miR-27b-3p Targeting BDNF Inhibits the TrkB/CREB Signaling Pathway and Improves IL-1 β -Induced Chondrocyte Inflammation

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Abstract

Background: Explore the mechanism of "miR-27b-3p targeting BDNF inhibits the TrkB/CREB signaling pathway and improves IL-1 β -induced chondrocyte inflammation".

Methods: The animal and cell models of arthritis were constructed, and various biochemical detection methods were used to detect the changes of apoptosis, inflammation and oxidative stress.

Results: The results showed that the expression of miR-27b-3p was downregulated in IL-1 β -treated chondrocytes and cartilage tissues isolated from a KOA rat model. miR-27b-3p overexpression notably reduced IL-1 β -induced chondrocyte apoptosis and the expression levels of caspase-3 and caspase-9. In addition, cell transfection with miR-27b-3p mimics increased the mRNA and protein expression levels of inducible nitric oxide synthase and cyclooxygenase-2. The levels of nitric oxide, prostaglandin E2 (PGE2), TNF- α and IL-6 were also reduced following cell transfection with miR-27b-3p mimics. Furthermore, bioinformatics analysis predicted that miR-27b-3p could directly target brain-derived neurotrophic factor (BDNF). Additionally, the present study suggested that the tropomyosin receptor kinase B (TrkB)/cAMP response-element binding protein (CREB) signaling axis could be a downstream pathway of the miR-27b-3p/BDNF axis. The percentage of apoptotic cells and the expression levels of nitric oxide, PGE2, TNF- α and IL-6 were enhanced in chondrocytes co-treated with BDNF + IL-1 β . However, these effects were restored following transfection of chondrocytes with miR-27b-3p mimics. Staining of cartilage tissues with safranin O showed that miR-27b-3p overexpression significantly attenuated KOA-induced cartilage degradation.

Conclusions: miR-27b-3p targeting BDNF inhibits the TrkB/CREB signaling pathway and improves IL-1 β -induced chondrocyte inflammation.

Introduction

The meniscus is the fibrous cartilage plate of the knee joint and is composed of "C"-shaped medial and "O"-shaped lateral parts. The outer edge of the meniscus is thick, while the inner edge is thin and concave, and composed of collagen fibers, which serve an important role in the structure and function of the knee joint. Meniscus serves a crucial role in relieving vibration, lubricating joints, reducing friction, increasing the stress area, conducting load and maintaining the stability of the knee joint (1). Trauma or natural aging can make the meniscus appear with varying degrees of deformation, subsequently causing surrounding tissue edema, abnormal proliferation, loss of normal function and stability of the knee joint and accelerated articular cartilage degeneration, eventually leading to the occurrence of knee osteoarthritis (KOA).

KOA is a common, chronic progressive disease characterized by articular cartilage degradation, subchondral bone sclerosis, hyperostosis, osteochondral vessel formation and inflammatory responses

in articular chondrocytes, which may eventually lead to disability (2). Patients with KOA suffer from joint pain, swelling and reduced quality of life (3). It has been reported that > 10% of adults aged > 60 suffer from KOA, while the incidence of the disease increases with age and body mass index (4). Currently, there are no effective treatment strategies to reverse KOA in the clinic. Therefore, determining the mechanisms underlying KOA pathogenesis is of great importance for the development of effective therapeutic strategies against KOA. MicroRNAs (miRNAs/miRs) are a cluster of non-coding RNAs of 15–18 nucleotides in length that can bind to the 3'-untranslated region (3'-UTR) of their target mRNAs (5, 6), thus promoting their degradation or inhibiting their translation (7, 8). It has been reported that miRNAs can regulate the cellular behavior in several diseases, including cancer, spinal cord injury and hepatitis (7, 9). Additionally, miRNAs can regulate the pathogenesis of KOA. For example, a previous study demonstrated that miR-140 was expressed in chondrocytes and could regulate cartilage development (10). Additionally, miR-26a could regulate KOA progression via targeting the fucosyltransferase IV/NF- κ B signaling pathway (11). Other studies showed that miR-489-3p could regulate tumorigenesis, bladder cancer cell migration and proliferation, and prostate cancer progression (12–14). However, to the best of our knowledge, the effect of miR-27b-3p on KOA has not been previously reported.

Emerging evidence has suggested that IL-1 β is upregulated in the cartilage tissues of patients with KOA (15, 16). IL-1 β is considered as one of the most important inflammatory factors involved in the occurrence and development of KOA (15). It has been also reported that IL-1 β can induce the expression of other cytokines, such as IL-6, and activate catabolism, thus supporting its implication in cartilage degradation. Therefore, inhibiting the effects of IL-1 β is critical for attenuating the development of KOA (17). Chondrocytes, the dominant cell type in the cartilage tissue, serve a key role in maintaining the metabolic balance of the cartilage matrix. Additionally, they secrete collagen and proteoglycan to preserve the integrity and function of cartilage. A study demonstrated that IL-1 β could also promote chondrocyte apoptosis to reduce the self-repair ability of cartilage tissue (18). Therefore, inhibiting chondrocyte apoptosis is of important significance for the treatment of KOA.

The tropomyosin receptor kinase B (TrkB)/cAMP response-element binding protein (CREB) signaling pathway is involved in several biological processes, including inflammatory responses, cell injury and apoptosis (19). It has been reported that in KOA, the TrkB/CREB signaling pathway can be activated by IL-1 β (20). Therefore, inhibition of TrkB/CREB signaling attenuates IL-1 β -induced chondrocyte apoptosis and provides beneficial effects in KOA (20). Furthermore, emerging evidence has shown that brain-derived neurotrophic factor (BDNF) is upregulated in the joint tissues of patients with KOA (21, 22). BDNF, an extracellular cytokine, can activate the TrkB/CREB signaling pathway via binding to its cell membrane receptor, namely the neurotrophin receptor. Therefore, blocking the expression of BDNF could be considered as an efficient approach for attenuating the development of KOA via inhibiting the activation of TrkB/CREB signaling.

The present study aimed to uncover the role of miR-489-3p in KOA both *in vitro* and *in vivo*. In addition, the current study investigated the effects of the BDNF/miR-27b-3p axis on regulating chondrocyte

apoptosis via regulating the BDNF/TrkB/CREB pathway. Overall, this study may identify novel therapeutic targets for KOA.

Materials And Methods

Animals and ethics statement. A total of 96 Sprague-Dawley male rats (age, 3 months; weight, 250 ± 0.05 g) were obtained from the Experimental animal center of Yan'an University. The rats were randomly divided into four groups, namely the control, KOA, KOA + lentivirus-negative control (len-NC) and KOA + len-miR-27b-3p groups. Each group consisted of 24 rats, whose tissues and cells were used for reverse transcription-quantitative PCR (RT-qPCR), histological examination, western blot analysis and ELISAs. The rats were housed under a 12-h light/dark cycle. All animal experiments were approved by the Ethics Committee of Yan'an University (approval no. SXYA201901) and lasted for 2 years from design to completion.

Establishment of KOA rat model. A total of 96 rats were randomly divided into the control group, the KOA group, the KOA + len-NC group and the KOA + len-miR-27b-3p group (24 mice/group). The KOA model was established by partially removing the medial meniscus of the right knee. Prior to surgery, rats were anesthetized with 1% sodium pentobarbital (40 mg/kg), while iodine was applied as a disinfectant. Subsequently, a lateral parapatellar skin incision was performed with ophthalmic scissors along the right knee joint at 1 cm proximal to the patella under a microscope. The patella was turned outward and secured to expose the joint capsule. The medial meniscus and tibial ligament were disconnected and the partial medial meniscus was then removed. Subsequently, the patella was placed in its original position and sealed with a scalpel. Following surgery, rats were intramuscularly injected with 1.5 mg/kg ampicillin for 3 days to prevent infection. In addition, to relieve surgery-induced pain, rats were intraperitoneally injected with 0.1 mg/kg tramadol upon awakening from anesthesia. The rats were allowed to fully recover from anesthesia in warmed cages and were closely monitored for pain, infections and other surgery-related complications. After 8 weeks, all rats were euthanized by CO₂ inhalation-mediated asphyxia with 10–30% volume/min gas displacement flow rate. Briefly, the rats were placed in a special closed container with air or oxygen supply. Subsequently, the concentration of CO₂ was increased until cardiac arrest occurred. Finally the knee tissues were harvested for further analysis.

Chondrocyte culture and transduction with oligonucleotides. To isolate chondrocytes, the knee cartilage from 3-month-old Sprague Dawley rats was excised under aseptic conditions and 1.0 cm of the knee joint meniscus from rats in each group was collected, cut into 1 mm×1 mm sized pieces, add 0.2% I collagen enzyme with 10x body mass for 4 h digestion at 37°C with a volume fraction of 5% CO₂ saturation humidity. By 120 mesh copper mesh overfiltration, absorb filter fluid, 1500g centrifugation 5min. The obtained cells are based on a DMEM medium containing 10% fetal bovine blood, at 37°C, with a volume fraction of 5% CO₂ Incubator. Change fluid every 3d once. When cells reach 80%–90% fusion, trypsin digestion 1:3 for transmission culture. Subsequently, the cartilage tissue was digested with 0.4% sterile II collagen enzym (12 ml/g of cartilage tissue) at 37°C for 90 min followed by centrifugation at 1,400 g for 5 min. Following removal of the supernatant, the cartilage tissue was digested overnight with 0.025%

sterile collagen II (12 g/ml of cartilage tissue). Reserve cartilage on liquid, centrifugal, frozen. Following counting (activity of fetal blue staining cells < 90%), cells were seeded into a 6-well plate at a density of 1×10^5 cells/ml in DMEM/F12 supplemented with 10% FBS and placed in an incubator at 37°C and 5% CO₂.

Once cell confluence reached 70–80%, cells were divided into four groups, namely the control, KOA, KOA + len-NC and KOA + len-miR-27b-3p groups. Cartilage chondrocytes in the KOA + len-NC and KOA + len-miR-27b-3p groups were transduced with the corresponding lentiviral particles for 24 h using Lipofectamine® 2000 (Invitrogen; Thermo Fisher Scientific, Inc.) according to the manufacturer's instructions. The transduction efficiency was verified by RT-qPCR.

RT-qPCR. To assess the mRNA expression levels of BDNF, inducible nitric oxide synthase (iNOS) and cyclooxygenase (COX-2) in cultured chondrocytes and those of miR-27b-3p in cartilage tissues, RT-qPCR was carried out as previously described. Briefly, chondrocytes and cartilage tissues were harvested and total RNA was extracted using TRIzol® (Invitrogen; Thermo Fisher Scientific, Inc.). The concentration of the isolated RNA was measured using a Nanodrop 2000 spectrophotometer (Thermo Fisher Scientific, Inc.). Subsequently, the cDNA of miR-489-3p was synthesized using the RevertAid First Strand cDNA Synthesis kit (Thermo Fisher Scientific, Inc.), while that of BDNF, iNOS and COX-2 was synthesized using a PrimeScript RT Master mix (Takara Bio, Inc.). qPCR (Applied Biosystems, USA) was performed using a MiScript SYBR® Green PCR kit (Thermo Fisher Scientific, Inc.). The expression levels of BDNF, iNOS and COX-2 and miR-27b-3p were normalized to the internal reference genes, GAPDH and U6, respectively. The relative gene expression was calculated using the $2^{-\Delta\Delta C_q}$ method. The primer sequences used are listed in Table I.

Western blot analysis. The protein expression levels of caspase-3, caspase-9, iNOS, COX-2, BDNF, TrkB, CREB and phosphorylated (p)-CREB in cultured chondrocytes and those of BDNF, p-TrkB and p-CREB in cartilage tissues were determined by western blot analysis. Total proteins were extracted from cells and tissues with RIPA lysis buffer (Beyotime Institute of Biotechnology). The protein concentration in each group was measured using a Nanodrop 2000 spectrophotometer (Thermo Fisher Scientific, Inc.). Subsequently, the protein extracts were separated by SDS-PAGE and were then transferred onto PVDF membranes. Membranes were blocked with 4% solution of BSA for 2 hrs and incubated with primary antibody at 4 °C overnight. Primary antibody was removed and the membranes were further incubated with respective secondary antibody for 1 hr at room temperature. Protein bands were visualized using ECL assay kit (Bio-Rad, USA). Image J software was used for the quantification of band densities.

ELISA. The samples for ELISA were prepared according to the manufacturer's instructions using the corresponding ELISA kits (DEIA048V). Briefly, 1 cm of the knee joint meniscus was removed from rats in each group. Following repeated washings with sterile PBS, the cartilage tissues were immersed in oxidizing acid aqueous solution for sterilization three times (30 min each time). Subsequently, the cartilage tissues were cut into small pieces with sterile ophthalmic scissors on a sterilized ultra-clean working bench, rinsed repeatedly in sterile distilled water, soaked in 3% hydrogen peroxide solution for 30

min and washed again with sterile distilled water for three times (30 min each time). Then, the cartilage pieces were supplemented with three volumes of sterile tri-distilled water and smashed into an articular cartilage tissue homogenate using an ultrasonic tissue pulverizer at 4°C. The homogenate was mixed well with 10 volumes of sterile tri-distilled water in a hypotonic environment followed by 4–5 cycles of freezing at -20°C and slowly thawing at room temperature. Following the above freezing-thawing cycles, the remaining cells were ruptured and the homogenate was centrifuged at 2,000 g for 30 min to harvest the supernatant. The isolated supernatant was centrifuged at 3,000 g for 30 min and the resulting supernatant was further subjected to centrifugation at 4,000 g for 30 min. The above steps were repeated for five cycles. Finally, the supernatant containing small particles was centrifuged at a high speed of 10,000 g for 30 min and the precipitate was used to prepare a decellularized nano-scale human articular chondrocytes-extracellular matrix (ECM) homogenate. The homogenate was transferred into Eppendorf tubes and stored at low temperature for subsequent experiments. The levels of nitric oxide, PGE2, TNF- α , IL-6, Bax, Bcl-2 and IL-6 in the above homogenates were determined using the ELISA method on a fully automatic biochemical instrument to detection of the supernatant of meniscus chondrocytes.

Flow cytometry. To evaluate the role of miR-27b-3p on chondrocyte apoptosis, a flow cytometry assay was carried out using a FITC-Annexin V apoptosis detection kit (Beyotime Institute of Biotechnology). Briefly, chondrocytes were harvested, rinsed in cold PBS and labeled with FITC-Annexin V solution for 4 min on ice. Then, the cells were rinsed in coupling solution and supplemented with a PI solution followed by incubation in the dark. Finally, the cell apoptosis rate was determined using a Fortessa flow cytometer (BD Biosciences) and the acquired data were analyzed with FlowJo software (FlowJo LLC10).

Dual luciferase reporter assay. To verify whether BDNF could be directly targeted by miR-27b-3p, a dual luciferase reporter assay was carried out. The binding sites of miR-27b-3p on the BDNF 3'-UTR were predicted using the TargetScan database(<https://www.mirnet.ca/miRNet/home.xhtml>). Subsequently, the BDNF 3'-UTR was subcloned into the p-MIR-GLO reporter vector (Promega Corporation) to construct the BDNF-wild-type (WT; concentration, 500 μ g/ μ l) and BDNF-mutant (MUT; concentration, 500 ng/ μ l) plasmids, encompassing the WT and MUT binding sites of miR-27b-3p, respectively. The cultured chondrocytes were co-transfected with miR-27b-3p mimics/NC and BDNF-WT/BDNF-MUT using Lipofectamine 2000. Following transfection for 48 h, the relative luciferase activity was measured using a Dual Luciferase Reporter assay system (Promega Corporation).

Histological analysis. The cartilage tissues were harvested, fixed with 4% paraformaldehyde, embedded into paraffin and cut into 5- μ m thick sections. The sections were then stained with Safranin O staining and images were captured under a microscope (Nikon Corporation).

Statistical analysis. All data were analyzed using GraphPad Prism 6.0 (GraphPad Software, Inc.). The results are expressed as the mean $\pm$ SD. The differences among multiple groups were analyzed using a one-way ANOVA followed by a Tukey's multiple comparison post-hoc test. $P < 0.05$ was considered to indicate a statistically significant difference.

Results

miR-27b-3p inhibits IL-1 β -induced chondrocyte inflammation and CH8 human articular chondrocyte apoptosis. To further clarify the role of miR-27b-3p in KOA, IL-1 β -treated chondrocytes were transfected with miR-27b-3p mimics. RT-qPCR analysis showed that the expression of miR-27b-3p was notably downregulated in the IL-1 β group compared with the control group. The inhibitory effect of IL-1 β on miR-27b-3p was markedly reversed following cell transfection with miR-27b-3p mimics (Fig. 1A). The current study hypothesized that treatment of chondrocytes with IL-1 β could promote chondrocyte inflammation, thus suggesting that IL-1 β could induce chondrocyte apoptosis. miR-27b-3p overexpression was discovered to reduce the apoptosis rate of IL-1 β -induced chondrocytes compared with the control group (Fig. 1B). Cell treatment with IL-1 β also increased the expression of caspase-3 and caspase-9, which was restored following miR-27b-3p overexpression (Fig. 1C). These findings indicated that miR-27b-3p could exert a protective effect on IL-1 β -induced chondrocyte apoptosis.

miR-27b-3p downregulates the expression of inflammatory factors in IL-1 β -treated chondrocytes. Since IL-1 β could induce the inflammatory responses and apoptosis of chondrocytes, the current study aimed to investigate whether there was an association between them and whether miR-27b-3p could exert its anti-apoptotic role via attenuating the inflammatory responses. Therefore, the mRNA expression levels of iNOS and COX-2 were determined by RT-qPCR. IL-1 β induced the expression of iNOS and COX-2, while miR-27b-3p overexpression exhibited the opposite effects (Fig. 2A). Similarly, cell transfection with miR-27b-3p mimics reduced the increased protein expression levels of iNOS and COX-2 in IL-1 β -treated chondrocytes (Fig. 2B). Additionally, cell treatment with IL-1 β upregulated nitric oxide, PGE₂, TNF- α and IL-6. This effect was reversed following cell transfection with miR-27b-3p mimics (Fig. 2C-F).

miR-27b-3p directly targets BDNF. To further investigate the mechanism underlying the effects of miR-27b-3p on KOA, the target genes of miR-27b-3p were predicted using the TargetScan database. BDNF was predicted to be a direct target gene of miR-27b-3p; the predicted binding sites of miR-27b-3p on the BDNF 3'-UTR are illustrated in Fig. 3A. Furthermore, dual luciferase reporter assays showed that the relative luciferase activity was significantly reduced in the BDNF-WT + miR-27b-3p mimics group compared with the other three groups (Fig. 3B). Subsequently, the mRNA expression levels of BDNF were determined in miR-27b-3p mimics-transfected cells. Treatment of chondrocytes with IL-1 β markedly upregulated BDNF, while cell transfection with miR-27b-3p mimics markedly abrogated the inhibitory effect of IL-1 β on the expression of BDNF mRNA (Fig. 3C). These results were consistent with those obtained at the protein level (Fig. 3D and E). Overall, the aforementioned findings verified that BDNF was directly targeted by miR-27b-3p.

miR-27b-3p targets BDNF to regulate the downstream TrkB/CREB pathway. To further evaluate the role of miR-27b-3p on KOA, the effects of the downstream pathways of the miR-27b-3p/BDNF axis were investigated. It has been reported that the TrkB/CREB signaling pathway is the downstream pathway of miR-27b-3p/BDNF (22). Herein, to further verify this finding, the expression levels of TrkB, p-TrkB, CREB and p-CREB were determined in cultured chondrocytes. The expression levels of p-TrkB and p-CREB were

notably upregulated following cell treatment with IL-1 β , and were decreased after transfection of chondrocytes with miR-27b-3p mimics (Fig. 4A). These results indicated that miR-27b-3p overexpression could inhibit the TrkB/CREB pathway triggered by IL-1 β . To further uncover the regulatory effect of miR-27b-3p on the BDNF-triggered TrkB/CREB pathway, cultured chondrocytes were treated with BDNF. The IL-1 β -mediated p-TrkB and p-CREB upregulation was further increased following co-treatment with BDNF. However, miR-27b-3p overexpression abrogated this effect (Fig. 4B). The aforementioned findings suggested that BDNF could be a direct target gene of miR-27b-3p.

miR-27b-3p attenuates IL-1 β -induced chondrocyte apoptosis and the secretion of inflammatory factors via targeting the BDNF/TrkB/CREB signaling pathway. To reveal whether the anti-apoptotic effect of miR-27b-3p on IL-1 β -treated chondrocytes was mediated via the BDNF/TrkB/CREB pathway, the cell apoptosis rate was measured in cells treated with BDNF and miR-27b-3p mimics. miR-27b-3p overexpression reduced the IL-1 β -induced cell apoptosis rate. Additionally, the apoptosis rate was elevated in the IL-1 β + BDNF group, which was restored in cells transfected with miR-27b-3p mimics (Fig. 5A). These results suggested that miR-27b-3p could attenuate IL-1 β + BDNF-induced apoptosis. Furthermore, the expression levels of the inflammatory factors, nitric oxide, PGE₂, TNF- α and IL-6, were enhanced in the IL-1 β + BDNF group. Cell transfection with miR-27b-3p mimics markedly downregulated the expression of these factors (Fig. 5B-E). Overall, these findings indicated that miR-27b-3p could inhibit IL-1 β -induced chondrocyte apoptosis and inflammatory factor secretion via targeting the BDNF/TrkB/CREB pathway.

miR-27b-3p attenuates the degradation of ECM, protects cartilage tissue and relieves KOA-triggered pain via inhibiting the expression of inflammatory factors in vivo. The aforementioned *in vitro* experiments revealed that miR-27b-3p could attenuate KOA-triggered pain via inhibiting chondrocyte apoptosis and inflammation. The current study aimed to evaluate the above effects of miR-27b-3p on KOA *in vivo*. Therefore, a KOA rat model was first established by knee meniscus dissection and KOA rats were then injected with len-miR-27b-3p into the knee lumen to overexpress miR-27b-3p in the cartilage tissues. The results revealed that the expression of miR-27b-3p was markedly downregulated in the KOA group compared with the control group. In addition, treatment with len-miR-27b-3p markedly enhanced the expression of miR-27b-3p compared with the len-NC group (Fig. 6A). These results showed that miR-27b-3p was downregulated in KOA cartilage tissues, while treatment with len-miR-27b-3p increased its expression. Subsequently, the cartilage tissues were harvested and stained with Safranin O/fast green staining solution. The cartilage tissues in the control group showed large red stained areas, while these areas were notably reduced in the KOA and KOA + len-NC group. Additionally, miR-27b-3p overexpression markedly increased the red-stained areas compared with the KOA + len-NC group (Fig. 6B). Furthermore, the expression levels of BDNF, p-TrkB and p-CREB were significantly reduced in the cartilage tissues of rats in the KOA and KOA + len-NC groups compared with the control group. By contrast, treatment with len-miR-27b-3p significantly increased their expression levels (Fig. 6C). In addition, treatment with len-miR-27b-3p decreased the expression of Bax and promoted that of Bcl-2 compared with the KOA and KOA + len-NC groups (Fig. 6D and E). Furthermore, TNF- α and IL-6 were significantly upregulated in the KOA and KOA-len-NC groups, while their expression was inhibited in the len-miR-27b-3p group (Fig. 6F and G).

These data suggested that miR-27b-3p could protect the cartilage tissues from degradation and inhibit the expression of inflammatory factors *in vivo*, thus providing a habitable environment for chondrocytes.

Discussion

KOA is mainly characterized by articular cartilage degeneration and destruction, due to the lack of blood vessels, nerves, and lymphatic vessels and the decreased congenital differentiation ability of cartilage cells. In addition, in KAO, chondrocytes are also characterized by poor migration and self-repair capacities. Therefore, the treatment of degenerative KOA remains a major medical problem. Currently, repairing the damaged articular cartilage tissues and maintaining their functions are considered as the main targets for treating osteoarthritis (23, 24).

KOA is the leading cause of disability among elderly individuals. There are currently no clinically available effective therapies to treat KOA (25, 26), which can be attributed to the lack of knowledge on the mechanisms underlying the development of the disease. Therefore, uncovering the mechanisms involved in the pathogenesis of KAO and developing novel treatment approaches are of great importance.

It has been recently reported that several miRNAs are involved in the development and pathogenesis of KOA (11, 26–29). The present study demonstrated that miR-27b-3p was downregulated in IL-1 β -treated chondrocytes. In addition, miR-27b-3p overexpression inhibited IL-1 β -induced chondrocyte apoptosis and secretion of inflammatory factors. Furthermore, bioinformatics analysis predicted that miR-27b-3p could directly target BDNF, which was further verified by dual luciferase reporter assays. Additionally, the mRNA and protein expression levels of BDNF were increased in chondrocytes transfected with miR-27b-3p mimics. The *in vivo* experiments revealed that miR-27b-3p overexpression attenuated cartilage degradation and the secretion of inflammatory factors. Therefore, the results of the current study suggested that miR-27b-3p could exert a positive effect on KOA.

Since chondrocytes are the only cell type in normal cartilage tissues, chondrocyte apoptosis in KOA significantly attenuates the self-repair capacity of cartilage tissues. Therefore, inhibiting chondrocyte apoptosis could reduce the degradation of the ECM and be considered as a novel treatment approach (30). Herein, miR-27b-3p overexpression attenuated chondrocyte apoptosis via targeting BDNF. It has been reported that BDNF is upregulated in the joint tissues in KOA, thus inducing KOA-related pain (31). As an extracellular cytokine, BDNF can promote the activation of intracellular pathways, such as the TrkB/CREB pathway (32). Therefore, IL-1 β -mediated activation of the TrkB/CREB pathway could induce chondrocyte apoptosis. Emerging evidence has suggested that the inhibition of the TrkB/CREB pathway exerts a beneficial role in KOA (33–35). Herein, cell transfection with miR-27b-3p mimics inhibited the activation of the TrkB/CREB pathway. This pathway was further activated following chondrocyte treatment with IL-1 β + BDNF. However, the effect of IL-1 β + BDNF treatment was abrogated by miR-27b-3p overexpression. These findings indicated that miR-27b-3p could target BDNF to inhibit the activation of the TrkB/CREB pathway and chondrocyte apoptosis.

KOA is also characterized by increased inflammatory reactions. In the present study, the levels of IL-6, nitric oxide, PGE2, COX-2, iNOS and TNF- α were increased in both KOA cartilage tissues and IL-1 β -treated chondrocytes. In addition, miR-27b-3p overexpression notably reduced the expression of these inflammatory factors. These data showed that miR-27b-3p could inhibit the inflammatory reactions in KOA, thus suggesting that miR-27b-3p overexpression could attenuate chondrocyte apoptosis via inhibiting the inflammatory reactions.

In conclusion, the present study demonstrated that miR-27b-3p was downregulated in KOA, while miR-27b-3p overexpression could protect chondrocytes from IL-1 β -induced cell apoptosis via regulating the BDNF/TrkB/CREB pathway. The development of a safe and effective treatment approach for KOA has become a hot research topic. The present study uncovered a novel mechanism underlying the onset of KOA and provided a novel therapeutic target for the disease. Overall, miR-27b-3p could promote cartilage repair in KOA via several mechanisms. However, further in-depth studies are needed to further verify the effectiveness and safety of miR-27b-3p on the treatment of KOA.

Declarations

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Availability of data and materials

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

Authors' contributions

CLR designed the study and revised the manuscript. RJC and MW drafted the manuscript. LJW and YY collected and analyzed the data. XJL and XFJ performed the statistical analyses.

Ethical approval and consent to participate

This study was approved by the Ethics Committee of the Yan'an University and it was in accordance with the Tenth People's Hospital Affiliated to Tongji University guidelines.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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Tables

Table I. Primer sequences used for reverse transcription-quantitative PCR.

Gene	Forward 5'	Reverse 3'
MicroRNA-27b-3p	GTGACATCACATATACGG	GTGACATCACATATACGG
BDNF	GGCTGACACTTTTGAGCACGTC	CTCCAAAGGCACTTGACTGCT
iNOS	GAGACAGGGAAGTCTGAAGCAC	CCAGCAGTAGTTGCTCCTCTTC
COX-2	GCGACATACTCAAGCAGGAGCA	GCGACATACTCAAGCAGGAGCA
GAPDH	AATGGACAACCTGGTCGTGGA	CCCTCCAGGGGATCTGTTTG

Figures

Figure 1

miR-27b-3p inhibits IL-1 β -induced chondrocyte apoptosis. (A) Expression levels of miR-27b-3p were detected by reverse transcription-quantitative PCR. (B) Cell apoptosis rate was determined using FITC-Annexin V flow cytometry assay. (C) Protein expression levels of caspase-3 and caspase-9 were measured using western blot analysis (n=8). The differences between groups were calculated using a one-way ANOVA followed by a Tukey's multiple comparison post-hoc test. *P < 0.05. miR, microRNA.

Figure 2

miR-27b-3p attenuates the expression of inflammatory factors in IL-1 β -treated chondrocytes. The mRNA and protein expression levels of iNOS and COX-2 were evaluated by (A) reverse transcription-quantitative PCR and (B) western blot analysis, respectively. The secretion levels of (C) nitric oxide, (D) PGE2, (E) TNF- α and (F) IL-6 were detected by ELISA (n=8). The differences between groups were calculated using a one-way ANOVA followed by a Tukey's multiple comparison post-hoc test. *P < 0.05. miR, microRNA; iNOS, inducible nitric oxide synthase; COX-2, cyclooxygenase 2; PGE2, prostaglandin E2.

Figure 3

BDNF is directly targeted by miR-27b-3p. (A) Predicted binding sites of miR-27b-3p on BDNF 3'-untranslated region are shown. (B) Dual luciferase reporter assay results are shown. (C) mRNA expression levels of BDNF were determined by reverse transcription-quantitative PCR. (D) Protein expression levels of BDNF were measured by western blot analysis (n=8). The differences between groups were calculated using a one-way ANOVA followed by a Tukey's multiple comparison post-hoc test. *P<0.05. BDNF, brain-derived neurotrophic factor; miR, microRNA.

Figure 4

mR-27b-3p targeting BDNF regulates the downstream TrkB/CREB pathway. (A and B) Protein expression levels of TrkB, p-TrkB, CREB and p-CREB in cultured chondrocytes were determined by western blot analysis (n=8). The differences between groups were calculated using a one-way ANOVA followed by a Tukey's multiple comparison post-hoc test. *P<0.05. p-, phosphorylated; TrkB, tropomyosin receptor kinase B; CREB, cAMP response-element binding protein; BDNF, brain-derived neurotrophic factor; miR, microRNA.

Figure 5

MicroRNA-27b-3p inhibits IL-1 β -induced chondrocyte apoptosis and inflammatory factor secretion via targeting the BDNF receptor kinase B/cAMP response-element binding protein pathway. (A) Cell apoptosis rate was evaluated by FITC-Annexin V flow cytometry assay. The secretory levels of (B) nitric oxide, (C) PGE2, (D) TNF- α and (E) IL-6 were detected using ELISAs (n=8). The differences between groups were calculated using a one-way ANOVA followed by a Tukey's multiple comparison post-hoc test. *P<0.05. PGE2, prostaglandin E2.

Figure 6

miR-27b-3p protects cartilage tissues from degradation and inhibits the expression of inflammatory factors in vivo. (A) Expression of miR-27b-3p was measured by reverse transcription-quantitative PCR. (B) Cartilage tissues stained with Safranin O are shown (magnification, x100). (C) Protein expression levels of BDNF, p- TrkB and p-CREB were detected by western blot analysis. The expression of (D) Bax, (E) Bcl-2, (F) TNF- α and (G) IL-6 were measured by ELISA (n=10). The differences between groups were calculated using a one-way ANOVA followed by a Tukey's multiple comparison post-hoc test. *P<0.05. miR, microRNA; BDNF, brain -derived neurotrophic factor; p-, phosphorylated; TrkB, tropomyosin receptor kinase B; CREB , cAMP response-element binding protein.