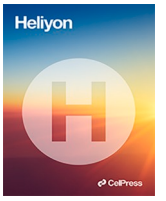
Heliyon 9 (2023) e17312

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon



CircSFMBT2-OA alleviates chondrocyte apoptosis and extracellular matrix degradation through repressing NF-κB/NLRP3 inflammasome activation

Axiang He, Yaru Liu, Renbo Zhang, Yanjie Mao, Wanjun Liu *

Department of Orthopedics, Shanghai Sixth People's Hospital Affiliated to Shanghai Jiao Tong University School of Medicine, Shanghai, China

ARTICLE INFO

Keywords:
Osteoarthritis
circSFMBT2-OA
Inflammation
Chondrocyte injury
NLRP3

ABSTRACT

Background: Intra-articular inflammation and cartilage degradation are the major pathological characteristics of osteoarthritis (OA). Mounting studies have revealed that circular RNAs (circRNAs) act as an important regulatory role in inflammatory diseases and are frequently dys-expressed in OA cartilage tissues.

Objective: Here, a dys-regulated cicrRNA (has_circ_0017636, termed circSFMBT2-OA) was identified, and its role in regulating lipopolysaccharide (LPS)-induced chondrocyte injury was next investigated.

Methods: CHON-001 chondrocytes were treated with LPS, and then the levels of circSFMBT2-OA, cartilage-related genes, and pro-inflammatory cytokines were measured using quantitative real-time PCR (qRT-PCR) and Western blot analysis. CHON-001 cell viability, proliferation, and apoptosis were assayed using Cell Counting Kit-8 (CCK-8), 5-Ethynyl-2′-deoxyuridine (EDU), and terminal deoxynucleotidyl transferase (TdT) dUTP nick-end labeling (TUNEL) assay, respectively.

Results: CircSFMBT2-OA level was significantly down-regulated in OA cartilage tissues and LPS-treated CHON-001 cells. Functionally, circSFMBT2-OA overexpression accelerated cell proliferation, and suppressed cell apoptosis, pro-inflammatory cytokines production, matrix-degrading enzymes expression, and ECM degradation in CHON-001 cells. Inversely, circSFMBT2-OA depletion decreased cell viability and increased matrix-degrading enzymes expression and ECM degradation. Mechanistically, circSFMBT2-OA inhibited LPS-induced NF-κB/NOD-like receptor family pyrin domain containing protein 3 (NLRP3) inflammasome activation in CHON-001 cells. Consequently, NLRP3 activator reversed the effect of circSFMBT2-OA on repressing LPS-induced CHON-001 cell injury.

Conclusion: These data reveal a vital effect of a novel circSFMBT2-OA on repressing OA progression and provide a promising target to treat OA.

1. Introduction

Osteoarthritis (OA) is the most frequent degenerative joint disorder in the world, particularly people aged 65 years or older [1–3]. Aging, mechanical injury, and obesity are the three main risk factors of OA, which results in cartilage degradation, joint loading, and

* Corresponding author. Department of orthopedics, Shanghai Sixth People's Hospital Affiliated to Shanghai Jiao Tong University School of Medicine, No.222, West Huanhu third road, Pudong New Area, Shanghai,201306, China.
E-mail address: 7250099781@shsmu.edu.cn (W. Liu).

https://doi.org/10.1016/j.heliyon.2023.e17312
Received 22 November 2022; Received in revised form 8 June 2023; Accepted 13 June 2023
Available online 16 June 2023

intra-articular inflammation [4,5]. Patients with OA commonly required medical intervention because articular cartilage has a poor intrinsic ability for self-healing [6]. Although nonsteroidal anti-inflammatory drugs (NSAIDs), opioids, and surgical operation are hopeful therapeutic strategy for pharmaceutical interventions in OA [7], the side effects and potential complication hamper their widespread application.

circRNA is a kind of circular non-coding RNA transcript with a covalently closed-loop structure and generated through back-splicing of precursor mRNA (pre-mRNA) [8]. CircRNA is stable and resistant to exonuclease due to lack of 5′ Cap and 3′ poly (A) tail [9]. With the progress of RNA sequencing and bioinformatical approache, plenty of functional circRNAs were identified, and circRNAs are no longer seemed as transcriptional noise due to lack of protein-coding potential [10]. CircRNAs act as an important role in a variety of physio-pathological processes including development, immunity, cell survival, cell apoptosis, tumorigenesis, cardiovascular diseases, and inflammation-related diseases [11–16]. CircRNAs have been found to frequently dys-regulated in OA cartilage tissues. For instance, Liu et al. revealed that 104 circRNAs were dys-regulated, of which 44 circRNAs were increased and 60 circRNAs were decreased in OA cartilage [17]. In IL-1β-treated chondrocytes, 255 circRNAs were dys-regulated, of which 119 circRNAs were significantly increased and 136 circRNAs were decreased [18]. The biological role of circRNA in OA progression has also been revealed. CircSERPINE2 level is remarkably decreased in OA tissues, and circSERPINE2 silencing accelerates chondrocyte apoptosis, while circSERPINE2 over-expression decreases IL-1β-induced chondrocyte apoptosis [19]. CircSERPINE2 also decreases IL-1β-induced matrix-degrading enzymes expression and ECM degradation [19].

According to the annotation of circBase, the alternative splicing pre-mRNA of Scm like with four mbt domains 2 (SFMBT2) generates many kinds of circRNAs. The functional of several SFMBT2-derived circRNAs have been identified. For example, SFMBT2-derived hsa_circ_0017639 facilitates gastric cancer progression by inhibiting miR-182-5p and thus increasing CREB1 expression [20]. Hsa_circ_0000212, derived from exon 2–4 of SFMBT2, accelerates vascular smooth muscle cell proliferation and migration [21]. SFMBT2-derived hsa_circ_0000211 promotes esophageal cancer cell proliferation through regulating miR-107/SLC1A5 axis [22].

In the study, a novel SFMBT2-derived circRNA, has_circ_0017636 (termed circSFMBT2-OA), was identified as down-regulated circRNA in OA tissues. CircSFMBT2-OA contributes to alleviate chondrocyte injury through suppressing NF-κB/NLRP3 inflammasome activation.

2. Materials and methods

2.1. OA cartilage tissues

Cartilage tissues were collected from 11 OA patients (age 59.4 ± 5.6 years) and 9 traumatic amputees without history of OA or rheumatoid disorder. These tissue specimens was collected with the approval of the Ethics Committee of Shanghai Sixth People's Hospital with informed consent (Approval No : 2021-001) .

2.2. Cell culture

A chondrocyte cell line, CHON-001, was obtained from the ATCC (VA, USA), used at passage 3–11, and maintained in DMEM (Beyotime, Shanghai, China) containing 10% FBS (Beyotime) in a humidified CO2 incubator at 37 °C.

2.3. Over-expression and knockdown

A recombinant plasmid, pcDNA-circSFMBT2-OA which contains Exon 3–7 of SFMBT2 gene and partial flanking intron, was constructed in our laboratory to over-express circSFMBT2-OA. Small interfering RNA (siRNA) against circSFMBT2-OA (si-circSFMBT2-OA, UUUGAUUGCUUUGGGAGGAGUdUdU) was used to silence circSFMBT2-OA in CHON-001 cells with Lipofectamine RNAiMAX reagent (Invitrogen, CA, USA).

2.4. qRT-PCR

RNA was isolated from CHON-001 cells or cartilage tissues using TRIzol reagent (Thermo Fisher Scientific, MA, USA). First-strand cDNA was synthesized using M-MLV reverse transcriptase (Invitrogen) and Oligo dT primers (or random primers) following the manufacturer's instructions. qRT-PCR was performed using Power SYBR™ Green PCR mix (Thermo Fisher Scientific) on 7500 Real-time qPCR System. β-actin was applied as internal reference, and relative mRNA level was calculated using 2-ΔΔCt equation. Primer sequences were showed in Supporting Table S1.

2.5. CCK-8

CHON-001 cell viability was measured using CCK-8 reagent (Beyotime). CHON-001 cells were plated into 96-well plates (~2 × 104 cells/well), and then were treated with LPS (5 μg/mL) and other indicated reagents (pcDNA-circSFMBT2-OA, si-circSFMBT2-OA, MCC950, etc.). After incubation for 48 h, cells were treated with 10 μL of CCK-8 for 60 min. Then absorbance at 450 nm was read with a BK-EL10C microplate reader (BioBase, Shandong, China).

2.6. TUNEL and Edu assay

TUNEL and Edu assay were applied to measure cell apoptosis and proliferation, respectively. In brief, CHON-001 cells were plated into 12-well plates and treated with LPS (5 μg/mL) for 48 h after circSFMBT2-OA over-expression. Then cells were fixed with 4% PFA for 15 min and stained with TUNEL or Edu reagent (Beyotime) for 55 min at room temperature. Nuclei were dyed with 4′,6-diamidino-2-phenylindole (DAPI, Beyotime). Fluorescence signal was collected with a XSPY-3201LED fluorescent microscope (Shanghai CSOIF Co., LTD, Shanghai, China).

2.7. Western blot

CHON-001 cells were lysed with RIPA buffer (Beyotime) and total protein was collected. Approximately 20 μg of protein were separated using 10% SDS-PAGE and then electro-transferred to PVDF membranes (Roche, Basel, Switzerland). After blocking with blocking buffer for western blotting (Thermo Fisher Scientific), membranes were incubated with primary antibodies against MMP-13 (1:2000, PA5-27242, Thermo Fisher Scientific), ADAMTS-5 (1:1000, PA5-14350, Thermo Fisher Scientific), Collagen II (1:2000, MA5-12789, Thermo Fisher Scientific), NLRP3 (1:1500, MA5-23919, Thermo Fisher Scientific), cleaved-caspase-1 (1:1000, PA5-105049, Thermo Fisher Scientific), and β-actin (1:4000, ab8226, Abcam, CA, USA) overnight at 4 °C. After washing thrice with PBST, membranes were incubated with HRP-labelled anti-mice/rabbit IgG secondary antibody. The protein blots were visualized with an enhanced chemiluminescence reagent (Pierce, IL, USA).

2.8. Fluorescence in situ hybridization (FISH)

RNA-FISH probes against circSFMBT2-OA (circSFMBT2-OA-probes) were synthesized by Sangon (Shanghai, China). CHON-001 cells were fixed by 4% PFA for 20 min and treated with proteinase K, glycine and acetic anhydride. Pre-hybridization was carried out for 50 min followed by hybridization with 300 ng/mL of circSFMBT2-OA-probes. Nuclei were dyed with DAPI. Fluorescence signal was collected with a XSPY-3201LED fluorescent microscope.

2.9. Statistics

Three independent experiments were carried out and data are represented as mean ± SD. GraphPad Prism 7.0 (CA, USA) was used

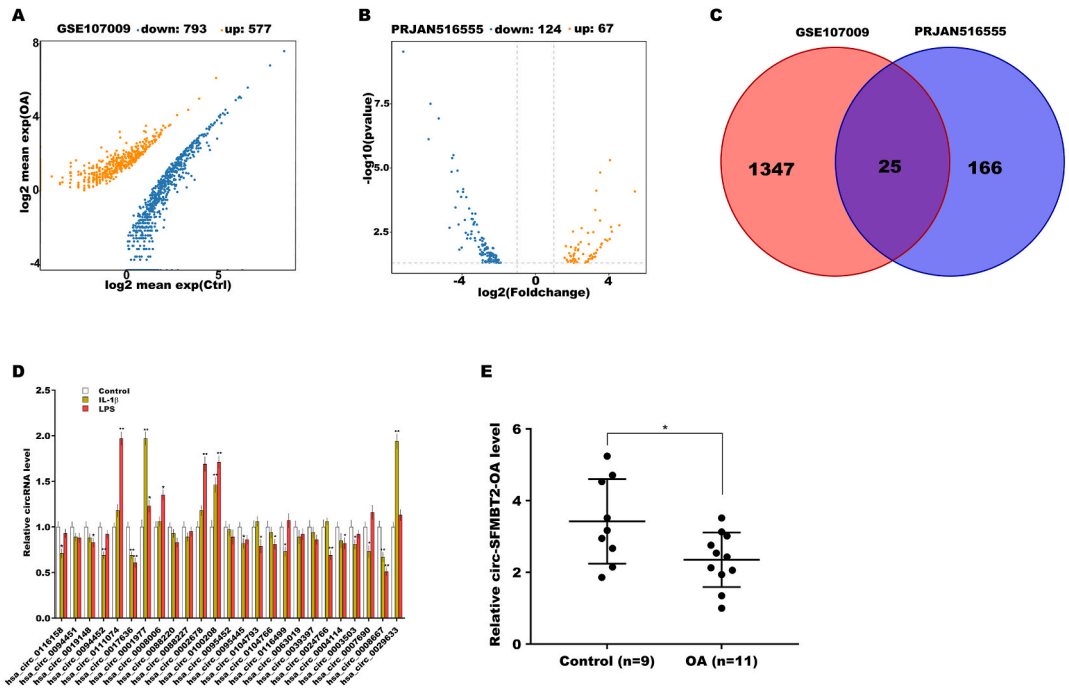


Fig. 1. CircSFMBT2-OA was down-regulated in OA cartilages. (A) Dys-regulated circRNAs in chondrocytes after IL-1β treatment were represented by volcano plots (prob ≥0.8, log2 |FC| ≥ 1). (B) Dys-regulated circRNAs between OA and normal cartilage tissues were represented by volcano plots ($p < 0.05$, |FC| ≥2). (C) Venn diagram analysis revealed 25 circRNAs appeared in GSE107009 and PRJAN516555 datasets. (D) CHON-001 cells were treated with LPS (5 μg/mL) or IL-1β (10 ng/mL) and relative circRNAs level was assessed after 24 h using qRT-PCR analysis. (E) qRT-PCR analysis of circSFMBT2-OA level in OA cartilage (n = 11) and normal cartilage (n = 9). *$p < 0.05$, **$p < 0.01$.

to compared the difference among groups using ANOVA followed by the Scheffé test or student's t-test using GraphPad Prism 7.0 (CA, USA). The difference was seemed as significant when $p < 0.05$.

3. Results

3.1. CircSFMBT2-OA level was decreased in OA cartilage tissues

To identify the differentially expressed circRNAs in OA progression, two datasets were downloaded from Gene Expression Omnibus database (GSE107009) and Sequence Read Archive (SRA) database (Accession No. PRJAN516555), respectively. In GSE107009, 1,370 circRNAs were dys-regulated, of which 577 circRNAs were increased and 793 circRNAs were decreased (Fig. 1A, Supporting Table S2 (prob $\geq$0.8, log2 |FC| $\geq$ 1). In PRJAN516555, 191 circRNAs were dys-regulated, of which 67 circRNAs were up-regulated and 124 circRNAs were down-regulated (Fig. 1B, Supporting Table S3 ($p < 0.05$, |FC| $\geq$2)). Venn diagram analysis showed that 25 circRNAs appeared in the two datasets, including 6 up-regulated and 19 down-regulated circRNAs (Fig. 1C). To validate the results, the levels of 25 circRNAs were assessed in in vitro model of OA using qRT-PCR. As shown in Fig. 1D, has_circ_0017636 and has_circ_00866 were down-regulated, and has_circ_0100208 was up-regulated in both LPS- and IL-1β-treated CHON-001 cells. In the study, has_circ_0017636, derived from exon 3–7 of SFMBT2 gene (Thereafter named circSFMBT2-OA), was chosen for further study because the circRNAs derived from SFMBT2 gene have been found to possess various biological functions [20,21,23,24]. CircSFMBT2-OA level was next assessed in OA tissues and normal cartilage tissues. Fig. 1E revealed that circSFMBT2-OA level was significantly decreased in OA tissues.

3.2. Characterization of circSFMBT2-OA

According to the annotation of circBase database [25], circSFMBT2-OA is derived from exon 3–7 of SFMBT2 gene. This is a novel circRNA transcript differs from previously reported circSFMBT2 [20,21,23,24]. Therefore it was named circSFMBT2-OA, and the back-splicing site of circSFMBT2-OA was further validated by Sanger sequencing (Fig. 1A). Then convergent primers were used to amplify SFMBT2 mRNA and divergent primers were used to amplify circSFMBT2-OA in cDNA and genomic DNA (gDNA). Fig. 2B revealed that circSFMBT2-OA can only be amplified by divergent primers in cDNA, indicating that linear-splicing site existed in both cDNA and gDNA, but the back-splicing site only existed in cDNA. As shown in Fig. 2C, circSFMBT2-OA was resistant to RNase R, suggesting that circSFMBT2-OA was a circRNA transcript because of the sensitivity of linear transcript to RNase R. Fig. 2D revealed that circSFMBT2-OA can only be amplified by random primers but not oligo (dT)18 primers, suggesting that circSFMBT2-OA didn't

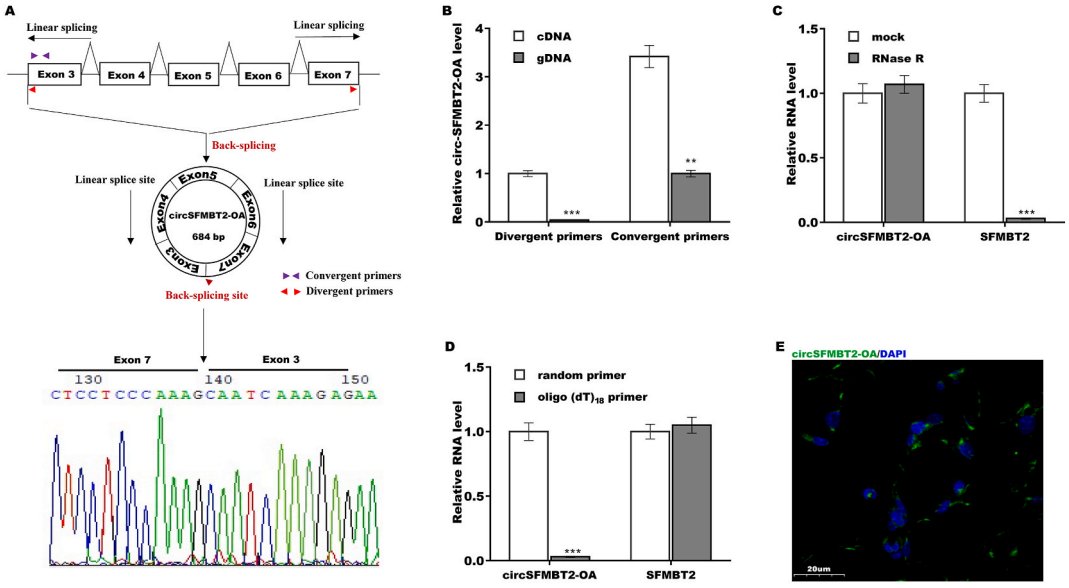


Fig. 2. Characterization of circSFMBT2-OA. (A) Schematic diagram illustrating the circularization of exon 3–7 of SFMBT2 to generate circSFMBT2-OA. Convergent primers (purple triangles) were applied to amplify linear sequence of circSFMBT2-OA, and Divergent primers (red triangles) were applied to amplify back-splicing site of circSFMBT2-OA. CircSFMBT2-OA was assessed using qRT-PCR, followed by Sanger sequencing. (B) cDNA was synthesized using Convergent primers and Divergent primers, respectively, and then CircSFMBT2-OA was assessed using qRT-PCR in gDNA and cDNA. (C) qRT-PCR analysis of circSFMBT2-OA and SFMBT2 levels after RNase R treatment. (D) cDNA was synthesized using Oligo dT primers and random primers, respectively, and then circSFMBT2-OA and SFMBT2 levels were assessed using qRT-PCR. (E) FISH were performed to measure the cellular localization of circSFMBT2-OA in CHON-001 cells. CircSFMBT2-OA-probes were dyed green and the nucleus was stained blue. **$p < 0.01$, ***$p < 0.001$. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

possess poly (A) tail (Fig. 2D). FISH assay revealed that circSFMBT2-OA mainly located in the cytoplasm of CHON-001 cells (Fig. 2E).

3.3. CircSFMBT2-OA decreased LPS-induced CHON-001 cell injury

To explore the biological function of circSFMBT2-OA on regulating chondrocyte injury, CHON-001 cells were over-expressed with circSFMBT2-OA and treated with LPS, and then cell viability, proliferation, apoptosis, pro-inflammatory cytokines production, matrix-degrading enzymes expression, and ECM degradation were assessed. PcDNA-circSFMBT2-OA transfection significantly up-regulated circSFMBT2-OA level, but not SFMBT2 (Fig. 3A). Functionally, LPS treatment accelerated CHON-001 cell apoptosis, whereas circSFMBT2-OA over-expression reversed the effect (Fig. 3B and C). The results from CCK-8 and Edu assay revealed that circSFMBT2-OA over-expression also blocked the inhibitory effect of LPS on cell viability (Fig. 3D) and proliferation (Fig. 3E and F) in CHON-001 cells. In an addition, the expression of cartilage-degrading enzymes (MMP-13 and ADAMTS-5) was up-regulated, while collagen II expression was decreased in CHON-001 cells after LPS treatment (Fig. 3G and H). Importantly, this impact was significantly reversed by circSFMBT2-OA over-expression (Fig. 3G and H). CircSFMBT2-OA over-expression further reversed the inhibitory role of LPS in pro-inflammatory cytokines production (Fig. 3I). These results demonstrate that circSFMBT2-OA alleviates LPS-induced CHON-001 cell injury.

3.4. CircSFMBT2-OA attenuated LPS-induced NF-κB/NLRP3 inflammasome activation

Given the vital role of NF-kB signaling in OA progression [26,27], the effect of circSFMBT2-OA on regulating LPS-induced NF-kB

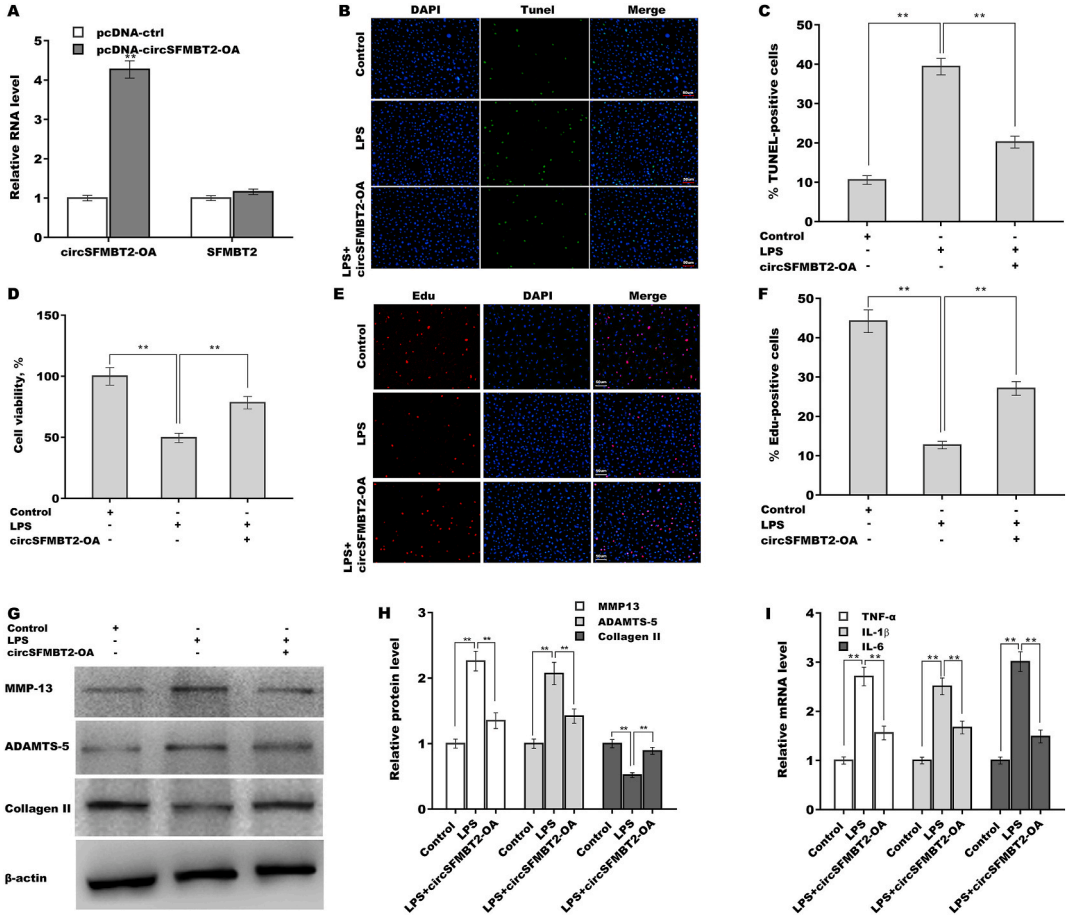


Fig. 3. CircSFMBT2-OA decreased LPS-induced CHON-001 cell injury. (A) CHON-001 cells were transfected with pcDNA-circSFMBT2-OA for 48 h, and then circSFMBT2-OA and SFMBT2 levels were assessed using qRT-PCR. CHON-001 cells were treated with LPS (5 μg/mL) after circSFMBT2-OA overexpression, and then TUNEL assay (B) and quantitative analysis (C) was assessed. (D) CHON-001 cells were treated with LPS (5 μg/mL) after circSFMBT2-OA overexpression, and then cell viability was assessed using CCK-8. CHON-001 cells were treated with LPS (5 μg/mL) after circSFMBT2-OA overexpression, and then Edu assay (E) and quantitative analysis (F) was assessed. Western blot (G) and quantitative analysis (H) of MMP-13, ADAMTS-5, and Collagen II protein expression in CHON-001 cells after treated with indicated reagents. (I) qRT-PCR analysis of TNF-α, IL-1β, and IL-6 mRNA level in CHON-001 cells after treated with indicated reagents. $^{**}p < 0.01$.

activation was next investigated. As shown in Fig. 4A and B, LPS treatment increased p-p65 level, whereas circSFMBT2-OA over--expression significantly reversed the effect, indicating that circSFMBT2-OA repressed LPS-induced NF-kB activation. NF-κB signaling activation is essential to NLRP3 inflammasome activation, which exerts a critical role in OA progression [28,29]. In CHON-001 cells, LPS treatment increased NLRP3 and activated caspase-1 protein expression, whereas circSFMBT2-OA over-expression reversed the effect (Fig. 4C and D). Moreover, circSFMBT2-OA also reversed the inhibitory role of LPS in IL-18 and IL-1β production (Fig. 4E). These data suggest that circSFMBT2-OA repressed LPS-induced NF-κB/NLRP3 inflammasome activation.

3.5. CircSFMBT2-OA attenuated CHON-001 cell injury through repressing NLRP3 activation

Finally, we explored whether circSFMBT2-OA attenuated chondrocyte injury through regulating NLRP3 activation. To this end, a NLRP3 activator, Nigericin, was applied to treat CHON-001 cells in the presence of circSFMBT2-OA over-expression. Fig. 5A and B showed that Nigericin treatment increased MMP-13 and ADAMTS-5 expression and decreased collagen II expression in circSFMBT2-OA-over-expressed CHON-001 cells. Nigericin also increased IL-18 and IL-1β expression in circSFMBT2-OA-over-expressed CHON-001 cells (Fig. 5C). In an addition, circSFMBT2-OA depletion increased NLRP3 and activated caspase-1 expression, whereas NLRP3 inhibitor, MCC950, reversed the effect (Fig. 5D–F). CircSFMBT2-OA depletion suppressed CHON-001 cell viability, whereas MCC950 reversed the effect (Fig. 5G). MCC950 further reversed the role of circSFMBT2-OA silencing in promoting ECM degradation (Fig. 5H). These results demonstrate that circSFMBT2-OA attenuated CHON-001 cell injury through negatively regulating NLRP3 activation.

4. Discussion

Although NSAIDs, opioids, and surgical operation are hopeful treatment for OA, the side effects and potential complication restrict their widespread application. To develop effective therapeutic strategy, it is essential to explore the mechanisms underlying OA progression. Mounting evidence has demonstrated that circRNAs exert a crucial role in the development and progression of OA [30]. In the current study, we identified the role of a novel circRNA, has_circ_0017636 (known also circSFMBT2-OA), in chondrocyte injury. The current results demonstrated that, i) CircSFMBT2-OA level was decreased in OA cartilage tissues, ii) CircSFMBT2-OA alleviated LPS-induced CHON-001 cell injury, iii) CircSFMBT2-OA repressed LPS-induced NF-κB/NLRP3 inflammasome activation, iv) CircSFMBT2-OA attenuated CHON-001 cell injury through repressing NLRP3 activation. These findings revealed the vital role of CircSFMBT2-OA/NLRP3 axis in chondrocyte injury, providing a promising opportunity to treat OA.

SFMBT2 dys-regulation is correlated with ECM degradation and OA. SFMBT2 expression is up-regulated in the early phases of chondrogenesis, and forced expression of SFMBT2 accelerates chondrocyte proliferation and increases SRY-box 9 (SOX9) expression

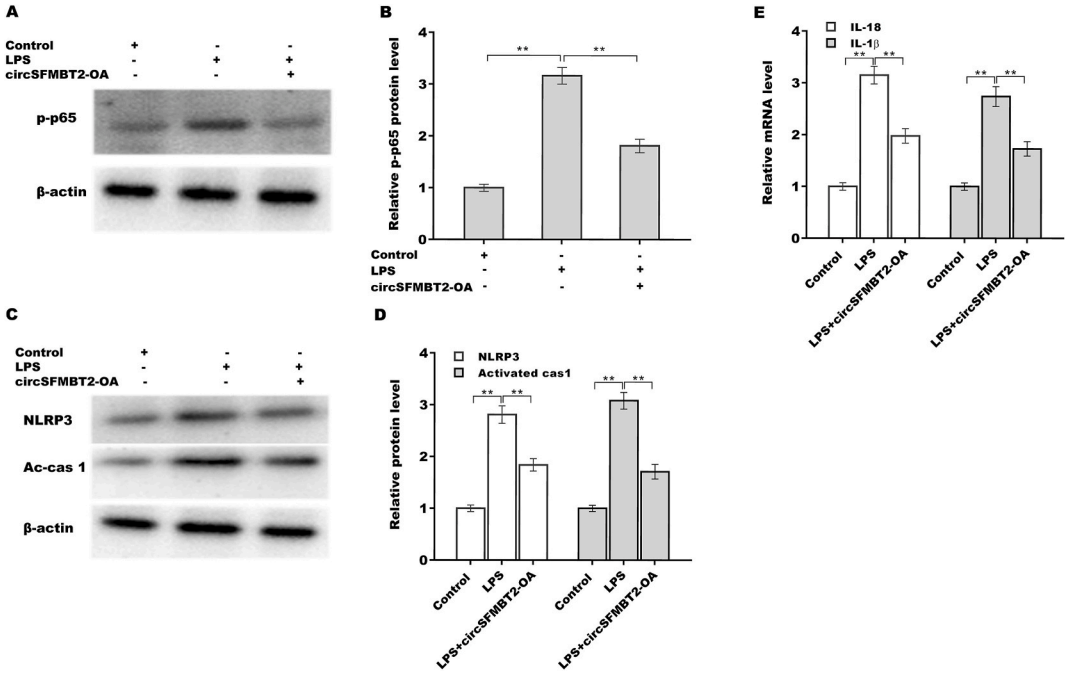


Fig. 4. CircSFMBT2-OA attenuated NF-κB/NLRP3 activation
Western blot (A) and quantitative analysis (B) of p-p65 level in CHON-001 cells after treated with LPS (5 μg/mL) in the presence or absence of circSFMBT2-OA overexpression. Western blot (C) and quantitative analysis (D) of NLRP3 and cleaved caspase-1 protein expression in CHON-001 cells after treated with indicated reagents. (E) qRT-PCR analysis of IL-18 and IL-1β mRNA level in CHON-001 cells after treated with indicated reagents. **$p < 0.01$.

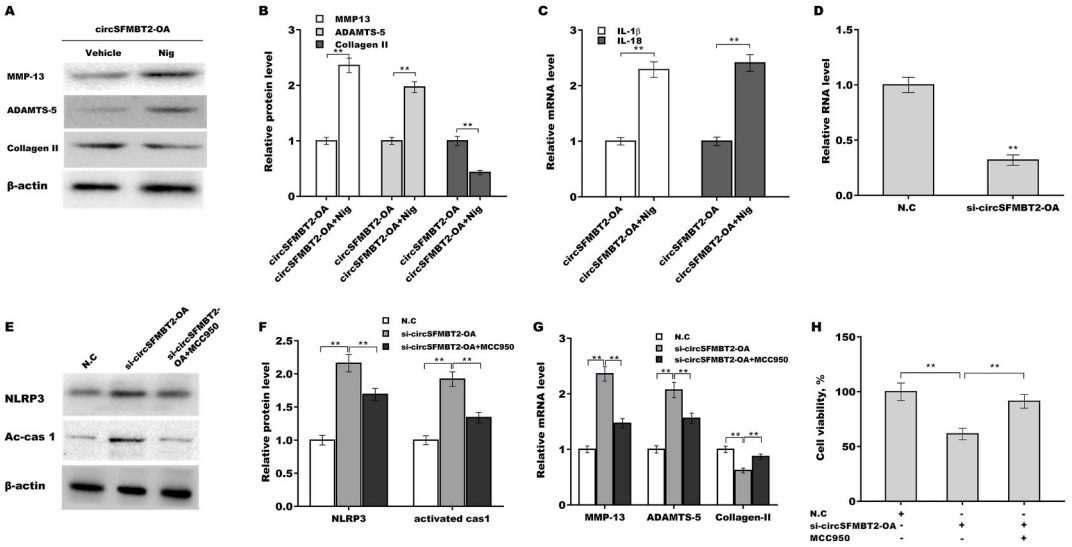


Fig. 5. CircSFMBT2-OA attenuated CHON-001 cell injury through repressing NLRP3 activation
Western blot (A) and quantitative analysis (B) of MMP-13, ADAMTS-5, and Collagen II protein expression in circSFMBT2-OA-overexpressed CHON-001 cells in the presence or absence of Nigericin (10 μM). (C) qRT-PCR analysis of IL-18 and IL-1β mRNA level in CHON-001 cells after treatment with indicated reagents. (D) qRT-PCR analysis of circSFMBT2-OA level in CHON-001 cells after treated with si-circSFMBT2-OA. Western blot (E) and quantitative analysis (F) of NLRP3 and cleaved caspase-1 protein expression in circSFMBT2-OA-depleted CHON-001 cells treated with MCC950 (8 μM). (G) CHON-001 cells were treated with si-circSFMBT2-OA and MCC950 (8 μM), and then cell viability was assessed using CCK-8. (H) qRT-PCR analysis of MMP-13, ADAMTS-5, and Collagen II mRNA level in CHON-001 cells after treated with indicated reagents. $^{*}p < 0.05$, $^{**}p < 0.01$.

[31]. Another study showed that SFMBT2 decreases ECM degradation through repressing NF-κB activation [32]. Given the vital role of SOX9 and NF-κB in maintaining chondrocyte proliferation and ECM production [33], SFMBT2 may be an important regulator in chondrocyte function and OA occurrence. Interestingly, the current study identified a novel alternative spliceosome of SFMBT2 gene, has_circ_0017636 (circSFMBT2-OA). CircSFMBT2-OA decreases chondrocyte apoptosis and ECM degradation through repressing NF-κB/NLRP3 activation.

CircRNA is generated by alternative splicing of pre-mRNA, which is a frequent way to increase transcriptome complexity [34]. Several alternative spliceosome, derived from SFMBT2 pre-mRNA, have been identified in many pathologic processes. Has_circ_0017639, derived from exon 5–8 of SFMBT2, promotes gastric cancer cell proliferation by increasing CREB1 expression [20]. Hsa_circ_0000212, derived from exon 2–4 of SFMBT2, accelerates vascular smooth muscle cell proliferation and migration [21]. Hsa_circ_0017620, derived from exon 5–15 of SFMBT2, contributes to lung cancer cell proliferation [35]. Hsa_circ_0000211, derived from exon 5–7 of SFMBT2, promotes lung adenocarcinoma cell invasion by increasing hypoxia-inducible factor (HIF)-1α expression [36]. At present, the role of these SFMBT2-derived circRNAs in OA remains unknown. In the study we demonstrated that circSFMBT2-OA is down-regulated in OA cartilages. Forced expression of circSFMBT2-OA increases chondrocyte viability, accelerates chondrocyte proliferation, and decreases cell apoptosis, inflammation, and ECM degradation through inhibiting NF-κB/NLRP3 inflammasome activation. Given the vital role of NF-kB/NLRP3 axis in OA progression [26–29], circSFMBT2-OA may be a promising target for pharmaceutical intervention in OA.

There are still several limitations in the study, i) Previous studies demonstrated that SFMBT2-derived circRNAs frequently exert their biological effect through acting as a competing endogenous RNA (ceRNA) to sponge miRNA via miRNA response elements (MREs). Therefore it is essential to further reveal the mechanism by which circSFMBT2-OA represses ECM degradation and OA progression. ii) It is necessary to explore the role of circSFMBT2-OA in repressing OA progression using an animal model of OA.

Declarations

Author contribution statement

Axiang He, Wanjun Liu: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

Yaru Liu: Conceived and designed the experiments; Performed the experiments; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Renbo Zhang: Performed the experiments.

Yanjie Mao: Performed the experiments; Contributed reagents, materials, analysis tools or data.

Funding statement

Dr. Wanjun Liu was supported by Shanghai Municipal Health Commission Health Aging Project (2020YJZX0120), Science and Technology Innovation Plan Of Shanghai Science and Technology Commission {21ZR1448900}.

Data availability statement

Data included in article/supp. material/referenced in article.

Ethics approval and consent to participate

Ethical approval for this study was obtained from the Ethics Committee of Shanghai Sixth People's Hospital with informed consent (Approval No : 2021-001). All donors provided written informed consent.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

Not Applicable.

Appendix ASupplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2023.e17312.

References

[1] S. Safiri, A.A. Kolahi, E. Smith, C. Hill, D. Bettampadi, M.A. Mansournia, D. Hoy, A. Ashrafi-Asgarabad, M. Sepidarkish, A. Almasi-Hashiani, G. Collins, J. Kaufman, M. Qorbani, M. Moradi-Lakeh, A.D. Woolf, F. Guillemin, L. March, M. Cross, Global, regional and national burden of osteoarthritis 1990-2017: a systematic analysis of the Global Burden of Disease Study 2017, Ann. Rheum. Dis. 6 (79) (2020) 819–828, https://doi.org/10.1136/annrheumdis-2019-216515.
[2] Global, regional, And national incidence, prevalence, and years lived with disability for 354 diseases and injuries for 195 countries and territories, 1990-2017: a systematic analysis for the Global Burden of Disease Study 2017, Lancet 10159 (392) (2018) 1789–1858, https://doi.org/10.1016/S0140-6736(18)32279-7.
[3] P. Kangeswari, J. Arulappan, Effectiveness of nurse-led intervention on quality of life of patients with knee osteoarthritis, J. Pat. Exp. 9 (2022), 23743735221098254, https://doi.org/10.1177/23743735221098254.
[4] B. Heidari, Knee osteoarthritis prevalence, risk factors, pathogenesis and features: Part I, Casp. J. Inter. Med. 2 (2) (2011) 205–212.
[5] S. Sudirman, P.S. Tseng, C.K. Chen, D. Tsou, Z.L. Kong, Seahorse protein hydrolysate ameliorates proinflammatory mediators and cartilage degradation on posttraumatic osteoarthritis with an obesity rat model, Biomed. Res. Int. (2022), 4117520, https://doi.org/10.1155/2022/4117520.
[6] N.K. Dubey, V.K. Mishra, R. Dubey, S. Syed-Abdul, J.R. Wang, P.D. Wang, W.P. Deng, Combating osteoarthritis through stem cell therapies by rejuvenating cartilage: a review, Stem Cells Int. (2018), 5421019, https://doi.org/10.1155/2018/5421019.
[7] D.J. Hunter, D.T. Felson, Osteoarthritis, BMJ 7542 (332) (2006) 639–642, https://doi.org/10.1136/bmj.332.7542.639.
[8] L.S. Kristensen, M.S. Andersen, L.V.W. Stagsted, K.K. Ebbesen, T.B. Hansen, J. Kjems, The biogenesis, biology and characterization of circular RNAs, Nat. Rev. Genet. 11 (20) (2019) 675–691, https://doi.org/10.1038/s41576-019-0158-7.
[9] C. Bao, D. Lyu, S. Huang, Circular RNA expands its territory, Mol. Cell. Oncol. 2 (3) (2016), e1084443, https://doi.org/10.1080/23723556.2015.1084443.
[10] H. Li, H.H. Yang, Z.G. Sun, H.B. Tang, J.K. Min, Whole-transcriptome sequencing of knee joint cartilage from osteoarthritis patients, Bone Joint Res. 7 (8) (2019) 290–303, https://doi.org/10.1302/2046-3758.87.BJR-2018-0297.R1.
[11] W. Chia, J. Liu, Y.G. Huang, C. Zhang, A circular RNA derived from DAB1 promotes cell proliferation and osteogenic differentiation of BMSCs via RBPJ/DAB1 axis, Cell Death Dis. 5 (11) (2020) 372, https://doi.org/10.1038/s41419-020-2572-3.
[12] A. Li, W.C. Wang, V. Mcalister, Q. Zhou, X. Zheng, Circular RNA in colorectal cancer, J. Cell Mol. Med. 8 (25) (2021) 3667–3679, https://doi.org/10.1111/jcmm.16380.
[13] F. Khanipouyani, H. Akrami, M.R. Fattahi, Circular RNAs as important players in human gastric cancer, Clin. Transl. Oncol. 1 (23) (2021) 10–21, https://doi.org/10.1007/s12094-020-02419-2.
[14] X. Gan, H. Zhu, X. Jiang, S.C. Obiegbusi, M. Yong, X. Long, J. Hu, CircMUC16 promotes autophagy of epithelial ovarian cancer via interaction with ATG13 and miR-199a, Mol. Cancer 1 (19) (2020) 45, https://doi.org/10.1186/s12943-020-01163-z.
[15] F. Wang, X. Chen, Y. Han, S. XI, G. Wu, circRNA CDR1as Regulated the Proliferation of Human Periodontal Ligament Stem Cells under a Lipopolysaccharide-Induced Inflammatory Condition, Mediators Inflamm, 2019, 2019, 1625381, https://doi.org/10.1155/2019/1625381.
[16] H. Li, J.D. Xu, X.H. Fang, J.N. Zhu, J. Yang, R. Pan, S.J. Yuan, N. Zeng, Z.Z. Yang, H. Yang, X.P. Wang, J.Z. Duan, S. Wang, J.F. Luo, S.L. Wu, Z.X. Shan, Circular RNA circRNA_000203 aggravates cardiac hypertrophy via suppressing miR-26b-5p and miR-140-3p binding to Gata4, Cardiovasc. Res. 7 (116) (2020) 1323–1334, https://doi.org/10.1093/cvr/cvz215.
[17] Q. Liu, X. Zhang, X. Hu, L. Yuan, J. Cheng, Y. Jiang, Y. Ao, Emerging roles of circRNA related to the mechanical stress in human cartilage degradation of osteoarthritis, Mol. Ther. Nucleic Acids 7 (2017) 223–230, https://doi.org/10.1016/j.omtn.2017.04.004.
[18] Z. Zhou, D. Du, A. Chen, L. Zhu, Circular RNA expression profile of articular chondrocytes in an IL-1beta-induced mouse model of osteoarthritis, Gene 644 (2018) 20–26, https://doi.org/10.1016/j.gene.2017.12.020.
[19] S. Shen, Y. Wu, J. Chen, Z. Xie, K. Huang, G. Wang, Y. Yang, W. Ni, Z. Chen, P. Shi, Y. Ma, S. Fan, CircSERPINE2 protects against osteoarthritis by targeting miR-1271 and ETS-related gene, Ann. Rheum. Dis. 6 (78) (2019) 826–836, https://doi.org/10.1136/annrheumdis-2018-214786.
[20] H. Sun, P. XI, Z. Sun, Q. Wang, B. Zhu, J. Zhou, H. Jin, W. Zheng, W. Tang, H. Cao, X. Cao, Circ-SFMBT2 promotes the proliferation of gastric cancer cells through sponging miR-182-5p to enhance CREB1 expression, Cancer Manag. Res. 10 (2018) 5725–5734, https://doi.org/10.2147/CMAR.S172592.

[21] Y. Luo, C. Huang, CircSFMBT2 facilitates vascular smooth muscle cell proliferation by targeting miR-331-3p/HDAC5, Life Sci. 264 (2021), 118691, https://doi.org/10.1016/j.lfs.2020.118691.
[22] Z. Chang, Y. Fu, Y. Jia, M. Gao, L. Song, W. Zhang, R. Zhao, Y. Qin, Circ-SFMBT2 drives the malignant phenotypes of esophageal cancer by the miR-107-dependent regulation of SLC1A5, Cancer Cell Int. 1 (21) (2021) 495, https://doi.org/10.1186/s12935-021-02156-8.
[23] H. Zhang, Y. Shen, Z. Li, Y. Ruan, T. Li, B. Xiao, W. Sun, The biogenesis and biological functions of circular RNAs and their molecular diagnostic values in cancers, J. Clin. Lab. Anal. 1 (34) (2020), e23049, https://doi.org/10.1002/jcla.23049.
[24] Y. He, Z. Zhang, Z. Wang, Y. Jiao, Q. Kang, J. Li, Downregulation of circ-SFMBT2 blocks the development of gastric cancer by targeting the miR-885-3p/CHD7 pathway, Anti Cancer Drugs 1 (33) (2022) e247–e259, https://doi.org/10.1097/CAD.0000000000001195.
[25] P. Glazar, P. Papavasileiou, N. Rajewsky, circBase: a database for circular RNAs, RNA 11 (20) (2014) 1666–1670, https://doi.org/10.1261/rna.043687.113.
[26] S. Rigoglou, A.G. Papavassiliou, The NF-kappaB signalling pathway in osteoarthritis, Int. J. Biochem. Cell Biol. 11 (45) (2013) 2580–2584, https://doi.org/10.1016/j.biocel.2013.08.018.
[27] J.A. Roman-Blas, S.A. Jimenez, NF-kappaB as a potential therapeutic target in osteoarthritis and rheumatoid arthritis, Osteoarthritis Cartilage 9 (14) (2006) 839–848, https://doi.org/10.1016/j.joca.2006.04.008.
[28] J. Chen, J. Yan, S. Li, J. Zhu, J. Zhou, J. Li, Y. Zhang, Z. Huang, L. Yuan, K. Xu, W. Chen, W. Ye, Atorvastatin inhibited TNF-alpha induced matrix degradation in rat nucleus pulposus cells by suppressing NLRP3 inflammasome activity and inducing autophagy through NF-kappaB signaling, Cell Cycle 20 (20) (2021) 2160–2173, https://doi.org/10.1080/15384101.2021.1973707.
[29] H. Xu, J. Zhang, X. Shi, X. Li, C. Zheng, NF-kappaB inducible miR-30b-5p aggravates joint pain and loss of articular cartilage via targeting SIRT1-FoxO3a-mediated NLRP3 inflammasome, Aging (Albany NY) 16 (13) (2021) 20774–20792, https://doi.org/10.18632/aging.203466.
[30] X. Mao, Y. Cao, Z. Guo, L. Wang, C. Xiang, Biological roles and therapeutic potential of circular RNAs in osteoarthritis, Mol. Ther. Nucleic Acids 24 (2021) 856–867, https://doi.org/10.1016/j.omtn.2021.04.006.
[31] S. Hussain, M. Sun, Y. Guo, N. Mushtaq, Y. Zhao, Y. Yuan, N. Hussain, E. Osoro, A. Suleiman, M. Sadiq, F. Zhang, Y. Han, J. Sun, S. Lu, SFMBT2 positively regulates SOX9 and chondrocyte proliferation, Int. J. Mol. Med. 6 (42) (2018) 3503–3512, https://doi.org/10.3892/ijmm.2018.3894.
[32] S. Hussain, M. Sun, Z. Min, Y. Guo, J. Xu, N. Mushtaq, L. Heng, H. Huang, Y. Zhao, Y. Yuan, N. Hussain, F. Zhang, Y. Han, P. Xu, J. Sun, S. Lu, Down-regulated in OA cartilage, SFMBT2 contributes to NF-kappaB-mediated ECM degradation, J. Cell Mol. Med. 11 (22) (2018) 5753–5758, https://doi.org/10.1111/jcmm.13826.
[33] S. Yamashita, M. Andoh, H. Ueno-Kudoh, T. Sato, S. Miyaki, H. Asahara, Sox9 directly promotes Bapx1 gene expression to repress Runx2 in chondrocytes, Exp. Cell Res. 13 (315) (2009) 2231–2240, https://doi.org/10.1016/j.yexcr.2009.03.008.
[34] Q. Liu, L. Fang, C. Wu, Alternative splicing and isoforms: from mechanisms to diseases, Genes 3 (13) (2022), https://doi.org/10.3390/genes13030401.
[35] S. Chen, K. Hong, L. Zhou, R. Ran, J. Huang, Y. Zheng, M. Xing, Y. Cai, Hsa_circRNA_0017620 regulated cell progression of non-small-cell lung cancer via miR-520a-5p/KRT5 axis, J. Clin. Lab. Anal. 4 (36) (2022), e24347, https://doi.org/10.1002/jcla.24347.
[36] D. Feng, Y. Xu, J. Hu, S. Zhang, M. Li, L. Xu, A novel circular RNA, hsa-circ-0000211, promotes lung adenocarcinoma migration and invasion through sponging of hsa-miR-622 and modulating HIF1-alpha expression, Biochem. Biophys. Res. Commun. 2 (521) (2020) 395–401, https://doi.org/10.1016/j.bbrc.2019.10.134.