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# CircVCAN regulates the proliferation and apoptosis of osteoarthritis chondrocyte through NF-κB signaling pathway

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**Abstract. – OBJECTIVE: Osteoarthritis is one of the chronic diseases with a high incidence. CircRNA is a circular non-coding RNA. Studies show that CircRNA is closely relevant to the pathogenesis of OA chondrocytes. However, the specific principle is still unclear.**

**PATIENTS AND METHODS: 38 patients with OA tissues and 38 patients with normal knee cartilage in our hospital were selected, respectively. The mRNA expression levels of CircVCAN were measured by quantificational real-time polymerase chain reaction (qRT-PCR). Cell proliferation was detected by the Cell Counting Kit (CCK8). Cell cycle and apoptosis of OA chondrocytes were measured by flow cytometry. qRT-PCR and western blot were used to detect PCNA, p50, p52, p65 mRNA and protein expression levels.**

**RESULTS: CircVCAN was highly expressed in OA tissues and OA chondrocytes. Cell proliferation and PCNA expression levels decreased significantly after transfection with si-CircVCAN in OA-chondrocytes. However, there was a significant increase on OA chondrocytes after transfection with LV-CircVCAN. Compared with the si-NC group, the apoptosis rate of OA chondrocytes was significantly increased after transfection with si-CircVCAN. The proportion of G0/G1 phase in the cell cycle was significantly reduced and the proportion of S phase was significantly increased. On the contrary, the apoptosis rate was significantly reduced after transfection with LV-CircVCAN. The proportion of G0/G1 phase in the cell cycle was significantly increased and the proportion of S phase was significantly reduced. The mRNA and protein levels of p50, p52 and p65 were significantly increased after transfection of LV-CircVCAN in OA-chondrocytes. Furthermore, PDTC (NF-κB inhibitor) transfection can significantly reverse the effect of overexpression of CircVCAN on the proliferation and apoptosis of OA chondrocytes.**

**CONCLUSIONS: CircVCAN is overexpressed in OA tissues and cells. CircVCAN can affect the proliferation and apoptosis of OA chondrocytes by blocking the activation of the NF-κB signaling pathway. Thus, CircVCAN may be an important target molecule for OA treatment.**

*Key Words:*
Osteoarthritis, CircVCAN, NF-κB, Proliferation, Apoptosis.

## Introduction

As the most common arthritis, osteoarthritis seriously affects the health and quality of life of middle-aged and elderly people[1,2]. The main clinical symptoms are joint pain, swelling, stiffness, deformity and dysfunction[3,4]. Osteoarthritis clinical symptoms are mainly manifested as joint pain, swelling, stiffness, deformity and dysfunction[5-7]. However, the pathogenesis of osteoarthritis has not been fully understood.

Circular RNA (circ-RNA) is a new type of non-coding RNA. It is a covalent closed-loop structure without 5′ to 3′ polarity but does not contain a polyadenylation tail[8-10]. CircRNA is closely related to the occurrence and development of OA chondrocyte proliferation and differentiation, inflammatory response, ECM degradation, and signaling pathways. In arthritis and normal cartilage, there are differences between the expression of 71 types of circRNA, sixteen of them including circRNA_10086, circRNA_10118 and cirRNA_101914 were up-regulated and fifty-five others were down-regulated[10]. They may play important roles in the development of cartilage dam-

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age and arthritis. The expression levels of CircSERPINE2 and ERG were significantly increased in OA. CircSERPINE2 served as a sponge of miR-1271-5p to regulate the expression levels of ERG in OA, thus promoting or alleviating the catabolism of ECM[11]. Wu et al[12] found that CircRNA hsa_circ_0005105 could upregulate the expression of NAMPT, which facilitates the matrix (ECM) degradation by sponging miR-26.

In the present study, we found that CircVCAN was significantly up-regulated in OA tissues and chondrocytes. CircVCAN could have an influence on the proliferation and apoptosis of OA chondrocytes by inhibiting the activation of NF-κB signaling pathway. Thus, CircVCAN may become an important target molecule for OA treatment.

## Patients and Methods

### Patients

Thirty-eight OA patients and Thirty-eight meniscal injury patients who were admitted to our hospital from May 2017 to September 2018 were selected. The diagnostic criteria for OA were from Guidelines for the diagnosis of osteoarthritis (2007) in China. 24 males and 14 females were selected, respectively, the average age of patients was 45.2 ± 8.7 years old. The average age of meniscus injury patients was 43.7 ± 9.8 years old, and 22 males and 16 females were included. None of the patients got diseases such as rheumatoid arthritis, rheumatoid arthritis, and secondary osteoarthritis. All patients signed the informed consent before surgery. The cartilage tissue was removed during the operation, the tissue samples were divided into the same size and stored in liquid nitrogen.

### qRT-PCR

The total RNA from OA tissues and OA chondrocytes were measured by TRIzol reagent (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions. The synthesis of cDNA for circRNA and mRNA was detected by reverse transcription kit (TaKaRa, Otsu, Shiga, Japan). SYBR Green PCR Kit (TaKaRa, Otsu, Shiga, Japan) was used to measure the quantification of CircRNA mRNA. The levels of mRNA and circRNA expression were normalized by GAPDH and U6. All primer sequences were synthesized and planed by Genery (Guangzhou, China). The results were processed by the 2-ΔΔCt methods. The primers used in our study were as follows: CircVCAN F 5'-GTATAGGTGGAACAGTCTTAA-3', R 5'-TTATATTCCTTCTTTAGAGTTTGG-3'. PCNA F 5'-GACACATACCGCTGCGATCG-3', R 5'-TCACCACAGCATCTCCAATAT-3'. β-actin F 5'-TGCAGCGACTAAGCAGGA-3', R 5'-TCACCAGCACGA AGGACA-3'. P50 F 5'-CAGGTCCACTGTCTGCCTCT-3', R 5'-GGAAGGATGT CTCCACACCA-3'. P52 F 5'-TCCATTTGTTCCTCCTGCTT-3', R 5'-GGTGGCCC TTGACAGTCTT-3'. P65 F 5'-GACCTGGAGCAAGCGATTAG-3', R 5'-CACTGT CACCTGGAAGCAGA-3'. GAPDH F 5'-ACCACAGTCCATGCCATCAC-3', R 5'-TCCACCACCCTGTTGCTGTA-3'. U6 F 5'-CGCTTCGGCAGCACATATAC-3', R 5'-AAATATGGAACGCTTCACGA-3'.

### Western Blot

The cells were collected, and the total protein was routinely extracted on 24 h after transfection. BCA kit (Pierce, Rockford, IL, USA) was used to measure the protein concentration. 40 μg of the protein was added to each well of the sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) gel (Millipore, Billerica, MA, USA). Experimental conditions are set to 110 V electrophoresis, 250 mA current was transferred to the polyvinylidene difluoride (PVDF) membrane and 5% skimmed milk powder was blocked at 37°C for 1 hour. PCNA, p50, p52, p65, β-catenin (Abcam, Cambridge, UK) and β-actin primary antibody were added and incubated for overnight at 4°C condition. TBST was washed for 3 × 10 min, secondary antibody was incubated for 1 h at 37°C, then TBST was washed for 3 × 30 mins, ECL was used for development. Quantity one software was used to analyze protein band gray value. β-actin was used as internal reference and calculation of expression levels was relative expression.

### CCK-8 Assay

The cells were taken in logarithmic growth phase, 3 × $10^5$ cells / well were inoculated in 6-well plates. Next, the cells were trypsinized and collected after transfection for 24 h and washed with PBS to prepare cell suspensions; pre-chilled 70% ethanol was added, and cells were fixed overnight at 4°C. After twice washing with PBS, the supernatant was discarded. Cell proliferation was assessed by a CCK-8 (CCK-8, CK04, Dojindo Molecular Technologies, Kumamoto, Japan). PI working solution was added, and the cells were incubated for 30 minutes in the dark at room temperature. Then the cell cycle was detected by flow cytometry. Cells were washed twice with pre-

chilled PBS, then 10 µl Annexin V-FITC and 5 µl propidium iodide staining solution were added. Cells were mixed gently and incubated for 20 min at room temperature in the dark.

### Cell Apoptosis Assay

The apoptosis of OA chondrocytes was measured by PE Annexin V apoptosis detection kits (BD Pharmingen, Franklin Lakes, NJ, USA) according to the manufacturer's instruction. CellQuest analysis software was used to analyze date of results by (BectonDickinson, Brea, CA, USA). All experiments were performed in triplicate.

### Statistical Analysis

All data were analyzed statistically using SPSS 22.0 statistical software (SPSS Inc., IBM, Armonk, NY, USA), which expressed as mean ± standard deviation. $p<0.05$ was considered statistically significant.

## Results

### CircVCAN Was Highly Expressed in OA Tissues and OA Chondrocytes

We examined the expression level of CircVCAN in OA tissues and OA chondrocytes. The results showed that the expression level of CircVCAN in cartilage tissue of OA patients was significantly higher than that of cartilage tissue in meniscus injury patients (Figure 1A). Similarly, the expression level of CircVCAN in OA chondrocytes was significantly higher than that of chondrocytes with meniscus injury (Figure 1B). When OA chondrocytes were transfected with si-CircVCAN, the relative expression level of CircVCAN was significantly reduced (Figure 1C). On the contrary, the relative expression level of CircVCAN was significantly increased in OA chondrocytes transfected with LV-CircVCAN (Figure 1D).

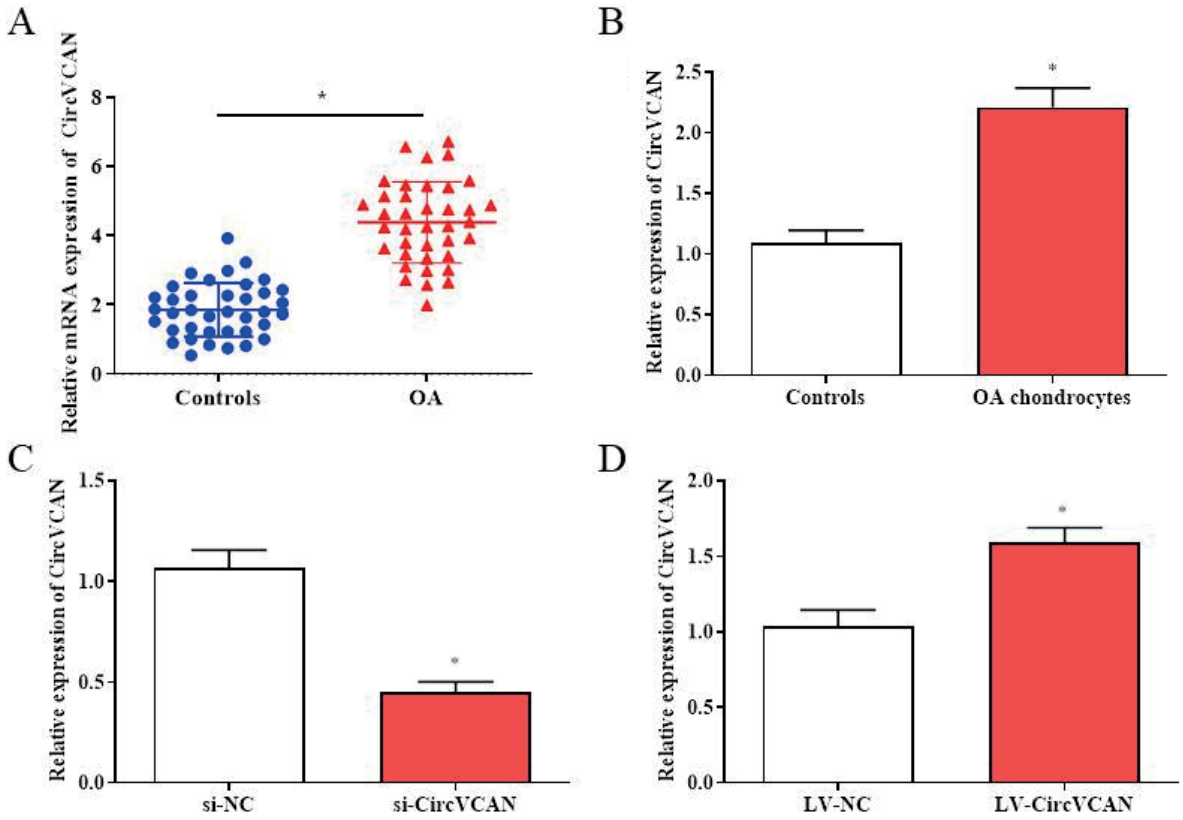


**Figure 1.** CircVCAN was highly expressed in OA tissues and OA chondrocytes**. A,** The expression levels of CircVCAN were detected by qRT-PCR in OA tissues and meniscus damaged tissues. **B,** CircVCAN expression was measured by qRT-PCR in OA chondrocytes and chondrocytes with meniscus injury. **C,** CircVCAN relative expression was measured by qRT-PCR after transfection with si-CircVCAN in OA chondrocytes. **D,** qRT-PCR was used to detect the relative expression of CircVCAN after transfection with LV-CircVCAN in OA chondrocytes. The data were expressed as mean ± SD.*$p<0.05$.

### Low Expression of CircVCAN Inhibited the Proliferation of OA Chondrocytes

In order to further explore the effect of CircVCAN on the proliferation of OA chondrocytes, OA chondrocytes were transfected with si-CircVCAN and LV-CircVCAN, respectively. PCNA mRNA and protein expression levels were detected by qRT-PCR and Western blot after three days of culture. The results showed that the expression levels of PCNA mRNA and protein in OA chondrocytes were significantly lower than the si-NC group after transfection with si-CircVCAN (Figure 2A-2C). Conversely, the expression levels of PCNA mRNA and protein in OA chondrocytes were significantly higher than the si-NC group after transfection with LV-CircVCAN (Figure 2D-2F).

### The Expression of CircVCAN Had a Great Influence on Cell Cycle of OA Chondrocytes

The effect of CircVCAN expression levels on the cell cycle was further explored, flow cytometry was used to detect the cell content at different stages in the cell cycle. The results showed that the proportion of cells in the G0/G1 phase of the cell cycle increased significantly, while the proportion of cells in the S phase decreased significantly when OA chondrocytes were transfected with si-CircVCAN (Figure 3A-3C). In contrast, when OA chondrocytes were transfected with LV-CircVCAN, the proportion of cells in the G0/G1 phase of the cell cycle decreased significantly, while the proportion of cells in the S phase increased significantly (Figure 3D-3F).

### Downregulation of CircVCAN Promoted Apoptosis of OA Chondrocytes

To further explore the effect of CircVCAN expression level on apoptosis of OA chondrocytes, flow cytometry was used to detect the apoptosis rate after transfection with si-CircVCAN and LV-CircVCAN, respectively. The results showed that the apoptosis rate of OA chondrocytes after transfection with si-CircVCAN was significantly increased compared with the group transfected with si-NC (Figure 4A-4C). In contrast, the apoptosis rate of OA chondrocytes after transfection with LV-CircVCAN was significantly reduced compared to the group transfected with si-NC (Figure 4D-4F).

### CircVCAN Had a Great Effect on NF-κB Pathway in OA Chondrocytes

The effect of circVCAN on NF-κB pathway in OA chondrocytes was further explored. The mRNA and protein expression levels of molecules related to NF-κB pathway were measured by qRT-PCR and Western blot. When OA chon-

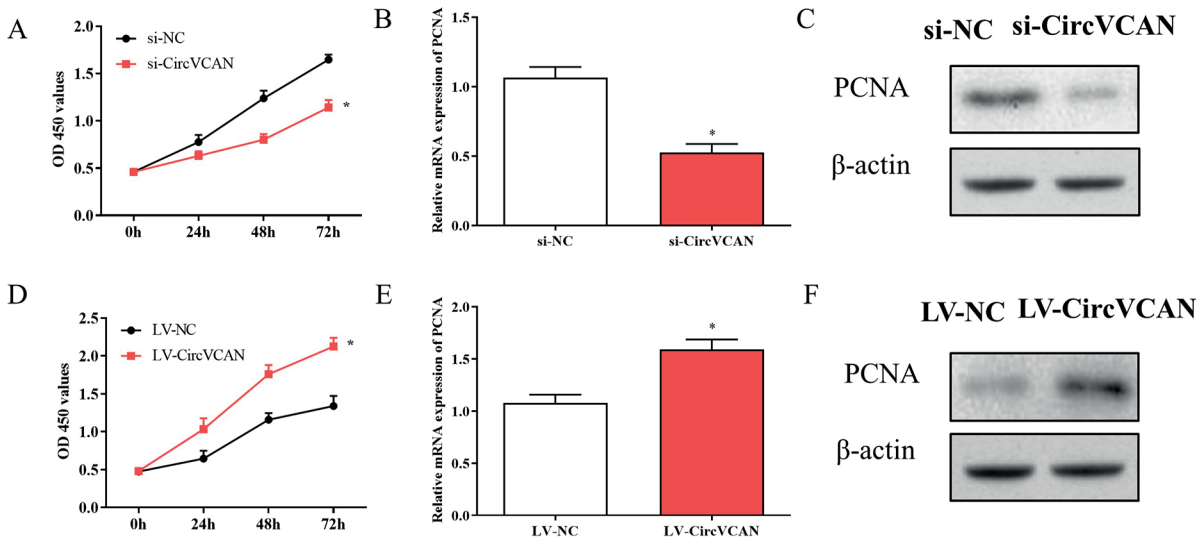


**Figure 2.** Low expression of CircVCAN inhibits the proliferation of OA chondrocytes. **A-B.** CCK-8 assay was used to detect the proliferation of OA chondrocytes after transfection with si-CircVCAN. **C.** PCNA and β-actin protein expression levels were measured by Western blotting after transfection with si-CircVCAN. **D-E.** The proliferation of OA chondrocytes was detected by CCK-8 assay after transfection with LV-CircVCAN. **F.** PCNA and β-actin protein expression levels were measured by Western blotting after transfection with LV-CircVCAN.

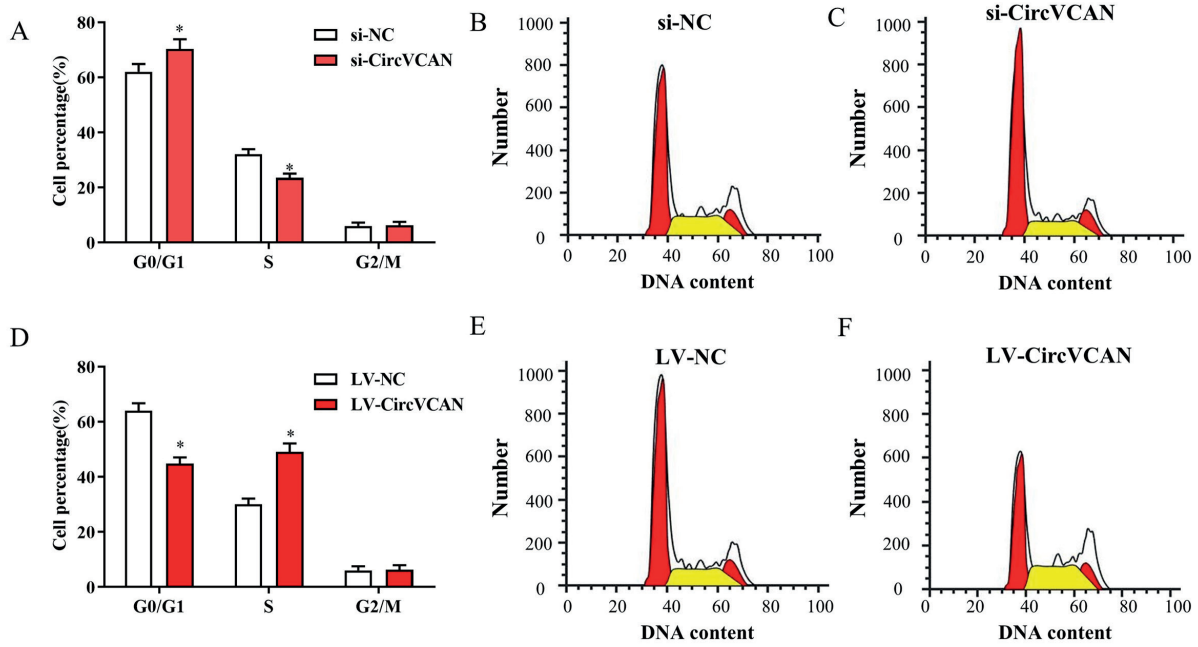


**Figure 3.** The expression of CircVCAN has a great influence on cell cycle of OA chondrocytes. **A-C.** The proportion of cells at different stages was detected by flow cytometry after transfection with si-CircVCAN. **D-F.** The proportion of cells at different stages was detected by flow cytometry after transfection with LV-CircVCAN.

drocytes were transfected with si-CircVCAN, the relative expression levels of molecules related to NF-κB pathway such as p50, p52 and p65 were significantly reduced (Figure 5A and 5C). On the contrary, when OA chondrocytes were transfected with LV-CircVCAN, the relative expression levels of NF-κB pathway-related molecules such as p50, p52 and p65 were significantly increased (Figure 5B and 5D).

### *CircVCAN Had a Great Effect on Proliferation and Apoptosis of OA Chondrocyte After Blocking NF-κB Signaling Pathway*

To further explore the effect of blocking the NF-κB signaling pathway on the proliferation and apoptosis of OA chondrocytes, we examined the proliferation and apoptosis of OA chondrocytes after transfection with LV-NC, LV-CircVCAN, LV-CircVCAN and PDTC. The results showed that the levels of PCNA mRNA and protein in OA chondrocytes transfected with LV-CircVCAN and PDTC were lower than those in LV-CircVCAN group alone (Figure 6A and 6C). At the same time, the proportion of G0/G1 phase cells transfected with LV-CircVCAN and PDTC was higher than that of LV-CircVCAN group, while S phase proportion cells was lower than transfection with LV-CircVCAN group (Figure 6D and 6H). Furthermore, the apoptosis rate of OA chondrocytes transfected with LV-CircVCAN and PDTC was higher than transfection with LV-CircVCAN alone (Figure 6I and 6L).

## Discussion

Osteoarthritis is the most common orthopedic disease, which not only affects the limb movement of patients, but also severely causes paralysis and even death[1,2]. Articular chondrocyte destruction, extracellular matrix degradation, and synthetic disorders are important factors in the occurrence of osteoarthritis[3,13]. However, its specific pathogenesis is unknown. CircRNA is a kind of non-coding circular RNA. More and more studies show that CircRNA is closely involved in the occurrence and development of osteoarthritis[10-12,14,15]. For example, has_circ_0005105 can promote the degradation of cartilage ECM by up-regulating the target gene of miR-26 NAMPT, thereby regulating the inflammatory response of OA chondrocytes[12]. Has_Circ_0045714 can up-regulate the expression of proteoglycan and type II collagen, thereby promoting the proliferation of OA chondrocytes[10].

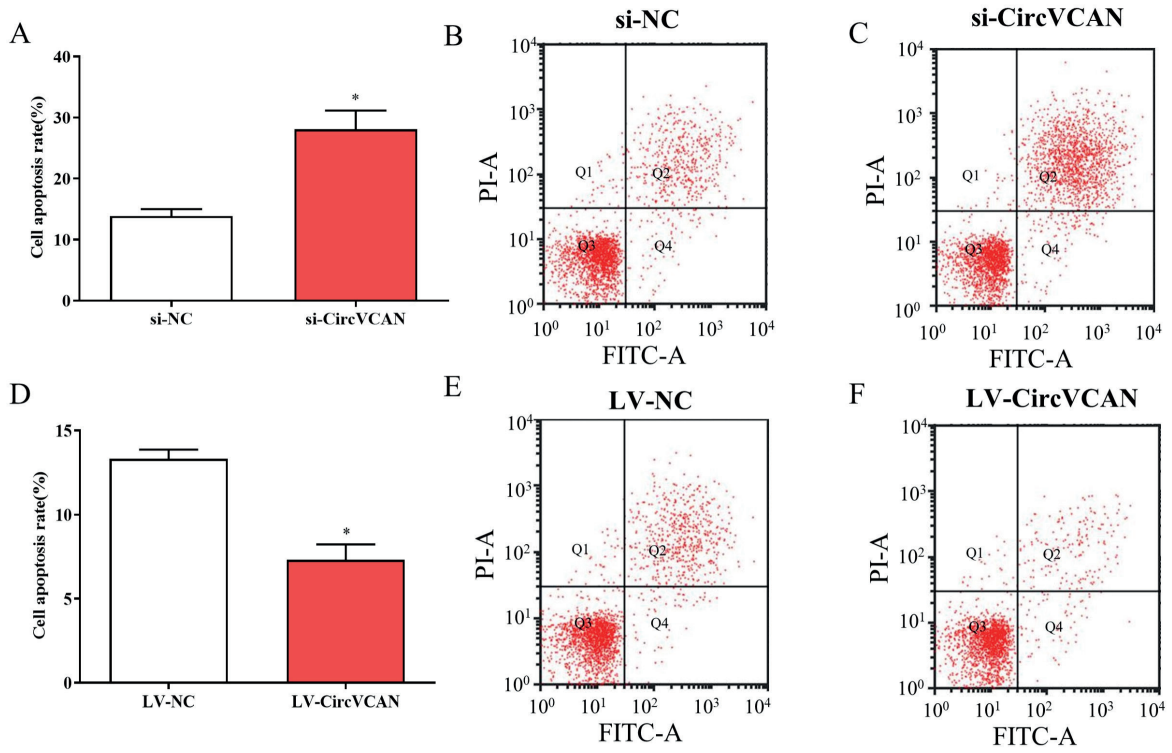


**Figure 4.** Downregulation of CircVCAN promoted apoptosis of OA chondrocytes. **A-C.** Flow cytometry was used to detect the apoptosis rates of OA chondrocytes after transfection with si-CircVCAN and si-NC. **D-F.** The apoptosis rates of OA chondrocytes were measured by flow cytometry after transfection with LV-CircVCAN and LV-NC.

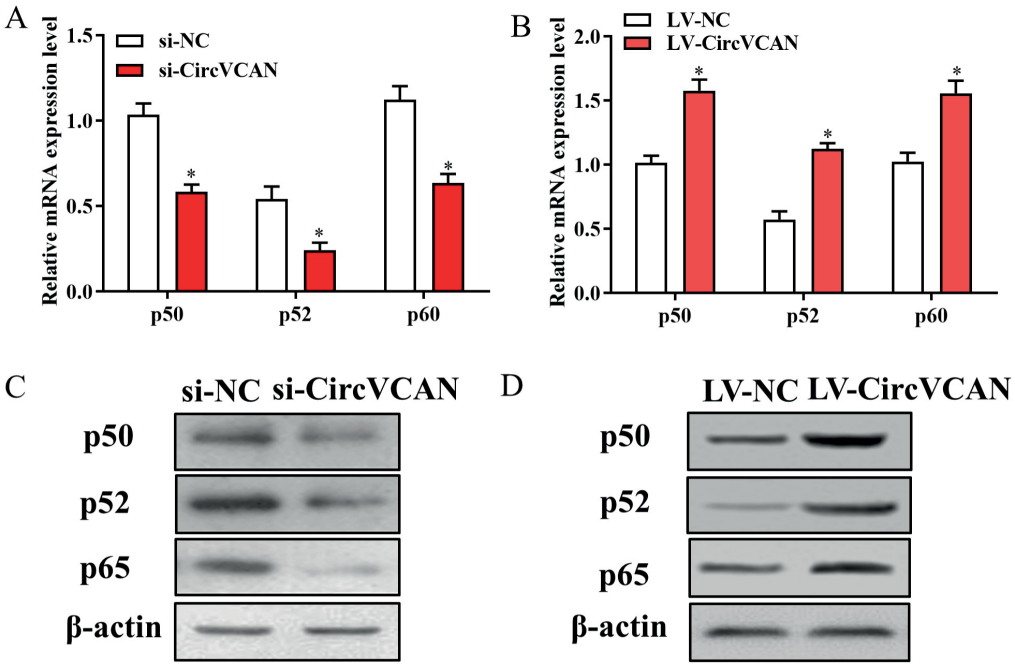


**Figure 5.** The expression of CircVCAN had a great effect on NF-κB pathway in OA chondrocytes**. A-B.** The relative expression levels of p50, p52 and p60 mRNA were measured by qRT-PCR after transfection with si-CircVCAN and LV-CircVCAN, respectively. **C-D.** The relative expression levels of p50, p52 and p60 protein were detected by Western blotting after transfection with si-CircVCAN and LV-CircVCAN, respectively.

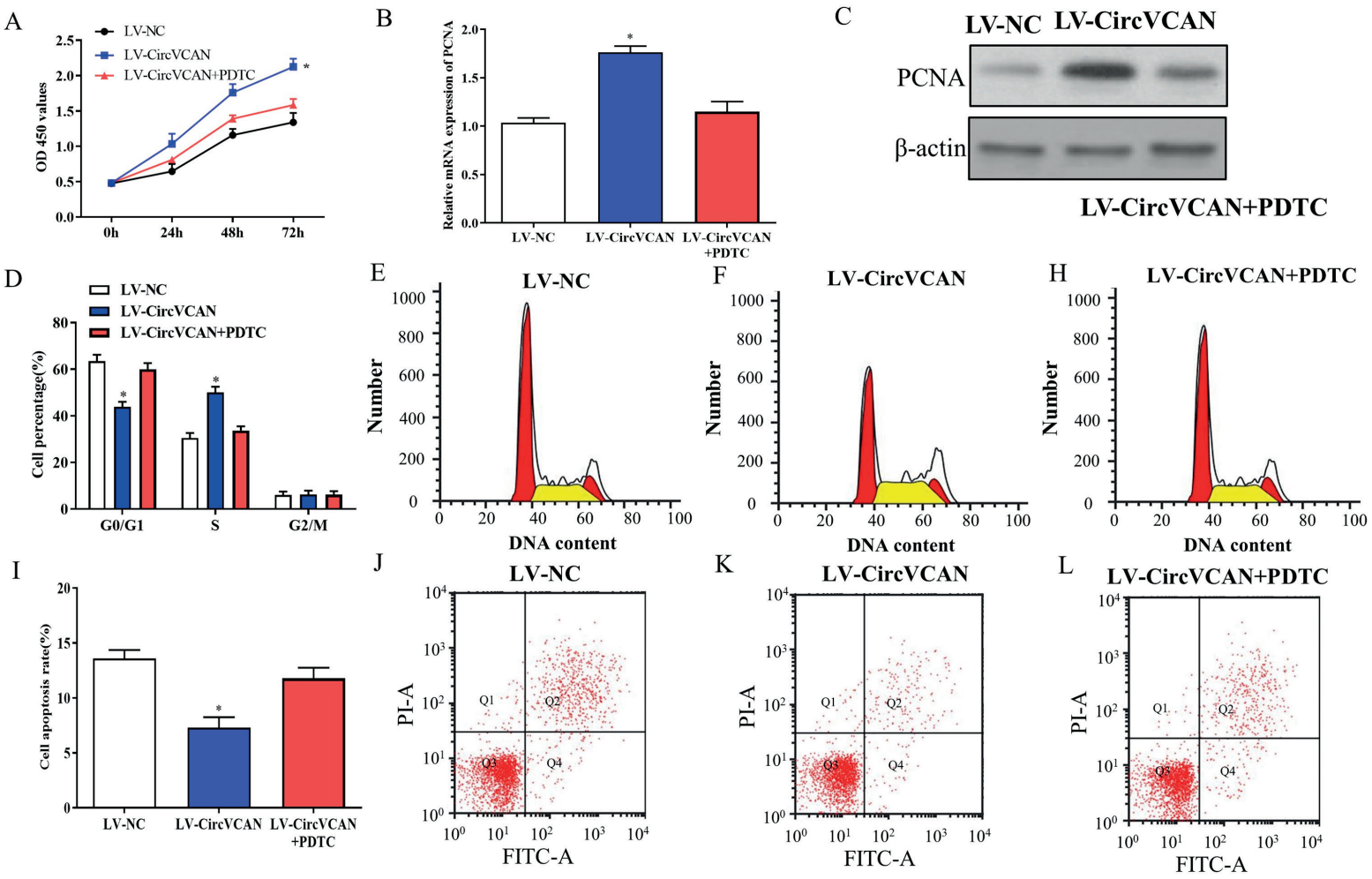


***Figure 6.*** CircVCAN expression levels had a great effect on OA chondrocyte proliferation and apoptosis after blocking NF-κB signaling pathway. **A-C.** PCNA mRNA and protein relative expression levels were measured by qRT-PCR and Western blotting after transfection with LV-NC, LV-CircVCAN, LV-CircVCAN and PDTC. **D-H.** Cell cycle was detected by flow cytometry after transfection with LV-NC, LV-CircVCAN, LV-CircVCAN and PDTC. **I-L.** Apoptotic rates were measured by flow cytometry after transfection with LV-NC, LV-CircVCAN, LV-CircVCAN and PDTC.

In our study, we found that the expression levels of CircVCAN were significantly higher in OA tissues and OA chondrocytes. When OA chondrocytes were transfected with si-CircVCAN, the relative expression level of CircVCAN was significantly reduced. However, the relative expression level of CircVCAN was significantly increased after transfection with LV-CircVCAN. This suggested that CircVCAN was abnormally expressed in OA chondrocytes, but the specific principle was not clear. Further research found that low expression of CircVCAN could effectively inhibit PCNA mRNA and protein expression levels in OA chondrocytes while high expression of CircVCAN could effectively promote PCNA mRNA and protein expression levels in OA chondrocytes. At the same time, low expression of CircVCAN could effectively increase the proportion of cells in the G0/G1 phase while reduce the proportion of cells in the S phase of the OA cell cycle. High expression could effectively reduce the proportion of cells in the G0/G1 phase of while increase the proportion of cells in the S phase of the OA cell cycle. The results further illustrated that CircVCAN had an important effect on the proliferation of OA chondrocytes. Low expression of CircVCAN inhibited cell proliferation and high expression promoted cell proliferation. At the same time, the apoptosis rate of group transfected with si-CircVCAN increased significantly while group transfected with LV-CircVCAN decreased significantly. The results indicated that CircVCAN participated in the apoptosis process of OA chondrocytes. Low expression of CircVCAN promoted apoptosis and high expression inhibited apoptosis.

NF-κB is a class of protein molecules that make up the transcription factor family. It was stimulated and activated by pro-inflammatory cytokines, chemokines, stress-related factors and ECM degradation products. NF-κB molecules were widely involved in immunity, stress response, inflammatory diseases, cell proliferation and cell death. The NF-κB signaling pathway was a key molecular pathway in the process of osteoarthritis cartilage degradation[16,17]. It promoted the secretion of multiple degradation enzymes such as MMP-1, MMP-2, MMP-3, thus aggravating the apoptosis and cartilage of osteoarthritis chondrocytes and inflammatory response[18]. The expressions of IL-1β, IL-6, TNF-α and MMP were reduced by inhibiting the NF-κB signaling pathway in the rat osteoarthritis model[19]. Tang et al[20] also found that modulation of the NF-κB signaling pathway could reduce IL-1β-induced ECM metabolic imbalance, proinflammatory cytokine production, cell viability and apoptosis. In this study, we revealed that low expression of CircVCAN could effectively inhibit the mRNA and protein levels of molecules including p50, p52 and p65 related to NF-κB pathway. Conversely, high expression of CircVCAN could effectively promote the mRNA and protein levels of 50, p52 and p65. This shows that CircVCAN is involved in the activation of NF-κB pathway in OA chondrocytes. Further research indicated that simultaneous transfection with PDTC (NF-κB inhibitor) and LV-CircVCAN could effectively reverse the effect of LV-CircVCAN on proliferation and apoptosis of OA chondrocyte. The results demonstrated that CircVCAN regulated proliferation and apoptosis of OA chondrocyte by blocking the NF-κB signaling pathway.

## Conclusions

For the first time, we discovered the effect of CircVCAN on the phenotype of osteoarthritis through the NF-κB signaling pathway. CircVCAN was demonstrated to be highly expressed in OA tissues and OA chondrocytes. Moreover, CircVCAN could regulate the proliferation and apoptosis of OA chondrocytes by blocking the activation of NF-κB signaling pathway. This study will provide new molecular mechanisms for CircRNA research in osteoarthritis and a new potential target for the treatment of osteoarthritis.

### Conflict of Interest

The Authors declare that they have no conflict of interests.

### Acknowledgement

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## References

1) Van Der Linden JC, Bank RA, Ding M, Hvid I, Sumner DR, Weinans H. Adaptation of subchondral bone in osteoarthritis. Biorheology 2004; 41: 359-368.

2) Wang Q, Wu Y, Li F, Zhu H, Zhou B, Qian Y, Ge C, Xu J, Chen L, Chu M, Jiang L, Peng J. Association of genetic polymorphisms in immune-related lncRNA with osteoarthritis susceptibility in Chinese Han population. Per Med 2018; 15: 103-110.

3) D'Lima D, Hermida J, Hashimoto S, Colwell C, Lotz M. Caspase inhibitors reduce severity of cartilage lesions in experimental osteoarthritis. Arthritis Rheum 2006; 54: 1814-1821.

4) Rong D, Lu C, Zhang B, Fu K, Zhao S, Tang W, Cao H. CircPSMC3 suppresses the proliferation and metastasis of gastric cancer by acting as a competitive endogenous RNA through sponging miR-296-5p. Mol Cancer 2019; 18: 25.

5) Adams ME. Cartilage hypertrophy following canine anterior cruciate ligament transection differs among different areas of the joint. J Rheumatol 1989; 16: 818-824.

6) Pelletier JP, Martel-Pelletier J, Mehraban F, Malemud CJ. Immunological analysis of proteoglycan structural changes in the early stage of experimental osteoarthritic canine cartilage lesions. J Orthop Res 1992; 10: 511-523.

7) Fitzgerald GK, Piva SR, Irrgang JJ. Reports of joint instability in knee osteoarthritis: Its prevalence and relationship to physical function. Arthritis Rheum 2004; 51: 941-946.

8) Xia L, Wu L, Bao J, Li Q, Chen X, Xia H, Xia R. Circular RNA circ-CBFB promotes proliferation and inhibits apoptosis in chronic lymphocytic leukemia through regulating miR-607/FZD3/Wnt/β-catenin pathway. Biochem Biophys Res Commun 2018; 503: 385-390.

9) Liu Q, Zhang X, Hu X, Dai L, Fu X, Zhang J, Ao Y. Circular RNA related to the chondrocyte ECM regulates MMP13 expression by functioning as a MiR-136 'sponge' in human cartilage degradation. Sci Rep 2016; 6: 22572.

10) Li BF, Zhang Y, Xiao J, Wang F, Li M, Guo XZ, Xie HB, Xia H, Chen B. Hsa_circ_0045714 regulates chondrocyte proliferation, apoptosis and extracellular matrix synthesis by promoting the expression of miR-193b target gene IGF1R. Hum Cell 2017; 30: 311-318.

11) Shen S, Wu Y, Chen J, Xie Z, Huang K, Wang G, Yang Y, Ni W, Chen Z, Shi P, Ma Y, Fan S. CircSERPINE2 protects against osteoarthritis by targeting miR-1271 and ETS-related gene. Ann Rheum Dis 2019; 78: 826-836.

12) Wu Y, Zhang Y, Zhang Y, Wang JJ. CircRNA hsa_circ_0005105 upregulates NAMPT expression and promotes chondrocyte extracellular matrix degradation by sponging miR-26a. Cell Biol Int 2017; 41: 1283-1289.

13) Li HZ, Lin Z, Xu XH, Lin N, Lu HD. The potential roles of CircRNAs in osteoarthritis: a coming journey to find a treasure. Biosci Rep 2018; 38(5). pii: BSR20180542.

14) Zhou Z, Du D, Chen A, Zhu L. Circular RNA expression profile of articular chondrocytes in an IL-1β-induced mouse model of osteoarthritis. Gene 2018; 644: 20-26.

15) Abramson SB, Attur M. Developments in the scientific understanding of osteoarthritis. Arthritis Res Ther 2009; 11: 227.

16) Hu J, Wang Z, Pan Y, Ma J, Miao X, Qi X, Zhou H, Jia L. MiR-26a and miR-26b mediate osteoarthritis progression by targeting FUT4 via NF-κB signaling pathway. Int J Biochem Cell Biol 2018; 94: 79-88.

17) Zhou B, Li H, Shi J. miR-27 inhibits the NF-κB signaling pathway by targeting leptin in osteoarthritic chondrocytes. Int J Mol Med 2017; 40: 523-530.

18) Ji B, Guo W, Ma H, Xu B, Mu W, Zhang Z, Amat A, Cao L. Isoliquiritigenin suppresses IL-1β induced apoptosis and inflammation in chondrocyte-like ATDC5 cells by inhibiting NF-κB and exerts chondroprotective effects on a mouse model of anterior cruciate ligament transection. Int J Mol Med 2017; 40: 1709-1718.

19) Dai T, Shi K, Chen G, Shen Y, Pan T. Malvidin attenuates pain and inflammation in rats with osteoarthritis by suppressing NF-κB signaling pathway. Inflamm Res 2017; 66: 1075-1084.

20) Tang J, Dong Q. Knockdown of TREM-1 suppresses IL-1β-induced chondrocyte injury via inhibiting the NF-κB pathway. Biochem Biophys Res Commun 2017; 482: 1240-1245.