human muscle stem cell dysfunction. FASEB J 2012;26:2509–21.
[28] LeBlanc L, Ramirez N, Kim J. Context-dependent roles of YAP/TAZ in stem cell fates and cancer. Cell Mol Life Sci 2021;78:4201–19.
[29] Fu L, Hu Y, Song M, Liu Z, Zhang W, Yu F-X, et al. Up-regulation of FOXD1 by YAP alleviates senescence and osteoarthritis. PLoS Biol 2019;17:e3000201.
[30] Li P, Chen Y, Mak KK, Wong CK, Wang CC, Yuan P. Functional role of Mst1/Mst2 in embryonic stem cell differentiation. PLoS One 2013;8:e79867.
[31] Qin H, Blaschke K, Wei G, Ohi Y, Blouin L, Qi Z, et al. Transcriptional analysis of pluripotency reveals the Hippo pathway as a barrier to reprogramming. Hum Mol Genet 2012;21:2054–67.
[32] Meng H, Fu S, Ferreira MB, Hou Y, Pearce OM, Gavara N, et al. YAP activation inhibits inflammatory signalling and cartilage breakdown associated with reduced primary cilia expression. S1063-4584(22)00913-X Osteoarthritis Cartilage 2022.
[33] Zhang X, Cai D, Zhou F, Yu J, Wu X, Yu D, et al. Targeting downstream subcellular YAP activity as a function of matrix stiffness with Verteporfin-encapsulated chitosan microsphere attenuates osteoarthritis. Biomaterials 2020;232:119724.
[34] Deng Y, Lu J, Li W, Wu A, Zhang X, Tong W, et al. Reciprocal inhibition of YAP/TAZ and NF-κB regulates osteoarthritic cartilage degradation. Nat Commun 2018;9:4564.
[35] Yui S, Azzolin L, Maimets M, Pedersen MT, Fordham RP, Hansen SL, et al. YAP/TAZ-Dependent reprogramming of colonic epithelium links ECM remodeling to tissue regeneration. Cell Stem Cell 2018;22:35–49.e7.
[36] Gregorieff A, Liu Y, Inanlou MR, Khomchuk Y, Wrana JL. Yap-dependent reprogramming of Lgr5(+) stem cells drives intestinal regeneration and cancer. Nature 2015;526:715–8.
[37] Kuyinu EL, Narayanan G, Nair LS, Laurencin CT. Animal models of osteoarthritis: classification, update, and measurement of outcomes. J Orthop Surg Res 2016;11:19.
[38] Yuan C, Pan Z, Zhao K, Li J, Sheng Z, Yao X, et al. Classification of four distinct osteoarthritis subtypes with a knee joint tissue transcriptome atlas. Bone Res 2020;8:38.
[39] Gil-Kulik P, Świstowska M, Kondracka A, Chomik P, Krzyżanowski A, Kwaśniewska A, et al. Increased expression of BIRC2, BIRC3, and BIRC5 from the IAP family in mesenchymal stem cells of the umbilical Cord Wharton's Jelly (WJSC) in younger women giving birth naturally. Oxid Med Cell Longev 2020;2020:9084730.
[40] Darling EM, Athanasiou KA. Rapid phenotypic changes in passaged articular chondrocyte subpopulations. J Orthop Res 2005;23:425–32.