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Blocking TXNIP reduced IL-1β Induced chondrocyte cell inflammation

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ABSTRACT

Osteoarthritis (OA) is the most common joint disease in the elderly and is characterized by progressive and irreversible degeneration of articular cartilage, particularly cartilage loss and callus formation. This study would like to investigate the important role and the molecular mechanism of OA progression following interleukin 1β (IL-1β)-induced chondrocyte injury regulated by TXNIP. In this study, high-purity mouse chondrocytes were obtained by enzymatic two-step digestion for primary culture. Toluidine blue staining and type II collagen immunofluorescence were used to identify cells through histochemical staining after slide mounting. The relative expression of TXNIP was detected by immunohistochemical staining and qRT-PCR.Aiming at the shRNA sequence of the TXNIP gene, the shRNA expression vector was constructed and packaged with lentivirus to form the lentiviral vector shTXNIP. After inhibiting the expression of TXNIP by transfecting shTXNIP into normal mouse chondrocytes, the CCK-8 kit was used for detecting its effect on cell proliferation after transfection, and the effect on chondrocyte apoptosis was detected by flow cytometry. The staining kit was used to detect the effect of TXNIP knockout on chondrocyte aging, and the differential expression of TNF, IL-6, MMP3, MMP13, ADAMTS-5 and type II collagen genes in chondrocytes was detected by RT-PCR and Western-bolt. Western blot was used to detect the expression of upstream-related protein P-ERK, downstream-related protein NLRP3 and Caspase1 after inflammatory injury of mouse articular chondrocytes. Results showed that the expression level of TXNIP in chondrocytes induced by different concentrations of il-1β was proportional to the concentration. After silencing TXNIP by shRNA, cell proliferation increased, chondrocyte apoptosis was weakened, and chondrocyte aging was weakened. The differential expression of genes such as TNF, IL-6, MMP3, MMP13, ADAMTS-5 and type II collagen and the differential expression of protein levels were relatively decreased. In addition, the expression of the upstream-related protein P-ERK did not change much when TXNIP was silenced, and the expression levels of the downstream-related proteins NLRP3 and Caspase1 were slightly reduced. In conclusion, silencing TXNIP can inhibit il-1β-induced chondrocyte apoptosis and aging, and has a positive effect on cell proliferation. However, this study has not clarified the molecular mechanism involved in TXNIP and the process of its signaling expression pathway.

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Introduction

Osteoarthritis (OA) is the most common musculoskeletal disorder and by leading the reason for pain and disability in modern society regarding bone disease (1). The main feature of OA is the gradual loss of function and degeneration of articular cartilage due to an imbalance between catabolic and anabolic processes, which eventually interferes with other tissues within the joint and leads to impairment of joint function. A major risk factor for osteoarthritis is aging (2).

However, the degeneration of cartilage joints in OA was found to be caused by a decrease in the only cell type of articular cartilage-the huge number of chondrocytes and growth factor-stimulated degradation of the extracellular matrix (ECM) and cytokine (3,4). Among them, chondrocytes (CC) play a very important role in bone development and growth. Therefore, we need to understand the molecular mechanism and cellular level of osteoarthritis to identify its early diagnostic markers and biological therapeutic targets.

Key mediators of OA pathology are known to be inflammatory mediators, such as IL-1β, which is dominated by catabolic processes initiated by pro-inflammatory cytokines. As we know, in humans and animals, the pro-inflammatory cytokines: IL-1β (interleukin 1β) and TNF-α (tumor necrosis factor α) are key players in the degradation of chondrocytes and Mediators of apoptosis in rheumatoid arthritis (RA) and osteoarthritis (OA) etc. (5–8).

In fact, many factors may affect the structural and functional changes of OA, including impaired mitochondrial activity, oxidative stress, etc. (9,10).

TXNIP (Thioredoxin-interacting protein) participates in the intracellular REDOX system and is an important mediator and gauger of inflammatory responses and oxidative stress (11). TXNIP, known as thioredoxin (TRX) interact-protein, interacts with an essential antioxidant protein TRX in the cell. TXNIP is also one of several α-arrestins involved in a lot of important cellular processes for example cell inflammation, metabolism and redox-independent pathways, and cell death through redox-dependent (12). At present, quite a few studies have shown that TXNIP is involved in the normal chondrocytes (CCs) metabolism by the regulation of autophagy(13–15). However, aberrant

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expression of TXNIP or abnormalities affected by various aging factors, infecting the CCs metabolism and degraded articular cartilage at specific body ages, ultimately affect the pathogenesis of OA. But the function of TXNIP is much more than these. There are also data showing that TXNIP is very closely related to the metabolism of bone, and the expression abnormal of TXNIP affects the differentia of osteoclasts (OCs), osteoblasts (OBs) and chondrocytes (CCs) by affecting inflammation and intracellular redox homeostasis. Furthermore, the TXNIP expression is low in cancer, and the overexpression of TXNIP inhibits the cancer cells' proliferation; thus, it is considered a potential tumor suppressor(16). TXNIP-deficient mice are at high risk of developing cancer. So TXNIP has shown strong importance due to its involvement in neurodegenerative diseases(17), glucose homeostasis and cancer(18), etc.

Caspase-1 is activated in a large multiprotein complex called the inflammasome. And caspase-1 cleavage of the inactive pro-IL-1β precursor can produce active, mature interleukin 1β (IL-1β)(19). The NLRP3 inflammasome, which is critical for producing mature IL-1β in response to various signals, consists of the Nod-like receptor protein NLRP3 (also known as cyopyrin or NALP3), the adapter proteins ASC, caspase-1, and CARDINAL(20).

There have Studies have shown that TXNIP binds to NLRP3 under conditions of oxidative stress and leads to its activation. NLRP3 and TXNIP mediate glucose-induced IL-1β secretion (11).

However, the cellular mechanism and molecular mechanism of TXNIP are still not clear enough. Therefore, in this study, IL-1β induced normal cells and shTXNIP-transfected chondrocytes to produce inflammatory damage to systematically analyze the role of TXNIP and its potential mechanism. And through the related proteins of TXNIP to explore the signaling pathways that TXNIP may participate in regulation. Finally, TXNIP was used as a target to explore its cellular and molecular mechanisms in chondrocyte inflammatory damage.

Materials and Methods

Cell culture and treatment

We euthanized 2-week-old mice and used these to isolate the primary chondrocytes. High-purity mouse chondrocytes were obtained by enzymatic two-step digestion for primary culture. The cartilage was isolated from the knee joints of these animals. After treating 4–6 h using 0.2% (2 mg/ml) collagenase II and putting it at 37°C.

We then centrifuged the digest at 1,000 rpm for 5 minutes, discarded the supernatant, resuspended the cells, plated them, and incubated them at 37°C in a humidified 5% CO2 incubator. Harvest cells when they have grown to 80%–90% confluency with 0.25% trypsin–EDTA solution. The second passage of cells thus obtained will be used for all experiments in this study.

Cell treatment

We cultured the chondrocyte cells of the second passage When they had reached 80% —90% confluency, they were cultured in serum-free medium for 24 hours and treated with IL-1β at a concentration of 10 ng/ml for 6 h, 24 h, 36 h, 48 h, and used 0.1 ng/ml, 0.5 ng/ml ,1 ng/ml, 5ng/ml,10ng/ml IL-1β for treating the CC cells in order to induce different degrees of chondrocyte inflammatory damage. 3 replicates were used for every group.

RT-PCR analysis

On the basis of the manufacturer's instruction (Qiagen, Germany) quantitative real-time PCR analyses for relative levels of collagen type II, TNF, Caspase-1, MMP3, TXNIP, MMP13, ADAMTS-5, and NLRP3, β-actin mRNA were determined by the QuantiFast SYBR Green RT-PCR kits. NLRP3, TXNIP, TNF, Caspase-1, MMP3, MMP13, ADAMTS-5 and β-actin primers were obtained from Sangon Biological Shanghai. Levels of the relative expression were calculated in the light of the 2-ΔΔCT standard method using the β-actin gene as a control for normalization. The primers table is as follows in Table 1.

Western blot analysis

The cultured CCs were lysed with the lysis buffer of Western (Beyotime, China). Whole-cell extracts were then subjected to electrophoretic separation by 10–12% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), and the bands were blotted on PVDF membranes (Millipore, USA). Nitrocellulose blots were incubated with primary antibodies diluted in primary antibody dilution buffer (Beyotime, China) for more than 12 h after blocking non-specific protein binding. Antibodies against NLRP3, MMP3, MMP13, ADAMTS-5, Caspase-1, TNF (Santa Cruze, USA) and TXNIP (Bios, China) were used at 1:200, β-actin (Bioworld, China) with 1:200-1000 used. After the incubation with the primary antibody, PVDF membranes could be washed four o five times in TBS/Tween-20 and incubated for one hour at a 1:10000 dilution in TBS/Tween20 by containing 5% skim milk. After the washes four times with TBS/Tween20, the western blot was detected using the ECL-chemiluminescence kit (ECL-plus, Thermo Scientific).

CCK-8 Assay

Use the cells that were grown in the logarithmic growth phase that were seeded in 6-well plates at a density of 1×100 cells/well for routine culture. Add 10 μl/well of CCK-8 solution for 4 hours at 37°C before washing. The absorbance of each well was then assessed by a microplate reader at 450 nm (Model 550, Bio-Rad, USA). Five replicate experiments were performed.

Construction of shRNA lentiviral vector

CCs cells were pretreated with 25 μM chloroquine for one hour to produce lentiviral particles, transfected with a shRNA which is a vector containing against TXNIP (Open Biosystems, Heidelberg) and a plasmid mixture of env, gag, VSV-G, and pol, with in pseudotyping. Eight hours after transfection, the medium was changed. After two days, It was passed the supernatant through a 0.45 μm filter supplemented with polybrene (8 μg/ml). 1 ml of viral supernatant was used to infect with $1x10^5$ target cells by spin bite method. Stably transduced Jurkat cells were selected by puromycin (1 μg/ml).

Assessment of cell death

Compared to living cells, human cell death was evaluated as the reduction in the forescatter spectrum and recalculated "specific cell death," by using flow cytometry.

Methamphetamine blue staining

The prepared second-generation cell climbing slices were removed, fixed in 95% ethanol 4℃ for 30 min, washed twice in PBS, stained with 1% volume fraction toluidine blue ethanol for 20 min, washed twice in PBS, rinsed in absolute ethanol, dried in air, sealed with neutral gum gel, and also observed and photographed under an inverted fluorescence microscope.

Immunofluorescence staining of type II collagen

The prepared second-generation cell climbing slices were incubated with cells in PBS-BSA (1% BSA prepared with PBS) for 15 min, washed twice in PBS, and immobilized with paraformaldehyde(4%) in 20 min. The paraformaldehyde was discarded, washed three or four times in PBS, and the cells were infiltrated with 0.2% Triton-X-100 for 10 min. They were washed twice with PBS and blocked with PBS-BSA for 15 min. Wash twice the cells in PBS and the cells were incubated with the antibody of type II collagen for 4℃ overnight, and after three washes in PBS the following day, goat anti-rabbit Dylight 488 fluorescent and add the secondary antibody, incubating for 1 hour at 25℃. After three times washes of PBS, nuclei were stained with DAPI for 30 min. Drop the antiquencher, seal the plate, observe and photograph under an inverted fluorescence microscope.

Results

Identification of the chondrocytes

Synthesis and secretion of proteoglycans and type II collagen are characteristic indicators of chondrocytes maintaining their differentiated phenotype and can be used for chondrocyte identification0. After the chondrocytes were adhered to the wall and fixed, stained with toluidine blue, intracellular blue-purple heterochromatic particles could be seen, the nuclei were stained blue-purple, and the cytoplasm was blue. Chondrocyte nuclei stained blue with DAPI and cytoplasm green with Dylight 488. It can be seen that pure chondrocytes were extracted in this experiment, and the characteristic collagen II mainly exists in the cell membrane and the cytoplasm using a fluorescent microscope to observe the cells.

Expression of TXNIP significantly increased after IL-1β induced inflammatory damage in chondrocytes

To observe the effects of IL-1β-induced inflammatory damage of mouse chondrocytes on the expression of TXNIP at different concentrations and at different times, we selected 10 ng/ml IL-1β to induce 6h, 24 h, 36 h, 48 h, and the concentration of IL-1β: 0.1 ng/ml-10ng/ml(0.1 ng/ml, 0.5 ng/ml, 1 ng/ml, 5 ng/ml and10ng/ml) IL-1β to study. The experimental results showed that the appearance of chondrocytes in the blank control group was mostly round or polygonal, converging into sheets. However, most of the chondrocytes in the induction group showed a long spindle shape, while the number of round or polygonal cells was significantly reduced. The number of round or polygonal chondrocytes is inversely proportional to the different treatment concentrations and time.

We have detected the expression of TXNIP by RT-PCR and Western-bolt. The experimental results (Fig.1a,b) show that with the increase of IL-1β induction time and the increase of induction concentration, the level of TXNIP also increases, the same to say, the induction time and concentration of IL-1β are directly proportional to the expression of TXNIP. The result of Western blot (Fig.1c,d) confirms again that the expression of TXNIP increases with the induction time and concentration of IL-1β.

The effect of inhibiting TXNIP expression on cell proliferation and apoptosis

To clarify the influence of TXNIP on IL-1β-induced inflammatory damage of CCs, we aimed at the shRNA sequence of the TXNIP gene, constructed the shRNA expression vector and packaged it with lentivirus to form lentiviral vector shTXNIP. After inhibiting the expression of TXNIP by transfecting shTXNIP into normal mouse chondrocytes, they were induced with 10ng/ml IL-1β for 24h, and then the effect on the cell proliferation after transfection was detected by using CCK-8 kit. The results (Fig. 2) proved that compared with the control group, the cell proliferation level of the shTXNIP group was significantly increased.

Then we used flow cytometry to detect its effect on chondrocyte apoptosis. The results (Table 2) showed that in the TXNIP-IL-1β group, the normal TXNIP was not induced by IL-1β, the apoptosis rate was (16.01 ± 0.25)%,

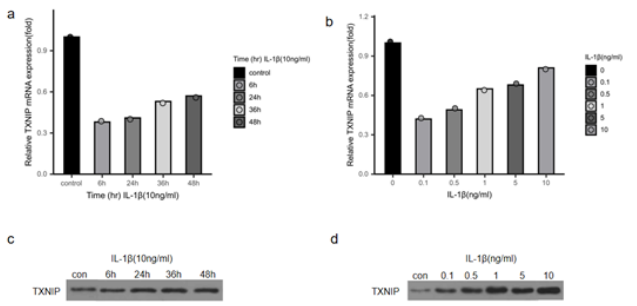

Figure 1. The effect of IL-1β on TXNIP expression level in chondrocytes. (a)Time course and (b) dose response of IL-1β affect the expression of TXNIP in chondrocyte cells as evaluated by RT-PCR. c, TXNIP protein levels measured by Western blot in chondrocyte cells incubated with IL-1β for 6h, 24 h,36h, 48h. d, Measure the TXNIP protein levels by Western blot in chondrocyte cells incubated with the concentration of 0.1 ng/ml, 0.5 ng/ml, 1 ng/ml, 5 ng/ml and 10ng/ml IL-1β. n 3–5 independent experiments, represent mean fold ± S.E.; con=control, *p<0.05.

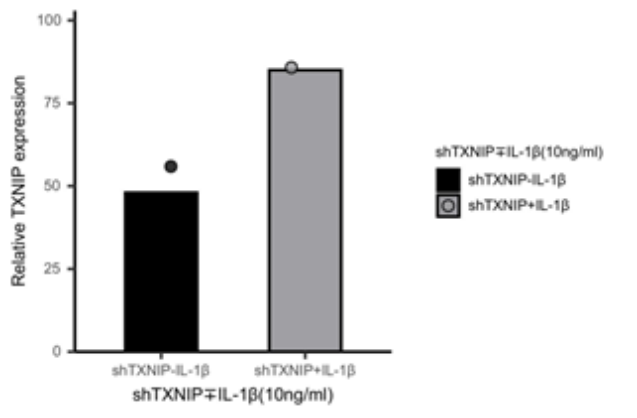

Figure 2. Use CCK-8 kit to detect the effect on chondrocyte cell proliferation after transfection. shTXNIP-IL-1β is the group without IL-1β induction, shTXNIP+IL-1β is the group with IL-1β induction. Every group has 5 duplications.

the cell apoptosis rate in the TXNIP+IL-1β group was (23.03 ± 3.01)%, and the apoptosis rate of the shTXNIP-IL-1β group was (7.07 ± 0.33)%, and the shTXNIP+IL-1β group was (11.46 ± 1.62)%. Compared with TXNIP-IL-1β, the apoptosis rate of shTXNIP-IL-1β group was significantly reduced; compared with TXNIP+IL-1β, the apoptosis rate of shTXNIP+IL-1β group was also obviously decreased. Compared with all groups, The apoptosis rate in the shTXNIP-IL-1β group was the lowest, and the difference was statistically significant ($P<0.05$). In conclusion, after the inhibition of the expression of TXNIP, the apoptosis time of chondrocytes was prolonged. This suggests that the presence of TXNIP accelerates apoptosis through some molecular mechanism.

We also used the β-Gal staining kit to detect the effect of TXNIP knockout on chondrocyte aging. Did the comparison with the control group (Fig. 3), we found that the aging speed of the cells in the shTXNIP group was slower. This means that the absence of TXNIP can also delay cell aging.

Similarly, we also used Western-bolt to detect the differential expression of TNF, IL-6, MMP3, MMP13, ADAMTS-5 and collagen II in chondrocytes and at the protein level. As shown in Fig 4, after the comparison between the two groups, we can clearly see that in the shTXNIP group which is the knockout of TXNIP, the relative expressions of TNF, IL-6, MMP3, MMP13, ADAMTS-5 and collagen II Reduced. This indicates that the reduction or deletion of TXNIP level does affect the expression of other related genes and proteins. However, the specific molecular mechanism of its influence needs to be explored more clearly.

Molecular mechanism of TXNIP regulating chondrocyte inflammatory damage

Although we have shown the effect of TXNIP on chondrocytes, we still don't know its specific molecular mechanism. In order to further clarify the mechanism molecular of TXNIP in IL-1β-induced cartilage inflammatory damage, on the basis of previous studies, we will use Western blot to detect the upstream related protein P-ERK, and downstream related protein NLRP3, Caspase-1 in mouse articular cartilage expression. The results (Fig. 5) showed no significant changes in the upstream-related protein P-ERK. However, we can see that the expression levels of downstream proteins NLRP3 and Caspase-1 were indeed reduced. However, the decrease in expression levels was not as low as expected. This means that the signaling pathway involved in the regulation of TXNIP must be relatively more complicated than we imagined.

Table 2. Detection results of apoptosis rate ($\bar{x} \pm s$, n=3).

Group	cell apoptosis rate (%)
TXNIP-IL-1β	16.01 ± 0.25
TXNIP+IL-1β	23.03± 3.01*
shTXNIP-IL-1β	7.07 ± 0.33*#
shTXNIP+IL-1β	11.46 ± 1.62*#

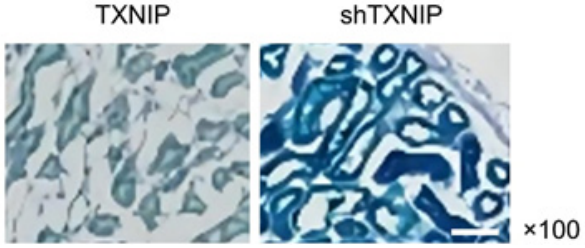

Figure 3. SA-β-Gal staining kit to detect the chondrocyte cells aging.

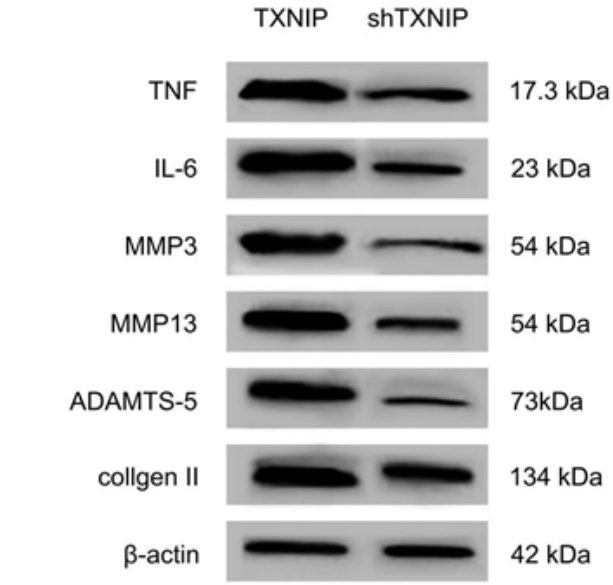

Figure 4. The relative expressions of TNF, IL-6, MMP3, MMP13, ADAMTS-5 and collagen II in the result of Western blot.

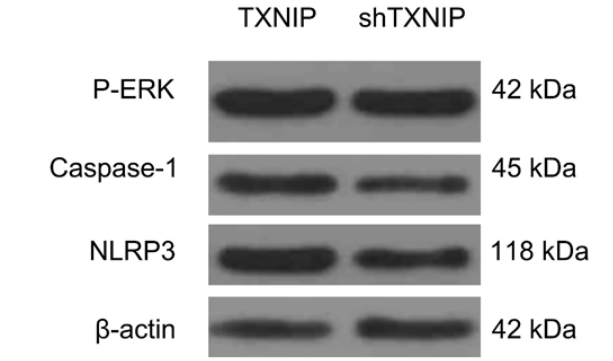

Figure 5. The expression level in the proteins P-ERK, NLRP3 and Caspase-1 in the experiment Western blot.

Discussion

As we all know, Osteoarthritis (OA) is a very common chronic joint disease in the world (21). It usually occurs in people 65 and older, and now it is a major and important public health problem (22). Unfortunately, because of the lack of knowledge and understanding of the maintenance and development of affected articular cartilage in OA, the comprehension of the mechanisms of osteoarthritis is still limited (23), so the study of the molecular mechanisms of OA has become all the more important. And TXNIP has been proved by many studies to play a very important role in OA. It has been reported that TXNIP is involved in the development and occurrence of various diseases(12). Previous researchers have proved that in OB, endogenous non-coding RNA regulates TXNIP, and downregulation of TXNIP can increase the expression of osteoprotegerin (OPG) not only in OB proliferation and differentiation(24). In other words, TXNIP also plays a very important role in osteoblasts.

However, not only in osteoblasts but also in osteoclasts. In particular, TXNIP is a vital key factor in the proliferation and differentiation of osteoclasts (OCs)(25). These are enough to prove the significance of the TXNIP that's why we choose to study according to the research background of TXNIP.

The major purpose of our study is to clarify the effect of TXNIP on IL-1β induced chondrocyte inflammatory injury and to explore its molecular mechanism in chondrocyte inflammatory injury by targeting TXNIP. In addition, we tried to figure out the molecular mechanisms of TXNIP in the progression of OA through in vivo or in vitro studies. Our findings allow us to conclude that the silencing of TXNIP does reduce the effects of IL-1β-induced inflammatory damage in chondrocytes.

First, we isolated and cultured mouse articular chondrocytes. Based on mouse chondrocyte cells, we induced chondrocyte inflammatory injury with different concentrations of IL-1β, and detected the expression of TXNIP, because its differential expression has statistical significance. TXNIP was highly expressed in affected cartilage in OA and chondrocytes treated with high concentrations of il-1β. Therefore, we judged that TXNIP may be involved in the occurrence and development of OA. Previous studies have shown that silencing of TXNIP can increase a large amount of osteoblasts (OBs) and the bone formation marker osteocalcin (OCN) expression, suggesting that TXNIP inhibits a lot of osteoblasts (OBs) and formation functions of bone and accelerates glucocorticoid-induced osteoporosis (OP)(26). Animal studies have confirmed that the antiaging protein Klotho and TRX expression family members were reduced and then induced apoptosis and IL-1β release in the articular cartilage of OA mice (27). So this is why our study wants to prove whether silencing of TXNIP can also have a positive effect on OA. Then we found that silencing TXNIP did have certain effects on TNF, IL-6, MMP3, MMP13, ADAMTS-5, collagen II, NLRP3 and Caspase-1.

But, unfortunately, we still don't know the specific molecular mechanism of TXNIP involvement. Because our study did not specifically show which genes TXNIP targets to inhibit the expression of osteoarthritis, we speculate that TXNIP may cooperate with other molecular mechanisms to positively affect chondrocyte inflammation. Other studies have shown that SIRT6 is a role key regulator of aging in CCs isolated from elderly samples. The results have shown that TXNIP levels are up-regulated in CCs of SIRT6 KO mice(28). We speculate that TXNIP in this study may be related to SIRT6 The relevant molecular mechanisms produced a certain synergy that led us to obtain such results.

Overall, we believe that TXNIP is closely related to various processes of chondrocytes and is very complex. Our study showed that silencing TXNIP had a partial positive effect on IL-1β-induced inflammatory damage of chondrocytes. However, we still need to do more research to clarify its specific molecular mechanism.

In conclusion, the silence TXNIP can inhibit il-1β induced chondrocytes(CCs) apoptosis and aging and has a positive effect on cell proliferation. However, this study has not clarified the molecular mechanism involved in TXNIP and the process of its signaling expression pathway. These findings suggest that the study of the specific molecular mechanism of TXNIP may become a viable target for the development of new therapeutic strategies.

Considering the versatility, necessity and variety of different TXNIP in osteocytes, signaling pathways or targeting molecules interacting with TXNIP is a strategy with a lot of potential for the prevention and treatment of the metabolic disorders of human bones. Therefore, further clinical and preclinical studies are fundamental to understanding specific inhibitors of TXNIP and developing new treatments to alleviate human health issues connected with the metabolic disorder of the human bone.

Data availability

The experimental data used to support the findings of this study are available from the corresponding author upon request.

Conflicts of interest

The authors declared that they have no conflicts of interest regarding this work.

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References

1. Hunter DJ, Schofield D, Callander E. The individual and socioeconomic impact of osteoarthritis. Nat Rev Rheumatolo 2014; 10(7):437–41.
2. Loeser RF, Goldring SR, Scanzello CR, Goldring MB. Osteoarthritis: a disease of the joint as an organ. Arthritis Rheum 2012;64(6):1697-707. doi: 10.1002/art.34453.
3. Aigner T, Söder S, Gebhard PM, McAlinden A, Haag J. Mechanisms of disease: role of chondrocytes in the pathogenesis of osteoarthritis--structure, chaos and senescence. Nat Clin Pract Rheumatol. 2007 Jul;3(7):391-9. doi: 10.1038/ncprheum0534.
4. Liu Q, Zhang X, Dai L, Hu X, Zhu J, Li L, Zhou C, Ao Y. Long noncoding RNA related to cartilage injury promotes chondrocyte extracellular matrix degradation in osteoarthritis. Arthritis Rheumatol. 2014 Apr;66(4):969-78. doi: 10.1002/art.38309.
5. Blanco FJ, Guitian R, Moreno J, de Toro FJ, Galdo F. Effect of antiinflammatory drugs on COX-1 and COX-2 activity in human articular chondrocytes. J Rheumatol. 1999 Jun;26(6):1366-73.
6. Heraud F, Heraud A, Harmand MF. Apoptosis in normal and osteoarthritic human articular cartilage. Ann Rheum Dis. 2000;59(12):959–65.
7. Aigner T, Kim HA. Apoptosis and cellular vitality: Issues in osteoarthritic cartilage degeneration. Arthritis Rheum 2002 Aug;46(8):1986–96.
8. Hashimoto S, Ochs RL, Komiya S, Lotz M. Linkage of chondrocyte apoptosis and cartilage degradation in human osteoarthritis. Arthritis Rheum. 1998 Sep;41(9):1632-8. doi: 10.1002/1529-0131(199809)41:9<1632::AID-ART14>3.0.CO;2-A.
9. Goldring MB. The role of the chondrocyte in osteoarthritis. Arthritis Rheum. 2000;43(9):1916–26.
10. Loeser RF, Collins JA, Diekman BO. Ageing and the pathogenesis of osteoarthritis. Nat Rev Rheumatol 2016;12(7):412–420.
11. Zhou R, Tardivel A, Thorens B, Choi I, Tschopp J. Thioredoxin-interacting protein links oxidative stress to inflammasome activation. Nat Immunol 2009;11(2):136–40.
12. Jiang N, Liu J, Guan C, Ma C, An J, Tang X. Thioredoxin-interacting protein: A new therapeutic target in bone metabolism disor-

ders? Front Immunol. 2022;13.
13. Su CJ, Feng Y, Liu TT, Liu X, Bao JJ, Shi AM, et al. Thioredoxin-interacting protein induced α-synuclein accumulation via inhibition of autophagic flux: Implications for Parkinson's disease. CNS Neurosci Ther. 2017;23(9):717–23.
14. Jin HO, Seo SK, Kim YS, Woo SH, Lee KH, Yi JY, et al. TXNIP potentiates Redd1-induced mTOR suppression through stabilization of Redd1. Oncogene 2011;30(35):3792–801.
15. Alvarez-Garcia O, Olmer M, Akagi R, Akasaki Y, Fisch KM, Shen T, Su AI, Lotz MK. Suppression of REDD1 in osteoarthritis cartilage, a novel mechanism for dysregulated mTOR signaling and defective autophagy. Osteoarthritis Cartilage. 2016 Sep;24(9):1639-47. doi: 10.1016/j.joca.2016.04.015. Epub 2016 Apr 23.
16. Sheth SS, Bodnar JS, Ghazalpour A, Thipphavong CK, Tsutsumi S, Tward AD, et al. Hepatocellular carcinoma in Txnip-deficient mice. Oncogene 2006;25(25):3528–36.
17. Tsubaki H, Tooyama I, Walker DG. Thioredoxin-Interacting Protein (TXNIP) with Focus on Brain and Neurodegenerative Diseases. Int J Mol Sci. 2020 Dec 8;21(24):9357. doi: 10.3390/ijms21249357.
18. Masutani H. Thioredoxin-Interacting Protein in Cancer and Diabetes. Antioxid Redox Signal 2022;36(13–15):1001–22.
19. Martinon F, Mayor A, Tschopp J. The Inflammasomes: Guardians of the Body. 2009;27:229–65.
20. Agostini L, Martinon F, Burns K, McDermott MF, Hawkins PN, Tschopp J. NALP3 Forms an IL-1β-Processing Inflammasome with Increased Activity in Muckle-Wells Autoinflammatory Disorder. Immunity 2004 Mar 1;20(3):319–25.
21. He L, Pan Y, Yu J, Wang B, Dai G, Ying X. Decursin alleviates the aggravation of osteoarthritis via inhibiting PI3K-Akt and NF-kB signal pathway. Int Immunopharmacol. 2021 Aug 1;97.
22. Su Y, Song X, Teng J, Zhou X, Dong Z, Li P, Sun Y. Mesenchymal stem cells-derived extracellular vesicles carrying microRNA-17 inhibits macrophage apoptosis in lipopolysaccharide-induced sepsis. Int Immunopharmacol. 2021 Jun;95:107408. doi: 10.1016/j.intimp.2021.107408.
23. Ma X, Zhang Z, Shen M, Ma Y, Li R, Jin X, et al. Changes of type II collagenase biomarkers on IL-1β-induced rat articular chondrocytes. Exp Ther Med. 2021 Apr 2;21(6).
24. Yang CS, Gu ZT, Ding R, Huang CQ, Li QC, Xie DH, et al. Retracted: Long non-coding RNA MEG3 silencing and microRNA-214 restoration elevate osteoprotegerin expression to ameliorate osteoporosis by limiting TXNIP. J Cell Mol Med. 2021;25(4):2025–39.
25. Lekva T, Ueland T, Byøum H, Evang JA, Godang K, Bollerslev J. TXNIP is highly regulated in bone biopsies from patients with endogenous Cushing's syndrome and related to bone turnover. Eur J Endocrinol. 2012;166(6):1039–48.
26. Lekva T, Ueland T, Byøum H, Evang JA, Godang K, Bollerslev J. TXNIP is highly regulated in bone biopsies from patients with endogenous Cushing's syndrome and related to bone turnover. Eur J Endocrinol. 2012 Jun;166(6):1039–48.
27. Gu Y, Ren K, Jiang C, Wang L, Yao Q. Regulation of cartilage damage caused by lack of Klotho with thioredoxin/peroxiredoxin (Trx/Prx) system and succedent NLRP3 activation in osteoarthritis mice. Vol. 11, Am J Transl Res. 2019.
28. Collins JA, Kapustina M, Bolduc JA, Pike JFW, Diekman BO, Mix K, et al. Sirtuin 6 (SIRT6) regulates redox homeostasis and signaling events in human articular chondrocytes. Free Radic Biol Med. 2021 Apr 1;166:90–103.