

Protective effects of hsa_circ_0072568 on interleukin-1 β -stimulated human chondrocytes are mediated via the miR-382-5p/TOP1 axis

QING CHANG, CHAO LI, JUNZHENG HU and RUI GENG

Department of Orthopaedic Surgery, Zhongda Hospital, School of Medicine, Southeast University, Nanjing, Jiangsu 210009, P.R. China

Received January 13, 2023; Accepted April 28, 2023

DOI: 10.3892/etm.2023.12082

Abstract. Circular RNA (circRNA) dysregulation has been linked to osteoarthritis (OA). The present study investigated the involvement of hsa_circ_0072568 (circ0072568) in OA. The expression of circ0072568 was detected in OA tissues and interleukin (IL)-1 β -stimulated human chondrocytes. After performing dual-luciferase reporter and RNA immunoprecipitation assays, MTT, enzyme-linked immunosorbent assay and western blot analysis were used to assess the functions of circ0072568 in IL-1 β -induced inflammation in chondrocytes *in vitro*. Circ0072568 was inhibited in OA tissues and the cell model *in vitro*. Circ0072568 overexpression protected the chondrocytes against IL-1 β -induced inflammation and extracellular matrix (ECM) breakdown. Circ0072568 directly attached to microRNA (miR)-382-5p and enhanced the production of topoisomerase 1 (TOP1). Furthermore, miR-382-5p overexpression or TOP1 knockdown attenuated the effects of circ0072568 in IL-1 β -stimulated human chondrocytes. On the whole, the present study demonstrates that the Circ0072568/miR-382-5p/TOP1 axis is involved in inflammation and ECM degradation in OA. These findings may contribute to the development of potential therapeutic strategies for OA.

Introduction

Osteoarthritis (OA), a chronic degenerative joint disease, is characterized by the growth of osteophytes, subchondral bone sclerosis and synovial hyperplasia (1). According to current research, a variety of risk factors, such as aging, hereditary

factors, previous joint injury and obesity are linked to the development of OA (2). However, the mechanisms underlying the pathogenesis of this disease remain to be fully elucidated.

Previous studies have demonstrated the involvement of circular RNAs (circRNAs) and microRNAs (miRNAs/miRs) in controlling the progression of OA, including processes such as chondrocyte death, oxidative stress, extracellular matrix (ECM) metabolism and autophagy (3,4). Some circRNAs are rich in miRNA binding sites, they act as 'miRNA sponges' (5). For instance, circSERPINE2 suppresses OA development by regulating the miR-495/TGFBR2 axis (6). On the other hand, circ33186 promotes the pathogenesis of OA by upregulating matrix metalloproteinase (MMP)-13 expression, which is targeted by miR-127-5p (7). This cross-interaction between circRNAs and miRNAs in the pathophysiology of OA suggested that they may be potential targets for OA therapy. hsa_circ_0072568 (circ0072568) is a circRNA derived from phosphodiesterase 4D that inhibits the tumorigenesis and progression of colorectal cancer and oxaliplatin-resistant colorectal cancer. RNA sequencing (RNAseq) analysis has revealed that circ0072568 is significantly downregulated in OA tissues and in pro-inflammatory factor-stimulated human chondrocytes (8). However, the role of circ0072568 in OA remains unclear. The present study aimed to investigate the effects of circ0072568 in interleukin (IL)-1 β -stimulated chondrocytes. It is hoped that the findings presented herein may provide novel insight into OA treatment by modulating the miR-382-5p/topoisomerase 1 (TOP1) axis.

Materials and methods

Collection of specimens. Cartilage tissues from the knee joints of patients with OA [7 males and 3 females; age range, 54–66 years; body mass index (BMI), 19.7–23.5 kg/m²] who had undergone total knee arthroplasty, and healthy cartilages from patients with femoral neck fracture (5 males and 5 females; age range, 50–52 years; BMI, 19.9–23.2) from January 2021 to January 2022 were obtained and used for PCR analysis. The study was approved by the Ethics Committee of Zhongda Hospital Southeast University and the signed informed consent was obtained from all subjects (no. ZDL22-21; date: April 23, 2022).

Correspondence to: Dr Qing Chang, Department of Orthopaedic Surgery, Zhongda Hospital, School of Medicine, Southeast University, 87 Ding Jia Qiao Road, Nanjing, Jiangsu 210009, P.R. China
E-mail: kbrcq2108@163.com

Key words: osteoarthritis, hsa_circ_0072568, inflammation, extracellular matrix, microRNA-382-5p, topoisomerase 1

Primary culture of chondrocytes and treatment. As previously described (9), cartilage tissues from the knee joints of OA patients were collected, and then cut into small sections. After washing five times with PBS (Beyotime Institute of Biotechnology), the specimens were digested in 0.25% trypsin-EDTA (Beyotime Institute of Biotechnology) solution for 30 min and supplemented with Dulbecco's modified Eagle's medium (DMEM; Thermo Fisher Scientific, Inc.) containing collagenase type II for 2 h at 37°C. The released chondrocytes were seeded in 25 cm² cell flasks. The cells were passaged at a ratio of 1:3, when they reached 80-90% confluency. For stimulation with IL-1 β , the isolated chondrocytes were maintained in DMEM (Thermo Fisher Scientific, Inc.) at 37°C, 5% CO₂ and incubated with IL-1 β (10 ng/ml; Abcam) for 24 h.

Cell transfection. Overexpression vector for circ0072568 (oe-circ0072568) was provided by Guangzhou BersinBio Biotechnology Co., Ltd.). miR-382-5p mimics and negative control (miR-NC) were obtained from RiboBio (Guangzhou RiboBio Co., Ltd.) and MyBioSource, respectively, without sequence information. miR-382-5p inhibitor (inhib-miR-382-5p) and negative control (inhib-NC) were obtained from Shanghai GenePharma Co., Ltd. Circ0072568 siRNA (si-circ0072568) and its corresponding control (si-nc), as well as TOP1 siRNA (si-TOP1) and its corresponding control (si-NC), were designed and synthesized by Shanghai GenePharma Co., Ltd. Primary chondrocytes, isolated from normal articular cartilage tissues, were cultured in 96-well plates at 37°C for 24 h and then transfected with 200 pmol/l oe-circ0072568 [or 200 pmol/l negative control (oe-NC)], 50 nM miR-382-5p mimics (or 50 nM miR-NC), 100 nM inhib-miR-382-5p (or 100 nM inhib-NC), or 200 pmol/l si-TOP1 (or 200 pmol/l si-NC) using Lipofectamine 3000[®] (Invitrogen; Thermo Fisher Scientific, Inc.). The aforementioned oligonucleotides or plasmids were transfected into chondrocytes using Lipofectamine[™] 2000 reagent (Invitrogen; Thermo Fisher Scientific, Inc.) following the manufacturer's instructions. Briefly, chondrocytes were cultured for 24 h, before the constructs were transfected into the cells using 500 μ l Opti-MEM containing 20 μ l Lipofectamine 2000 (1 mg/ml) in a humidified atmosphere with 5% CO₂ at 37°C. After incubating for 6 h, culture medium was replaced by fresh medium. Subsequently, at 48 h following transfection, the cells were harvested for use in subsequent experiments. The sequences of the siRNAs, mimics and inhibitors used were as follows: miR-382-5p mimics forward, 5'-GAAGUUGUUCGU GGUGGAUUGG-3' and reverse, 5'-AAUCCACCACGAACA ACUUCUU-3'; miR-NC forward, 5'-CTCGCTTCGGCAGCA CA-3' and reverse, 5'-AACGCTTCACGAATTTGCGT-3'; miR-382-5p inhibitor, 5'-CGAAUCCUAGUACAACUU C-3'; inhib-NC, 5'-CAGUACUUUUGUGUAGUACA-3'; si-TOP1, 5'-GCAUAAAGACAAACAUAAGA-3'; si-NC, 5'-UUCUCCGAACGUGUCACGUTT-3'. si-circ0072568, 5'-AACAGUUUUGAUGUGGACAAU-3'; si-nc, 5'-UUCUCC GAACGUGUCACGU-3'.

Reverse transcription-quantitative PCR (RT-qPCR). Total RNA extracted from tissues and cells was incubated in the presence or absence of RNase R (3 U/ μ g, Epicentre) at 37°C for 20 min. After TRIzol[®] reagent (Invitrogen; Thermo Fisher

Scientific, Inc.) and the miRNA isolation kit (Thermo Fisher Scientific, Inc.) to extract the total RNA, reverse transcription was performed using a miRNA cDNA kit (Invitrogen; Thermo Fisher Scientific, Inc.), TaqMan[™] MicroRNA Assays (Applied Biosystems; Thermo Fisher Scientific, Inc.) and PrimeScript RT Master Mix (Takara Bio, Inc.). The PCR thermal cycling parameters were as follows: 95°C (5 min), 45 cycles of 94°C (30 sec) and 60°C (30 sec). Gene expression was quantified using the 2^{- $\Delta\Delta$ C_q} method (10), using GAPDH and U6 as internal genes for circ0072568 and TOP1, as well as miR-382-5p and miR-545-3p, respectively. The primer sequences used are listed in Table I.

MTT assay. Chondrocytes (1x10⁴ cells/well) were cultured in the 96-well plates. 10 μ l MTT stock solution (Beijing Solarbio Science & Technology Co., Ltd.) was added to the culture medium according to the manufacturer's instructions, and the plate was incubated for 4 h at 37°C. Following the addition of dimethyl sulfoxide (DMSO; Beijing Solarbio Science & Technology Co., Ltd.), the absorbance of the cells was read at 570 nm using a Multiska FC microplate reader (Thermo Fisher Scientific, Inc.).

Flow cytometry. The apoptotic rate of the chondrocytes (2x10⁵ cells/well) at 80% confluency was analyzed using an apoptosis detection kit [Annexin V-FITC/propidium iodide (PI); Beyotime Institute of Biotechnology] and a BD FACS flow cytometer (BD Biosciences).

Enzyme-linked immunosorbent assay (ELISA). The secretion of pro-inflammation factors was detected using corresponding IL-6 (cat. no. PI325), TNF- α (cat. no. PT518) and monocyte chemoattractant protein-1 (MCP-1; cat. no. PC130) ELISA kits (Beyotime Institute of Biotechnology).

Western blot analysis. After using the bicinchoninic acid (BCA) method (Beyotime Institute of Biotechnology) to quantify total proteins, which were extracted from the cell using radio immunoprecipitation assay buffer (cat. no. BP0013B; Beyotime Institute of Biotechnology), the proteins were separated by 10% SDS-PAGE, transferred onto PVDF membranes. Thereafter, the membranes were blocked with 5% skimmed milk at room temperature for 2 h and then incubated with primary antibodies at 4°C overnight. Subsequently, the membranes were incubated with secondary antibody IgG (1:2,000; cat. no. ab205718; Abcam) at room temperature for 2 h. The primary antibodies used were as follows: Anti-collagen II (1:1,000; cat. no. ab188570; Abcam), anti-MMP-13 (1:1,000; cat. no. ab51072; Abcam), anti-TOP1 (1:1,000; cat. no. ab131166; Abcam) and anti-GAPDH (1:1,000; cat. no. ab8245; Abcam). Blots were developed using an enhanced chemiluminescence system (Beyotime Institute of Biotechnology). The density of each protein blot was compared with that of GAPDH using ImageJ software (version 1.46r; National Institutes of Health) and is presented as a ratio to the endogenous control.

Nuclear-cytoplasmic fractionation. The PARIS kit (cat. no. AM1921; Invitrogen; Thermo Fisher Scientific, Inc.) was used for nuclear-cytoplasmic fractionation. Briefly, the nuclei were lysed using cell disruption buffer and the

Table I. Primer sequences used in the present study.

Gene	Sequence
circ0072568	Forward: 5'-GCAAGATCGAGCACCTAGCA-3' Reverse: 5'-GCTTGGAGAATTAGCCCCGGA-3'
TOP1	Forward: 5'-ACGAATCAAGGGTGAGAAGG-3' Reverse: 5'-CGATACTGGTTCCGGATCTT-3'
GAPDH	Forward: 5'-GGGAAACTGTGGCGTGAT-3' Reverse: 5'-GAGTGGGTGTCGCTGTTGA-3'
miR-382-5p	Forward: 5'-ACACTCCAGCTGGGAAAGTGCTTCCC-3' Reverse: 5'-CTCAACTGGTGTCTGTGGA-3'
miR-545-3p	Forward: 5'-TCGGCAGGTCAGCAAACATTT-3' Reverse: 5'-CAGTGCCTGTCGTGGAGT-3'
U6	Forward: 5'-CGCTTACGAATTTGCGT-3' Reverse: 5'-CTCGCTTCGGCAGCACA-3'

TOP1, topoisomerase 1.

supernatant of nuclear and mitochondrial DNA extracted from chondrocytes were mixed with a 2X lysis binding solution, followed by eluting the cytoplasm and nucleus RNA using the corresponding kit solutions.

Luciferase reporter assay. The downstream target miRNAs of circ0072568 were predicted using the online website StarBase v2.0 (<http://starbase.sysu.edu.cn/index.php>) and circBank (<http://www.circbank.cn/searchCirc.html>). Direct targets of miR-382-5p were predicted using the online databases StarBase, miRTarBase (<http://mirtarbase.mbc.nctu.edu.tw/index.html>), TargetScan (https://www.targetscan.org/vert_80/) and miRDB (<https://mirdb.org/>). Primary chondrocytes (1×10^4) were cultured to 70% confluency in 24-well plates. Either wild-type or mutant circ0072568 (circ0072568-wt or circ0072568-mut) and TOP1 (TOP1-wt or TOP1-mut) fragments were inserted into the pGL3-firefly luciferase vectors (Shanghai Genechem Co. Ltd.). Primary chondrocytes were cultured for 24 h before being co-transfected with 600 ng circ0072568-wt or 600 ng TOP1 3'UTR-wt luciferase reporter gene plasmid and 20 nmol miR-382-5p mimics or 20 nmol miR-NC using Lipofectamine 3000 reagent for 48 h at 37°C, and the dual-luciferase System (Promega Corporation) was used to determine the luciferase activity. The results were normalized to *Renilla* luciferase activity.

RNA immunoprecipitation (RIP) assay. The Ago-RIP test (MilliporeSigma) was performed using the the Magna RIP RNA-Binding Protein Immunoprecipitation kit and RNeasy MinElute Cleanup kit (Qiagen GmbH). The cells were lysed in RIP buffer (Beyotime Institute of Biotechnology) containing a protease inhibitor cocktail and RNase inhibitors, and incubated at 4°C with anti-Ago2 (cat. no. ab186733; 1:50; Abcam) or anti-IgG antibodies (cat. no. PP64B; 1:20; EMD Millipore) overnight. RNA was quantified by qRT-PCR.

Statistical analysis. SPSS v22.0 software was used for statistical analyses. An unpaired two-tailed Student's t-test

and one-way ANOVA (for ≥ 3 groups) followed by post hoc (Tukey's or Dunnett's) tests was used. Data are presented as the mean \pm SD of three repeats. The correlation between circ0072568 and miR-382-5p expression in OA tissues was assessed using Pearson's correlation analysis. A value of $P < 0.05$ was considered to indicate a statistically significant difference.

Results

Effects of circ0072568 on IL-1 β -stimulated human primary chondrocytes. As shown in Fig. 1A, circ0072568 expression was decreased in OA-affected cartilage, as well as in IL-1 β -stimulated primary human chondrocytes (Fig. 1B). As demonstrated by the results of RT-qPCR, transfection with circ0072568 overexpression vector (oe-circ0072568) successfully increased the circ0072568 level in chondrocytes isolated from normal articular cartilage tissues (Fig. 1C). Additionally, the downregulation of circ0072568 induced by exposure to IL-1 β for 24 h was restored by transfection with circ0072568 overexpression vector (Fig. 1D). The decrease in the viability of IL-1 β -stimulated primary human chondrocytes was abolished by the overexpression of circ0072568 (Fig. 1E). The increased apoptosis in IL-1 β -induced human primary chondrocytes was also abolished by the overexpression of circ0072568 (Fig. 1F). In addition, circ0072568 overexpression led to the decreased secretion of pro-inflammatory factors (Fig. 1G-I), and to decreased MMP-13 expression and enhanced collagen II expression (Fig. 1J and K).

ECM degradation by IL-1 β -stimulated chondrocytes was assessed by detecting the expression of proteins related to ECM catabolism, including collagen II and MMP-13. Western blot analysis revealed that IL-1 β stimulation decreased the expression of collagen II and increased the expression of MMP-13, indicative of the increased ECM degradation potential (Fig. 1J and K). By contrast, circ0072568 overexpression led to an increase in collagen II expression and a decrease in MMP-13 expression in IL-1 β -stimulated chondrocytes.

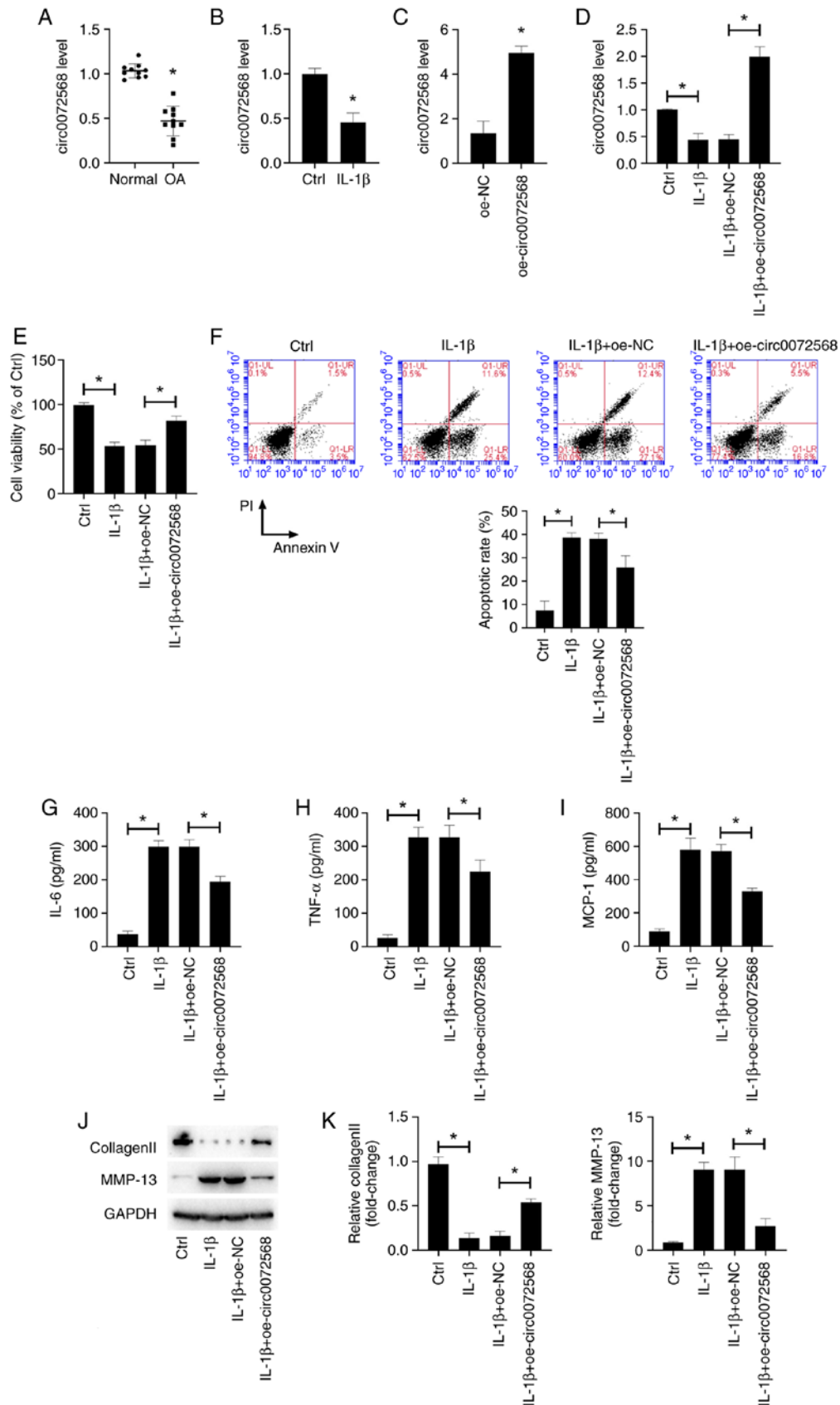


Figure 1. Circ0072568 protects chondrocytes from IL-1 β -induced inflammation and extracellular matrix degradation. (A) Expression of circ0072568 in the cartilage tissues of patients with OA and normal controls (n=10). (B) Expression of circ0072568 in chondrocytes isolated from normal articular cartilage tissues treated with or without IL-1 β stimulation; *P<0.05, vs. respective control. (C) The expression of circ0072568 in chondrocytes transfected with oe-circ0072568 or oe-NC was examined using reverse transcription-quantitative PCR. (D) The expression of circ0072568 in chondrocytes transfected with oe-circ0072568 was examined in the presence or absence of IL-1 β . (E) The viability of chondrocytes was analyzed using MTT assay. (F) The apoptosis of chondrocytes was analyzed using flow cytometry. The levels of (G) IL-6, (H) TNF- α and (I) MCP-1 in the supernatant of chondrocytes were measured using ELISA. (J and K) The expression of collagen II and MMP-13 was examined using western blot analysis; n=3. *P<0.05. OA, osteoarthritis; IL, interleukin; MMP-13, matrix metalloproteinase 13; MCP-1, monocyte chemoattractant protein-1.

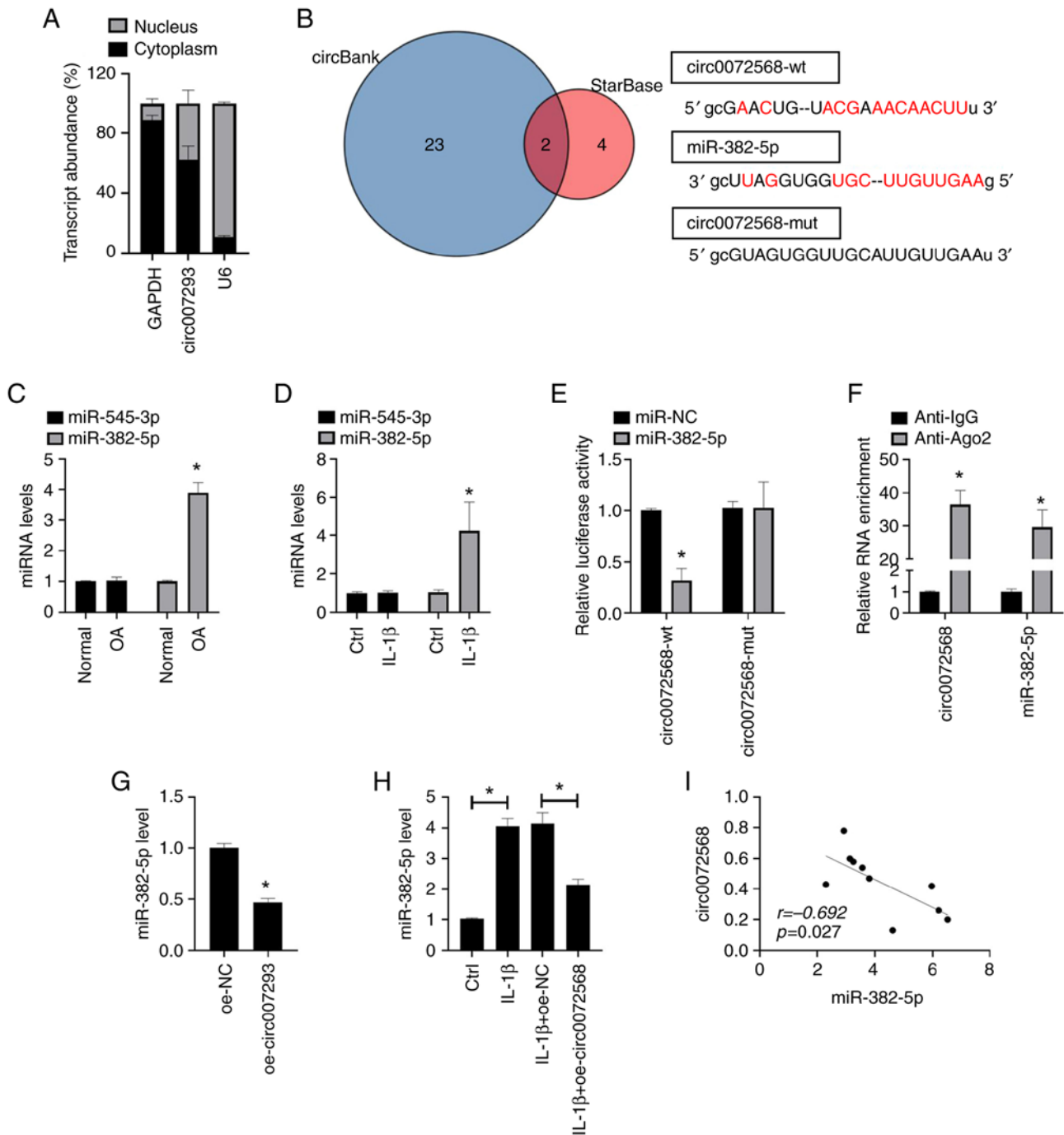


Figure 2. circ0072568 serves as a sponge for miR-382-5p, and miR-382-5p directly targets TOP1. (A) Subcellular fractionation for circ0072568 in the cytoplasm and nucleus of chondrocytes. (B) Left panel, Venn diagram illustrating miRNAs targeted by circ0072568; right panel, prediction binding sites of circ0072568 and miR-382-5p. (C) Expression of miR-545-3p and miR-382-5p in the cartilage tissues of patients with OA and normal controls (n=10). (D) Expression of miR-545-3p and miR-382-5p in chondrocytes isolated from normal articular cartilage tissues with or without IL-1 β stimulation. (E) Luciferase activity in chondrocytes co-transfected with circ0072568-wt or -mut vectors and miR-382-5p or miR-NC. (F) Reverse transcription-quantitative PCR analysis of circ0072568 and miR-382-5p enrichment in RIP precipitates of anti-Ago2 in chondrocytes. (G) Expression of miR-382-5p in chondrocytes transfected with oe-circ0072568 or si-circ0072568. (H) Expression of miR-382-5p in IL-1 β -treated chondrocytes transfected with or without oe-circ0072568. (I) Analysis of the correlation between circ0072568 and miR-382-5p in cartilage tissues from patients with OA (n=10). *P<0.05, vs. respective control.

Taken together, these data suggest that circ0072568 inhibits IL-1 β -induced inflammation and ECM degradation by chondrocytes.

circ0072568 functions as a sponge for miR-382-5p. The regulatory mechanism of circRNAs as miRNA sponges has been widely confirmed. The present study we identified

the distribution of circ0072568 in chondrocytes by subcellular fractionation and found that circ0072568 was mainly enriched in the cytoplasm of chondrocytes (Fig. 2A). The analysis of public databases (StarBase v2.0 and circBank) identified miR-545-3p and miR-382-5p as target miRNAs of circ0072568 (Fig. 2B). However, the expression of miR-382-5p and miR-545-3p did not differ significantly

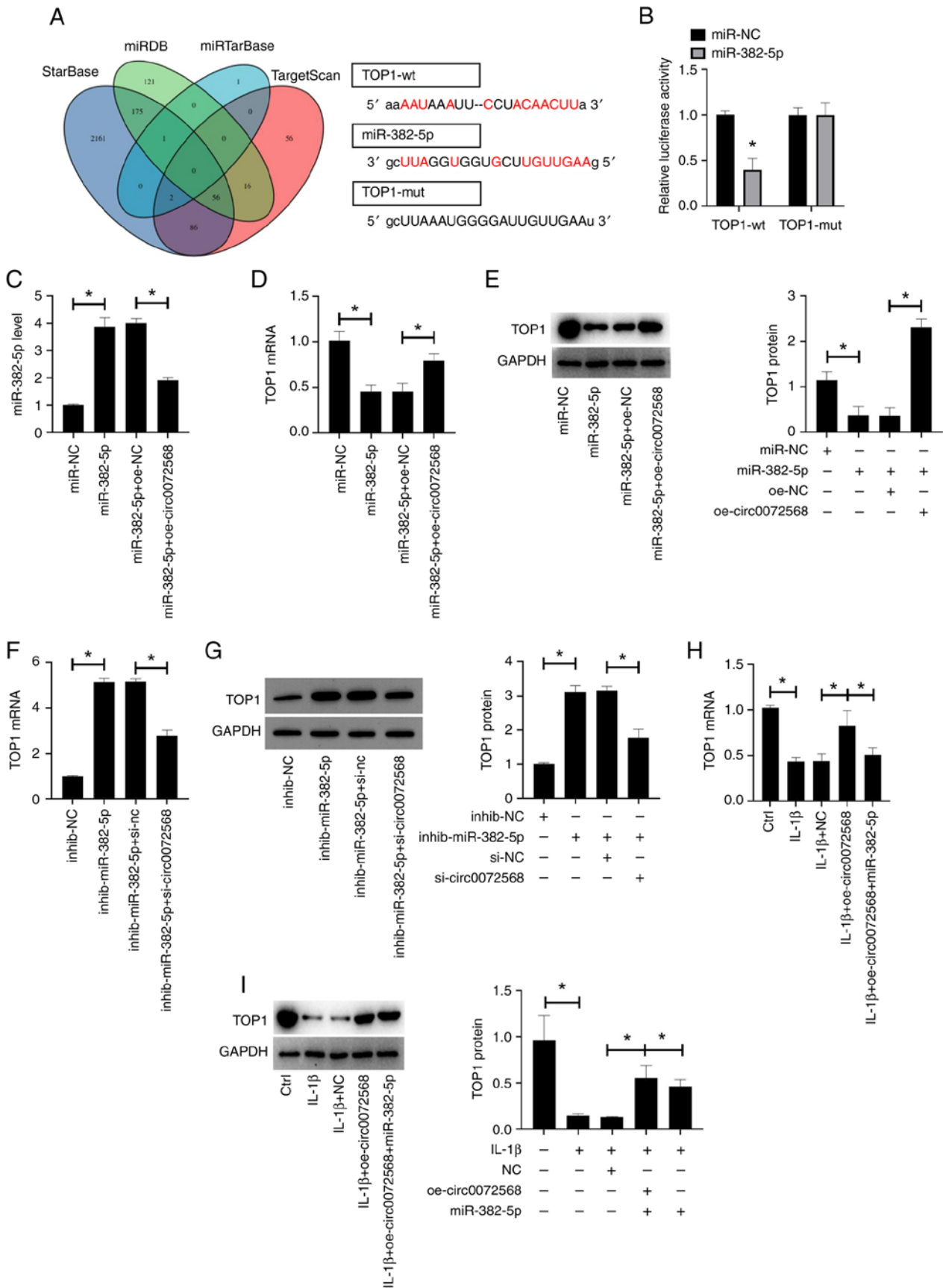


Figure 3. miR-382-5p directly targets TOP1. (A) Left panel, Venn diagram illustrating mRNAs targeted by miR-382-5p; right panel, prediction binding sites of miR-382-5p and TOP1 mRNA. (B) Luciferase activity in chondrocytes co-transfected with TOP1-wt or -mut vectors and miR-382-5p or miR-NC. (C) Expression of miR-382-5p in chondrocytes co-transfected with miR-382-5p mimic and oe-circ0072568. Expression of TOP1 mRNA (D) and protein (E) in chondrocytes co-transfected with miR-382-5p mimic and oe-circ0072568. Expression of TOP1 (F) mRNA and (G) protein in chondrocytes co-transfected with miR-382-5p inhibitor and si-circ0072568. Expression of TOP1 (H) mRNA and (I) protein in IL-1 β -stimulated chondrocytes co-transfected with miR-382-5p mimic and oe-circ0072568; n=3. *P<0.05. OA, osteoarthritis; IL, interleukin; TOP1, topoisomerase 1.

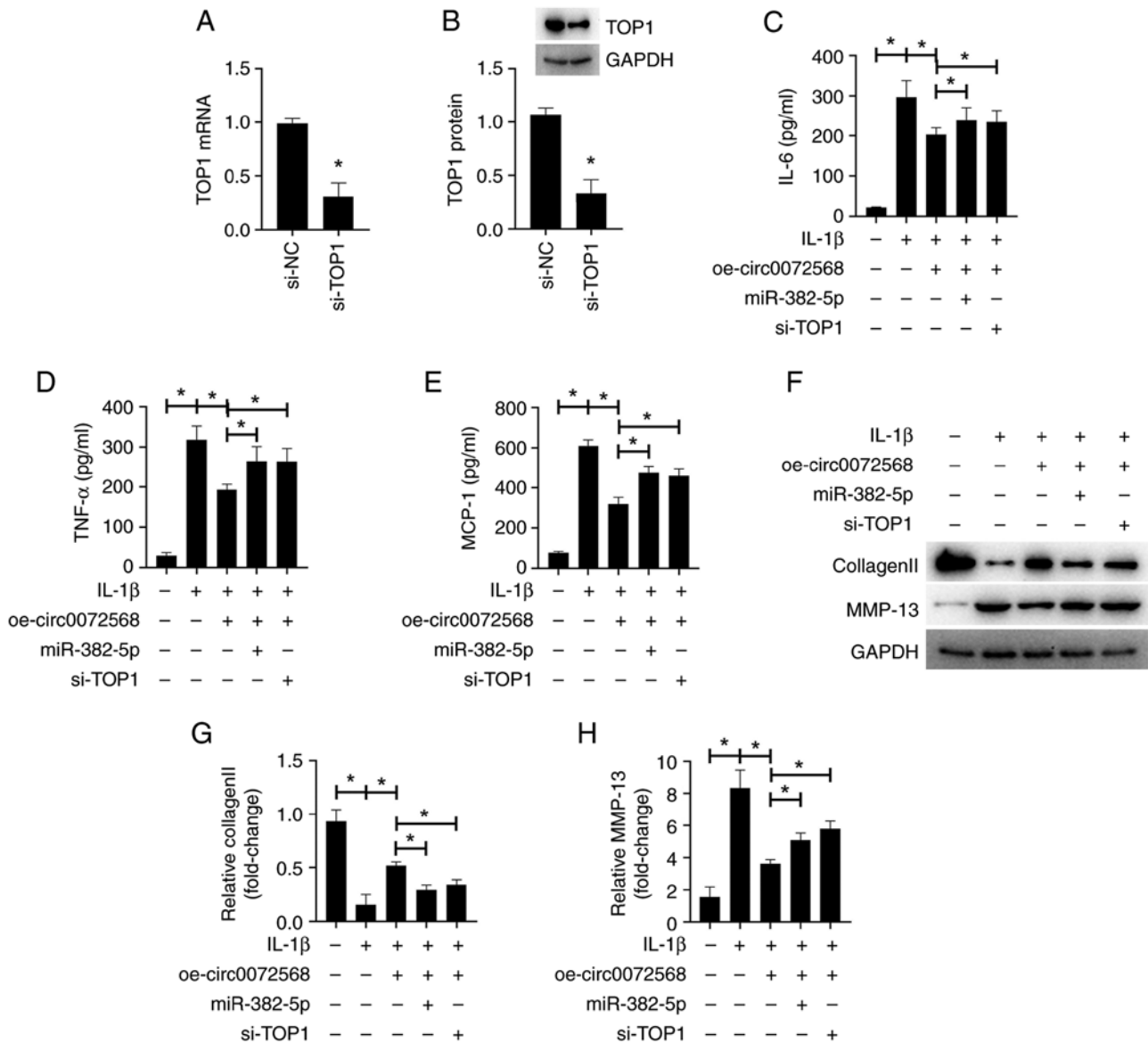


Figure 4. circ0072568 protects chondrocytes from IL-1 β -induced inflammation and extracellular matrix degradation via the miR-382-5p/TOPI axis. The expression of TOPI (A) mRNA and (B) protein in chondrocytes transfected with si-TOP1. The levels of (C) IL-6, (D) TNF- α and (E) MCP-1 in the supernatant of chondrocytes were measured using ELISA. (F-H) The expression of collagen II and MMP-13 was examined using western blot analysis; n=3. *P<0.05. OA, osteoarthritis; IL, interleukin; MMP-13, matrix metalloproteinase 13; MCP-1, monocyte chemoattractant protein-1.

between OA tissues and normal tissues, and the stimulation of human chondrocytes with IL-1 β did not result in significant changes in their expression levels (both P>0.05; Fig. 2C and D). In addition, miR-382-5p decreased the relative luciferase activity of circ0072568-wt, but not that of circ0072568-mut vectors (Fig. 2E). Furthermore, the enrichment of circ0072568 in beads was found to conjugate to the Ago2 antibody compared with the IgG controls (Fig. 2F). oe-circ0072568 and its negative control (oe-NC) were then transfected into chondrocytes (Fig. S1), and the results revealed that the overexpression of circ0072568 in chondrocytes led to a decreased expression of miR-382-5p (Fig. 2G), abolishing the IL-1 β -induced upregulation of miR-382-5p expression (Fig. 2H). Pearson's correlation analysis further confirmed the negative correlation between the expression of circ0072568 and miR-382-5p in the cartilage tissues of patients with OA (Fig. 2I).

miR-382-5p directly targets TOPI. TOPI was found to be one of the target genes of miR-382-5p, as predicted using the StarBase, TargetScan and miRDB databases (Fig. 3A). The relative luciferase activity of TOPI-wt vectors was decreased in the chondrocytes overexpressing miR-382-5p (P<0.05; Fig. 3B). The overexpression of miR-382-5p in human chondrocytes transfected with miR-382-5p mimic was abolished following transfection of the cells with circ0072568 overexpression vector (all P<0.05; Fig. 3C). While the transfection of chondrocytes with miR-382-5p mimic decreased the expression of TOPI in chondrocytes, this effect was reversed by co-transfection with circ0072568 overexpression vector (all P<0.05; Fig. 3D and E). In addition, transfection of the chondrocytes with miR-382-5p inhibitor increased TOPI expression in chondrocytes, whereas this effect was reversed by co-transfection with si-circ0007256 (all P<0.05; Fig. 3F and G). IL-1 β stimulation markedly decreased the

expression of TOP1 in chondrocytes (all $P < 0.05$), and this effect was reversed by the overexpression of circ0072568 (all $P < 0.05$). Additionally, circ0072568 regulated TOP1 expression via miR-382-5p in chondrocytes (Fig. 3H and I).

Protective effects of circ0072568 on IL-1 β -stimulated chondrocytes are mediated via the regulation of the miR-382-5p/TOP1 axis. miR-382-5p overexpression or TOP1 knockdown abolished the anti-inflammatory effects of circ0072568 overexpression on IL-1 β -stimulated chondrocytes. miR-382-5p overexpression or TOP1 knockdown abolished the suppressive effects of circ0072568 overexpression on the levels of pro-inflammatory factors (Fig. 4A-E), and reversed the inhibition of MMP-13 expression and the increase in collagen II expression, which was induced by circ0072568 overexpression (Fig. 4F-H).

Discussion

Currently, alleviating pain and improving function is the main treatment strategy for OA. However, this treatment is purely symptomatic, and does not affect the progression of OA (11). Since there is no effective therapy that can completely cure OA, the elucidation of the underlying mechanisms of OA has obvious clinical significance. As a type of endogenous non-coding RNAs, circRNAs have been increasingly recognized for their role in the development of tumors (12), as well as in the pathogenesis of degenerative diseases, including intervertebral disc degeneration and OA (13). In the present study, circ0072568 was inhibited in the cartilage tissue of individuals with OA and was linked to the onset and development of OA. Circ0072568 was also shown to prevent OA by inhibiting miR-382-5p and TOP1.

The PDE4E gene is the source of circ0072568, and has been linked to tumor invasion, and cell proliferation and death (14). In the present study, circ0072568 expression was decreased in IL-1 β -stimulated chondrocytes. Moreover, it was found that the overexpression of circ0072568 affected ECM metabolism and the inflammatory response in chondrocytes. These data suggest that circ0072568 may be a novel and effective molecular target in OA.

As a basic building block of the ceRNA network, circRNAs have been found to function as 'miRNA sponges' (15). miR-382-5p functions as an oncogene or cancer suppressor gene (16) is also involved in the regulation of inflammation (17,18). In the present study, miR-382-5p expression was significantly increased in OA tissues and IL-1 β -stimulated human chondrocytes, suggesting that it has a pro-inflammatory effect in OA.

It has been demonstrated that TOP1 is downregulated in the synovium and blood samples of patients with OA (19). This is consistent with the findings of the present study. miRNAs can directly target the mRNAs of the target gene via complementary base pairing (20). Previous studies have confirmed that TOP1 can be regulated by miRNAs, including miR-24 (21), miR-21-5p (22) and miR-9 (23). The present study found that TOP1 may be one of the potential targets of miR-382-5p. There is increasing evidence to indicate that the regulatory network of the circRNAs/miRNAs/mRNAs, such as circ0092516/miR-337-3P/PTEN (9) and circHIPK3/

miR-124/SOX8 (24), plays a vital role in regulating the progression of OA. As demonstrated herein, the protective effects of circ0072568 against the inflammatory response and ECM degradation in IL-1 β -stimulated chondrocytes were achieved, at least in part, by the regulation of hte miR-382-5p/TOP1 axis. Future studies are required however, to further explore whether IL-1 β regulates the chondrocyte phenotype by mediating TOP1.

However, both miR-382-5p and TOP1 can only partially explain the role of circ0072568 in OA. Further regulatory networks for circ0072568 need to be studied *in vitro* and *in vivo*, using animal models. Previous research has indicated that inhibiting ECM degradation and the secretion of inflammatory factors may delay the progression of OA to a certain extent (25,26). In this study, we mainly used chondrocytes as the modeling cell line. Further studies are required to explore the role of circ0072568 in OA from the perspective of macrophage infiltration and its role in OA progression.

In conclusion, the present study demonstrated a novel regulatory network, namely the circ0072568/miR-382-5p/TOP1 axis, which promoted the development of OA by accelerating ECM degradation and inflammation. These results may provide aid the development of novel potential treatment strategies for OA.

Acknowledgements

Not applicable.

Funding

Not applicable.

Availability of data and materials

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

Author's contributions

QC, CL and JH conducted the experiments. QC and RG were involved in the writing, data analysis and revision of the manuscript. CL and JH were involved in data analysis. All authors have read and approved the final manuscript. QC and CL confirm the authenticity of all the raw data.

Ethics approval and consent to participate

The present study was approved by the Ethics Committee of Zhongda Hospital Southeast University of Science and Technology (approval no. ZDL22-21).

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

References

- Sacitharan PK: Ageing and Osteoarthritis. *Subcell Biochem* 91: 123-159, 2019.
- Geyer M and Schönfeld C: Novel insights into the pathogenesis of osteoarthritis. *Curr Rheumatol Rev* 14: 98-107, 2018.
- Mumtaz PT, Taban Q, Dar MA, Mir S, Haq ZU, Zargar SM, Shah RA and Ahmad SM: Deep insights in circular RNAs: From biogenesis to therapeutics. *Biol Proced Online* 22: 10, 2020.
- Ehrlich GD: Circular RNAs as diagnostic biomarkers for osteoarthritis. *Genet Test Mol Biomarkers* 23: 701-702, 2019.
- Patop IL, Wüst S and Kadener S: Past, present, and future of circRNAs. *EMBO J* 38: e100836, 2019.
- Zhang Q, Qiao X and Xia W: CircSERPINE2 weakens IL-1 β -caused apoptosis and extracellular matrix degradation of chondrocytes by regulating miR-495/TGFBR2 axis. *Biosci Rep* 40: BSR20201601, 2020.
- Zhou ZB, Huang GX, Fu Q, Han B, Lu JJ, Chen AM and Zhu L: circRNA.33186 contributes to the pathogenesis of osteoarthritis by sponging miR-127-5p. *Mol Ther* 27: 531-541, 2019.
- Shen S, Wu Y, Chen J, Xie Z, Huang K, Wang G, Yang Y, Ni W, Chen Z, Shi P, *et al*: CircSERPINE2 protects against osteoarthritis by targeting miR-1271 and ETS-related gene. *Ann Rheum Dis* 78: 826-836, 2019.
- Huang Z, Ma W, Xiao J, Dai X and Ling W: CircRNA_0092516 regulates chondrocyte proliferation and apoptosis in osteoarthritis through the miR-337-3p/PTEN axis. *J Biochem* 169: 467-475, 2021.
- Livak KJ and Schmittgen TD: Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. *Methods* 25: 402-408, 2001.
- Sharma L: Osteoarthritis of the Knee. *N Engl J Med* 384: 51-59, 2021.
- Zhang HD, Jiang LH, Sun DW, Hou JC and Ji ZL: CircRNA: A novel type of biomarker for cancer. *Breast Cancer* 25: 1-7, 2018.
- Marques-Rocha JL, Samblas M, Milagro FI, Bressan J, Martínez JA and Martí A: Noncoding RNAs, cytokines, and inflammation-related diseases. *FASEB J* 29: 3595-3611, 2015.
- Liu F, Ma J, Wang K, Li Z, Jiang Q, Chen H, Li W and Xia J: High expression of PDE4D correlates with poor prognosis and clinical progression in pancreatic ductal adenocarcinoma. *J Cancer* 10: 6252-6260, 2019.
- Zang J, Lu D and Xu A: The interaction of circRNAs and RNA binding proteins: An important part of circRNA maintenance and function. *J Neurosci Res* 98: 87-97, 2020.
- Sun LP, Xu K, Cui J, Yuan DY, Zou B, Li J, Liu JL, Li KY, Meng Z and Zhang B: Cancer-associated fibroblast-derived exosomal miR-382-5p promotes the migration and invasion of oral squamous cell carcinoma. *Oncol Rep* 42: 1319-1328, 2019.
- Xiang W, Jiang L, Zhou Y, Li Z, Zhao Q, Wu T, Cao Y and Zhou J: The lncRNA Ftx/miR-382-5p/Nrg1 axis improves the inflammation response of microglia and spinal cord injury repair. *Neurochem Int* 143: 104929, 2021.
- Zhang X, Zhang Y, Meng Q, Sun H, Wu S, Xu J, Yun J, Yang X, Li B, Zhu H, *et al*: MicroRNA-382-5p is involved in pulmonary inflammation induced by fine particulate matter exposure. *Environ Pollut* 262: 114278, 2020.
- Rialdi A, Campisi L, Zhao N, Lagda AC, Pietzsch C, Ho JSY, Martínez-Gil L, Fenouil R, Chen X, Edwards M, *et al*: Topoisomerase 1 inhibition suppresses inflammatory genes and protects from death by inflammation. *Science* 352: aad7993, 2016.
- Krol J, Loedige I and Filipowicz W: The widespread regulation of microRNA biogenesis, function and decay. *Nature reviews Genetics* 11: 597-610, 2010.
- Bu H, Baraldo G, Lepperdinger G and Jansen-Dürr P: mir-24 activity propagates stress-induced senescence by down regulating DNA topoisomerase 1. *Exp Gerontol* 75: 48-52, 2016.
- Chen JC, Hsieh YY, Lo HL, Li A, Chou CJ and Yang PM: In vitro and in silico mechanistic insights into miR-21-5p-Mediated topoisomerase drug resistance in human colorectal cancer cells. *Biomolecules* 9: 467, 2019.
- Kania EE, Carvajal-Moreno J, Hernandez VA, English A, Papa JL, Shkolnikov N, Ozer HG, Yilmaz AS, Yalowich JC and Elton TS: hsa-miR-9-3p and hsa-miR-9-5p as post-transcriptional modulators of DNA topoisomerase II α in human leukemia K562 cells with acquired resistance to etoposide. *Mol Pharmacol* 97: 159-170, 2020.
- Wu Q, Yuan ZH, Ma XB and Tang XH: Low expression of CircRNA HIPK3 promotes osteoarthritis chondrocyte apoptosis by serving as a sponge of miR-124 to regulate SOX8. *Eur Rev Med Pharmacol Sci* 24: 7937-7945, 2020.
- Guilak F, Nims RJ, Dicks A, Wu CL and Meulenbelt I: Osteoarthritis as a disease of the cartilage pericellular matrix. *Matrix Biol* 71-72: 40-50, 2018.
- Singh P, Marcu KB, Goldring MB and Otero M: Phenotypic instability of chondrocytes in osteoarthritis: On a path to hypertrophy. *Ann N Y Acad Sci* 1442: 17-34, 2019.



Copyright © 2023 Chang et al. This work is licensed under a Creative Commons Attribution-NonCommercial-NoDerivatives 4.0 International (CC BY-NC-ND 4.0) License.