Research Article

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miR-22-3p relieves the osteoarthritis by targeting to inflammasome *in vivo* and *in vitro*

miR-22-3p, *in vivo* ve *in vitro* olarak iltihaplanmayı hedefleyerek osteoartriti hafifletir

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Abstract

Objectives: Osteoarthritis (OA) is a common degenerative disease of the joints. It has become one of the main diseases that cause the disability of the elderly in the world, and it has a severe impact on the quality of life of patients. It has been reported that miRNAs are involved the occurrence and development of OA. In the current work, we evaluated the effects of miR-22-3p on osteoarthritis *in vivo* and *in vitro*.
Methods: Confocal Laser Scanning Microscope (CLSM), flow cytometry analysis, indirect immunofluorescence (IFA) and Western-blot assays were performed to study the effect of miR-22-3p on osteoarthritis (OA).
Results: An LPS-induced osteoarthritis cell model was first constructed on C28/I2 cells (*in vitro*), and the model of mice OA was established by operation (*in vivo*). The results form RT-qPCR indicated that miR-22-3p expression was reduced by LPS (lipopolysaccharides) stimulation. Additionally, inflammatory cytokines and apoptosis-related markers were also obviously elevated with LPS treatment. Furthermore, up-regulation of miR-22-3p relieved the osteoarthritis *in vivo* and *in vitro*. Additionally, up-regulation of miR-22-3p obviously reduced the production of proinflammatory factors and cell apoptosis induced by LPS. Further research indicated that miR-22-3p may alleviate osteoarthritis by targeting inflammasome.
Conclusions: In conclusion, the upregulation of miR-22-3p could effectively alleviate osteoarthritis *in vivo* and *in vitro*, suggesting that miR-22-3p can be used to treat OA.

Keywords: inflammasome; LPS; micro-RNA; miR-22-3p; osteoarthritis.

ÖZ

Amaç: Osteoartrit (OA), eklemlerin sık görülen dejeneratif bir hastalığıdır. Dünyada yaşlıların sakatlığına neden olan başlıca hastalıklardan biri haline gelmiştir ve hastaların yaşam kalitesini ciddi şekilde etkilemektedir. OA'nın oluşumunda ve gelişiminde çeşitli miRNA'ların rol oynadığı bildirilmiştir. Bu çalışmamızda miR-22-3p'nin osteoartrit üzerindeki etkilerini *in vivo* ve *in vitro* olarak değerlendirdik.
Gereç ve Yöntem: miR-22-3p'nin Osteoartrit (OA) üzerindeki etkisini incelemek için Konfokal Lazer Tarama Mikroskobu (CLSM), Flowsitometri analizi, dolaylıimmünflorasan (IFA) ve Western-blot yöntemleri uygulandı.
Bulgular: LPS ile indüklenen osteoartrit modeli, *in vitro* olarak C28/I2 hücrelerinde ve *in vivo* olarak da farelerde oluşturuldu. RT-qPCR'den elde edilen sonuçlar, miR-22-3p ekspresyonunun LPS uyarımı ile azaldığını gösterdi. Ek olarak, LPS tedavisi ile inflamatuar sitokinler ve apoptoz önemli ölçüde arttı. Ayrıca miR-22-3p'nin upregülasyonu, LPS tarafından indüklenen hücre apoptozu ve inflamatuar faktörlerin üretimini önemli ölçüde azaltarak *in vivo* ve *in vitro* olarak osteoartriti rahatlattı. Daha fazla araştırma, miR-22-3p'nin iltihaplanmayı hedefleyerek osteoartriti hafifletebileceğini belirtmektedir.

Bai Lu and Li Qingbo contributed equally to this work.

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Sonuç: Özetle, miR-22-3p'nin upregülasyonu, osteoartriti *in vivo* ve *in vitro* olarak etkili bir şekilde hafifletebilir, bu da miR-22-3p'nin OA tedavisinde kullanılabileceğini düşündürmektedir.

Anahtar kelimeler: miR-22-3p; Kireçlenme; iltihaplı; LPS.

Introduction

Osteoarthritis (OA) is a degenerative non-specific inflammation [1, 2]. OA is a common degenerative disease of the joints. It has become one of the main diseases that cause the disability of the elderly in the world, and it has a severe impact on the quality of life of patients [3]. During the progression of OA, bone joints can undergo a variety of pathological changes: such as the production of pro-inflammatory factors, the degradation of cartilage matrix, the apoptosis of chondrocytes, and the activation of macrophages in the synovium [4]. The etiology of OA is complex and involves many aspects, such as aging, obesity, genetics, and trauma. At present, the pathogenesis of OA has not been fully elucidated, and it is believed to be caused by a combination of multiple factors. The main purpose of non-surgical treatments for OA is to relieve pain, and it can hardly slow the progression of OA [4]. Therefore, exploring the pathogenesis of OA and finding effective treatment measures are of great significance for the prevention and treatment of OA.

The occurrence and development of OA are closely related to chondrocyte inflammation and apoptosis [3]. Studies have shown that some microRNAs (microRNAs, miRNAs) has the regulating effect on the cell proliferation and apoptosis. Studies has also showed that microRNAs exhibit the important function on the progress of OA [5–7]. For example, microRNA-34a is a major small RNA that causes apoptosis, which specifically expressed in cartilage and is involved in chondrocyte apoptosis during the progression of OA. Silencing the miRNA-34a gene via *in vitro* cell transfection could reduce the apoptosis of OA cells, and significantly alleviate the early symptoms of OA [4, 8, 9]. Inflammasome is a complex composed of multiple proteins with a molecular weight of about 700 KDa [10]. This concept was first proposed by the Tschopp research team in 2002 [11]. Inflammasome could modulate the activation of caspase-1 to promote the maturation and secretion of the cytokine precursors in the process of natural immune defense. It can also modulate caspase-1-dependent form of apoptosis (pyroptosis), and induce cell death under inflammatory and stress pathological conditions [12]. Recently, researchers have conducted intensive research on Mir-22-3p, they found that Mir-22-3p is involved in a series of physiological and pathological processes. It has been reported that Mir-22-3p is involved in the occurrence and development of different types of tumors (e.g. lung cancer and thyroid cancer) [13]. Furthermore, Mir-22-3p also shows important biological regulatory functions, Mir-22-3p was also involved in regulating neuropathic pain and protecting the endothelium against oxidative stress. More importantly, the researchers found that Mir-22-3p is involved in the process of inflammation regulation, Mir-22-3p exhibited the protective effects against gouty inflammation and retinal pigment epithelial inflammatory damage [14, 15].

Studies have shown that inflammasome can be used as a target to treat OA. Therefore, in this work, based on previous research and bioinformatics, we have studied the relationship between miR-22-3p and osteoarthritis. We established *in vitro* chondrocyte and *in vivo* osteoarthritis models, and the results indicated that miR-22-3p exhibits the therapeutic potential on OA. Further *in vivo* and *in vitro* experiments showed that inflammasome is the potential target of miR-22-3p. In summary, this study suggests that miR-22-3p can be used to treated osteoarthritis (OA).

Materials and methods

Materials

The fetal bovine serum was purchased from Thermo Fisher Scientific (Waltham, MA, USA). DMEM 1640 medium and phosphate buffered saline (PBS) were purchased from Thermo Fisher Scientific (Waltham, MA, USA). Lipopolysaccharide (LPS) was purchased from Solarbio (Beijing, China). IL-1β ELISA detection kit was purchased from Thermo Fisher (Waltham, MA, USA). NF-κB p65 (1:1,000 dilution), phosphorylated NF-κB p65 (1:500 dilution), IκBα (1:2,000 dilution), phosphorylated IκBα (1:1,000 dilution), and NLRP3 (1:1,500 dilution) antibodies were purchased from Cell Signaling Technology (Danvers, MA, USA). Anti-caspase-1 (1:2,000 dilution) and anti-ASC (1:1,000 dilution) antibodies were purchased from Abcam (Cambridge, UK). Unless otherwise specified, other reagents were purchased from Sigma-Aldrich company (St. Louis, Missouri, USA).

Cell culture

Human cartilage cell line C28/I2 were purchased from ATCC. The cells were cultured in DMEM medium supplemented with 10% FBS at 37 °C humidified incubator with 5% CO_2.

RT-PCR

Total RNAs were extracted from C28/I2 cells or tissue using TRIzol Reagent kit (Invitrogen™, Cat:10296028) following the manufacture's protocol. The total RNAs were subsequently subjected to cDNA synthesis by using superscript II reverse transcriptase (Invitrogen™, Cat:18064071). RT-PCR was conducted using the following primers: NLRP3 (forward, 5′-CACCTGTTGTGCAATCTGAA -3′; reverse, 5-TCCTGACAACATGC TGATGTGA)-3′, human capsase1 (forward, 5′-TTT

CCGCAAGGTTCGATTTTCA-3′; reverse, 5′-TGGGCATCTGCGCTCTACCATC-3′), human IL-1β (forward, 5′-TCCAGGGACAGGATATGGAG-3′; reverse, 5′-TCTTTCAACACGCAGGACAG-3′). miR-22: 5′-GTCGTATCCAGTGCGTTGCGTGGAGTC, GGCAATTGCACTGGATACGACAACTGT-3′. The mRNA levels were normalized to the levels of β-actin mRNA. GAPDH: F: forward, 5′-TGTGTCCGTCGTGGATCTGA-3′, Reverse:5′ -CCTGCTTCACCACCTTCTTGA-3′ [16].

Construction of adeno-associated virus (AAV) vector expressing miR-22-3p

Adeno-associated virus (AAV) is a vector widely used in gene therapy. In the current study, adeno-associated virus (AAV) vector expressing miR-22-3p was constructed by Gene Pharma Co., Ltd.

Establishment of OA model

Male C57BL/6J mice aged 10 weeks were used to establish a mouse OA model [17–19]. After the mice were anesthetized with chloral hydrate (400 mg/kg) in the abdominal cavity, the right knee joints of the mice were disinfected with alcohol, and one centimeter was cut adjacent to the patella to expose the joint cavity, and the anterior meniscus ligament was cut. After flushing the joint cavity with sterile saline, the incision was sutured and the skin was disinfected. The experiments were divided into four groups: Group A, control operation group; Group B, operation group; Group C, AVV-mic-22-3p treatment group; Group D, adeno-associated virus vector control group.

Safranin O-fast green stain

The tissue sections were routinely deparaffinized, and then the sections were soaked in xylene solution for 10 min. After treatment with the ethanol, the sections were stained with fast-green. After washing, the sample was stained with safranin O solution, the sections were then observed using a light microscope.

Intra-articular injection of AAV virus

One week after the establishment of the OA model, the AAV virus vector expressing miR-22-3p or control miRNA was injected into the joint cavity, after miR-22-3p treatment for four weeks, the cartilage tissue of the mouse knee joint was extracted for HE and Safranin O-fast green stain analysis.

Cell viability assay

Cell counting kit-8 was used to test the cell proliferation. In brief, C28/I2 cell (1×10^4 cells/mL) were seeded into 96-well plate for 24 h. Cells were pre-treated with different concentrations of LPS for 12 h, 10 μL CCK-8 solution was added and incubated at 37 °C for 4 h, after which, absorbance at 450 nm wavelength was detected using a microplate reader (Bio-rad).

Western-blot

After treatment with LPS for 12 h, total protein was extracted using RIPA lysis buffer. Protein concentrations were determined using the bicinchoninic acid (BCA) kit. Equal amounts of protein (30 μg/Lane) from each sample were subjected to SDS-PAGE and transferred onto a polyvinylidene fluoride (PVDF) membrane. The membranes were then treated with 5% skim milk powder at 4 °C overnight. After washing, the PVDF membranes were treated with the primary antibodies (NF-κB, 1:500 dilution; NLRP3, 1:1,000) at 4 °C overnight, followed by incubation with HRP-conjugated secondary antibodies (1:2,000 dilutions) for 1 h at RT. The immunoreactive bands were visualized by ECL detection system.

Fluorescence-activated cell sorting (FACS) analysis

After miR-22-3p or control miRNA transfection for the indicated time points, a single cell suspension was prepared. The cells were fixed, blocked and stained with the indicated antibodies, the flow cytometer was then used to analyze the cell samples.

Analysis of ROS content

C28/I2 cells were cultured in 6-well plates for 48 h. The cells were then stimulated with LPS for 12 h. After three washes, C28/I2 cells were treated with 10 uM DCFH-DA for 0.5 h. After washing, cells were collected and determined by Flow Cytometer (BD). ROS levels were determined by BD CellQuest software.

Statistical analysis

All data are expressed as mean ± SD. Three independent experiments were performed. Statistical analysis was performed using GraphPad Prism 8.0 (GraphPad Software Inc). $p<0.05$ was identified as significantly difference.

Results

Effect of LPS on human cartilage cell line C28/I2

We used LPS to treat the chondrocyte line (C28/I2) to establish inflammation model. Firstly, the cell viability of C28/I2 was determined by CCK-8 Kit. Figure 1A showed that the relative viability of C28/I2 cells was obviously decreased after treatment with 5–10 ug of LPS for 12 h. However, LPS (5 ug/mL) did not lead to the NLRP3 up-regulation. However, LPS (10 μg/mL) not only could obviously down-regulate the cell viability, but also elevate the NLRP3 expression. Furthermore, the ROS level was *evidently* enhanced (it has been demonstrated that ROS leads to the NLRP3 inflammasome activation) (Figure 1B). Additionally, the results form RT-PCR and Flow Cytometry showed that the expression levels of NLRP3, ACS, Caspase-1, and IL-1β were also elevated (Figure 1C). The proportion of cell apoptosis was obviously enhanced in LPS-treated group (Figure 1D). These observations suggests that the LPS-induced arthritis cell model was successfully established. Therefore, LPS (10 μg/mL) was used in the subsequent experiments (*in vitro*).

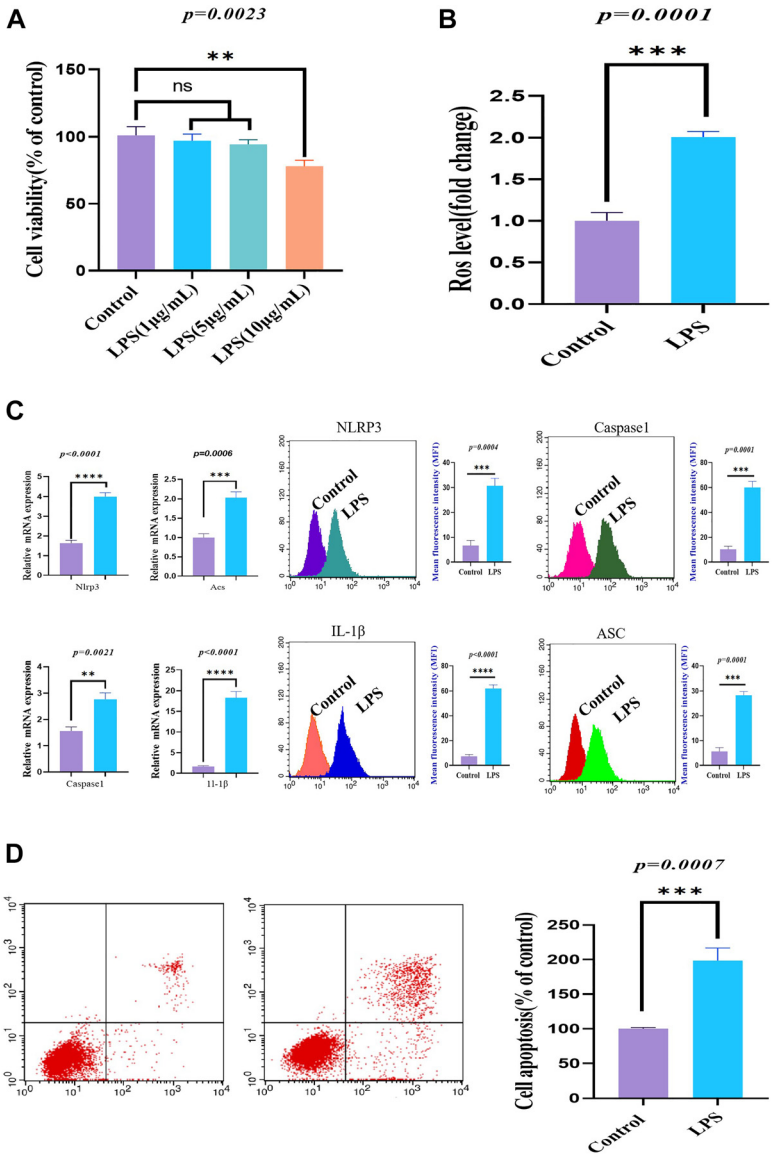

Figure 1: (A) The effect on LPS on the viability of C28/I2 cells. C28/I2 cell (1×10^4 cells/mL) were seeded into 96-well plate for 24 h. Cells were pre-treated with different concentrations of LPS, 10 μL CCK-8 solution was added and incubated at 37 °C for 4 h, after which, absorbance at 450 nm wavelength was detected using a microplate reader. (B) The ROS level was obviously enhanced by LPS treatment. C28/I2 cells were cultured in 6-well plates for 48 h. The cells were then stimulated with LPS for 12 h. After three washes, C28/I2 cells were treated with 10 uM DCFH-DA for 0.5 h. After washing, cells were collected and determined by flow cytometer (BD). ROS levels were determined by BD CellQuest software. (C) NLRP3, ACS, Caspase-1, and IL-1β were up-regulated by LPS treatment. (D) The proportion of apoptosis was obviously elevated in LPS group. The asterisk (*) indicates a statistically significantdifference (p-value<0.05).

The expression of MiR-22-3p was down-regulated in LPS-treated C28/I2 cells

RT-PCR was performed to test the expression of MiR-22-3p in LPS-treated C28/I2 cells. Figure 2 showed that the expression level of MiR-22-3p was obviously down-regulated compared to control group.

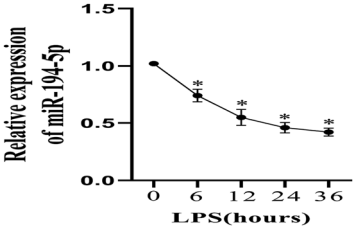

Figure 2: (A) The expression of MiR-22-3p was down-regulated in LPS-treated C28/I2 cells. Total RNAswere extracted from C28/I2 cells or tissue using TRIzol Reagent kit (Invitrogen™) following the manufacture's protocol. The total RNAs were subsequently subjected to cDNA synthesis by using superscript II reverse transcriptase. RT-PCR was performed in the materials and methods section. The asterisk (*) indicates a statistically significant difference (p-value<0.05).

The effect of up-regulation of miR-22-3p on the expression of NLRP3 and downstream cytokines

Bioinformatics analysis (MirDB: http://www.mirdb.org/, miRWalk: http://mirwalk.umm.uni-heidelberg.de/) showed

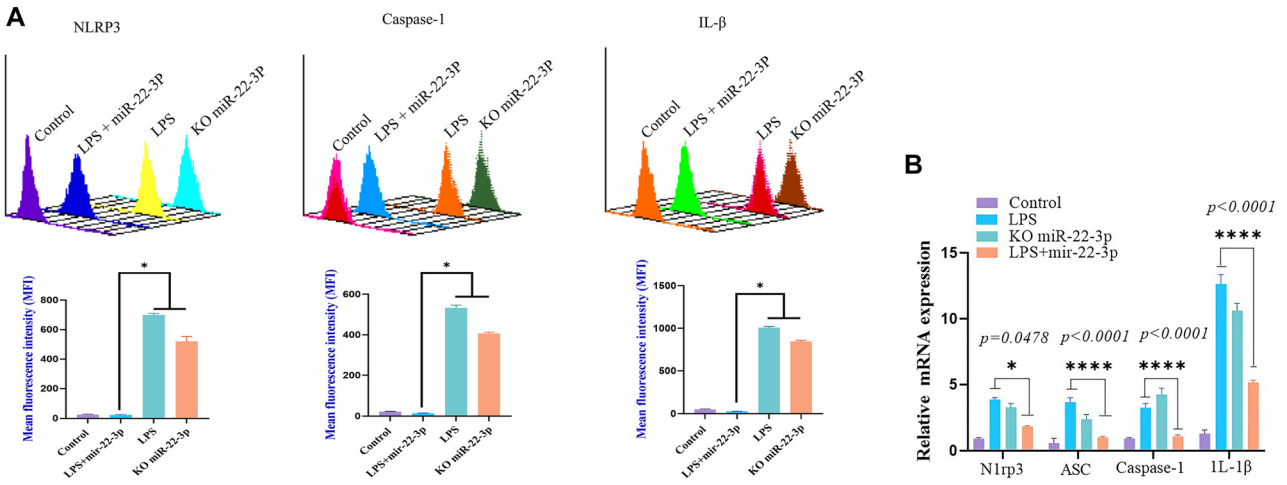

Figure 3: (A) The effect of up-regulation of miR-22-3p on the expression of NLRP3, IL-1β or Caspase-1. Total RNAswere extracted from C28/I2 cells or tissue using TRIzol Reagent kit (Invitrogen™) following the manufacture's protocol. The total RNAs were subsequently subjected to cDNA synthesis by usingsuperscript II reverse transcriptase. RT-PCR was performed in the Materials and Methods section. (B) Inhibition of miR-22-3p enhanced the expression of NLRP3, IL-1β and Caspase-1.

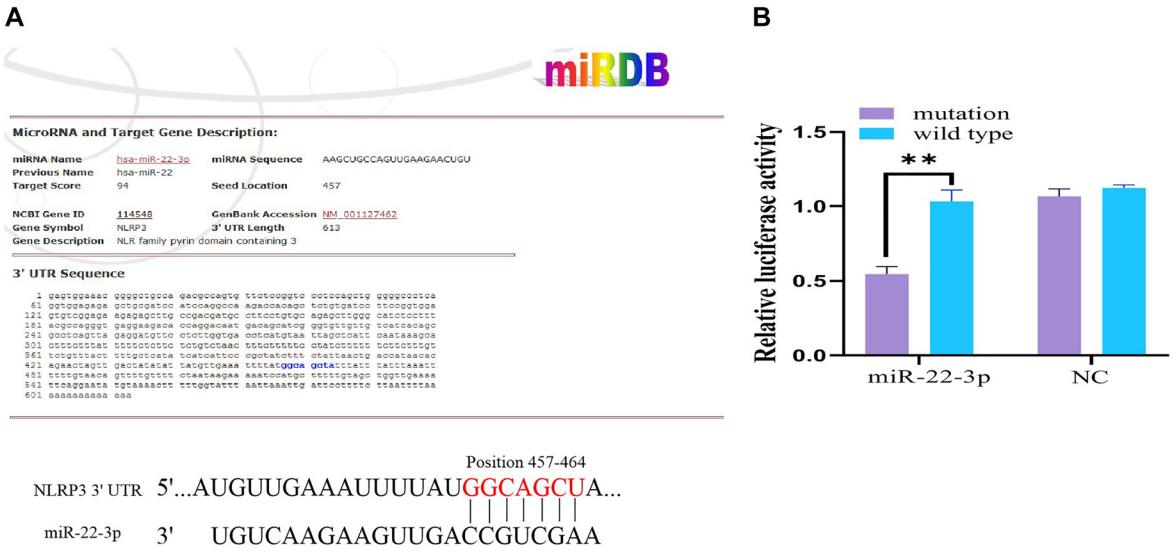

Figure 4: (A) The predicted binding sites of miR-22-3p on 3′-UTR of NLRP3 mRNA by MirDB and miRWalk (MirDB: http://www.mirdb.org/, miRWalk: http://mirwalk.umm.uni-heidelberg.de/). (B) miR-22-3p significantly inhibited luciferase activity compared with the mutant binding sites. The asterisk indicates thatthe difference is statistically significant (p-value <0.05).

that the miR-22-3p could target to NLRP3 (Figure 4A), the 3′-UTR of NLRP3 mRNA may has a direct target site of miR-22-3p. Therefore, we studied the effect of over-expression of miR-22-3p on NLRP3. It can be found that miR-22-3p over-expression remarkably blocked the expression of NLRP3, IL-1β and Caspase-1 (Figure 3A). In contrast, inhibition of miR-22-3p enhanced the expression of NLRP3, IL-1β and Caspase-1 (Figure 3B). These observations indicated that up-regulation of miR-22-3p could inhibit inflammatory responses via targeting NLRP3.

Identification of NLRP3 mRNA as the target gene of miR-22-3p

Here, we analyzed the relationship between NLRP3 and miR-22-3p. Bioinformatics software analysis (MicroRNA Target Prediction Database, MirDB and miRWalk) has showed that the miR-22-3p could bind to 457–464 of the 3′-UTR of NLRP3 mRNA (Figure 4A). Furthermore, a dual-fluorescent reporter assay system was conducted to confirm the result of bioinformatics analysis. As indicated

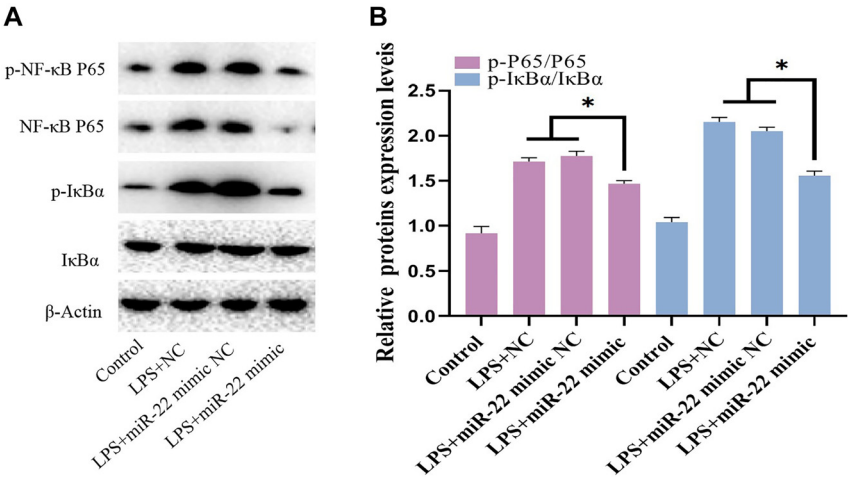

Figure 5: Effects of miR-22-3p on the NF-κB signaling pathway.
Total protein was extracted using RIPA lysis buffer. Protein concentrations were determined using the bicinchoninic acid (BCA) kit. Equal amounts of protein (30 μg/Lane) from each sample were subjected to SDS-PAGE and transferred onto apolyvinylidene fluoride (PVDF) membrane. The membranes were then treated with 5% skim milk powder at 4 °C overnight. After washing, the PVDF membranes were treated with the primary antibodies (NF-κB, 1:500 dilution; NLRP3, 1:1,000) at 4 °C overnight, followed by incubation with HRP-conjugated secondary antibodies (1:2,000 dilutions) for 1 h at RT. The immunoreactive bands were visualized by ECL detection system.

in Figure 4B, miR-22-3p over-expression remarkably reduced the luciferase levels of the wild-type (WT) 3′-UTR fragment of NLRP3 (but not mutated 3′-UTR fragment of NLRP3), these results suggested that NLRP3 may be a direct target gene of miR-22-3p (Figure 5).

miR-22-3p over-expression inhibited the activation of NF-κB -associated signaling pathway

Studies has indicated that the activation of NLRP3 is closely related to NF-κB signaling pathway. Therefore, we analyzed the activity of the NF-κB signaling pathway. It can be found that p-p65 and p-IκBα expression was evidently enhanced in inflammatory groups compared to control group. However, miR-22-3p transfection decreased the expression levels of p-p65 and p-IκBα. These results indicate that miR-22-3p suppressing inflammatory injury may be though NF-κB signaling pathway.

miR-22 over-expression relieved osteoarthritis *in vivo*

A lentiviral vector for the over-expression of miR-22-3p was constructed and injected into the joint cavity of osteoarthritis model mice. After treatment for four weeks, we analyzed the effect of over-expression of miR-22-3p on osteoarthritis (OA). Safranin-fast green stain was performed to observe the morphological changes of articular cartilage. As shown in Figure 6A, compared with the sham operation group (Sham), the surface of the knee joint cartilage in the operation group was significantly destroyed, and the cartilage matrix content was reduced; however, the cartilage damage was significantly reduced in AAV-miR22-3p treatment group, while the adeno-associated virus control group (AAV-miR22-NC) had no significant difference compared to operation group.

Additionally, HE staining of knee joint sections showed that in sham operation group, the surface cells of cartilage tissue were well-arranged, the surface of cartilage tissue was smooth, and the matrix staining was uniform. In the OA group, the cells on the surface of cartilage arranged disorderly, and the matrix staining was uneven. Compared with the operation group, the surface cells of cartilage in the operation group were well-arranged by AAV-miR-22-3p treatment (Figure 6B), there was no significant difference in Markin score between AAV-miR22-NC treatment group and operation group (OA group) (Figure 6C).

Discussion

OA is a degenerative joint disease, the main characteristics are chondrocyte apoptosis and cartilage degradation [1–3]. OA is more common in middle-aged and elderly people, and the proportion of female patients is higher than that of males. The Pathogenic factors of OA are complex (such aging and traumais), within which aging is an important

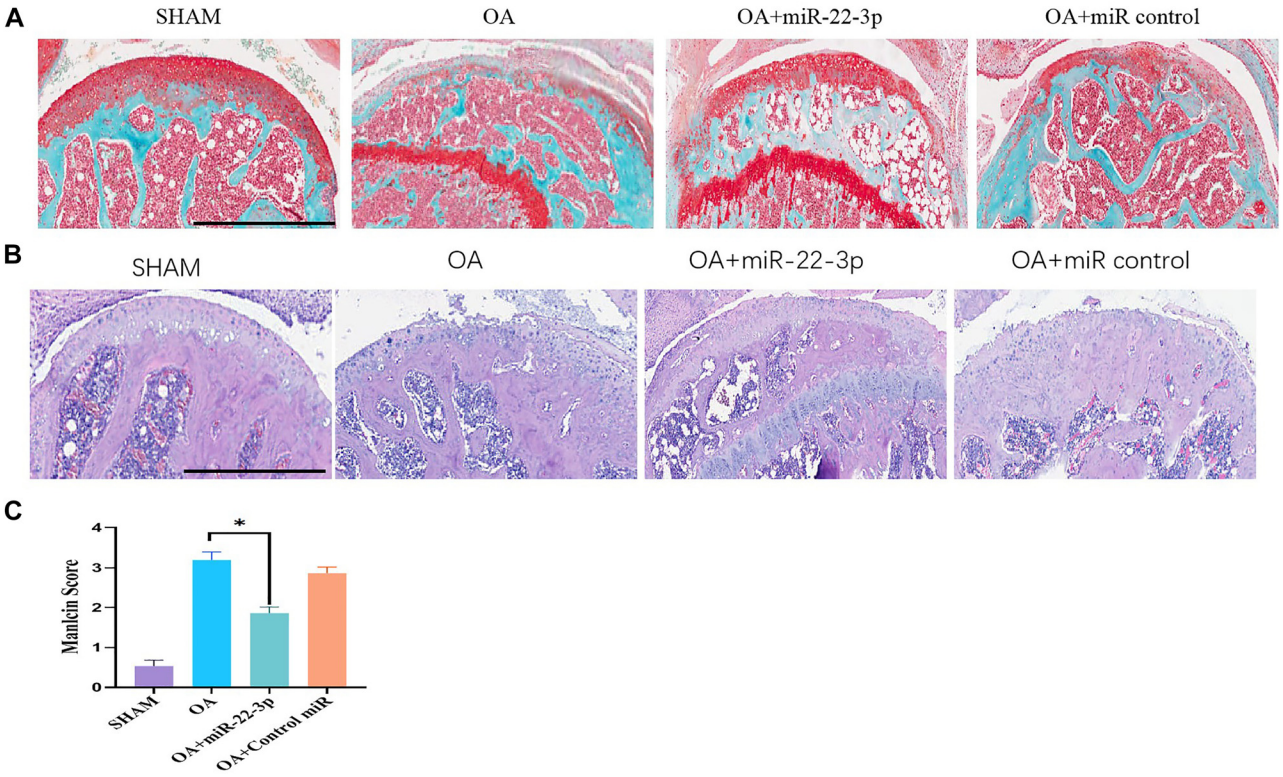

Figure 6: (A) AAV-miR22-3p treatment relieved osteoarthritis *in vivo* evaluated by Safranin O-fast green staining. The tissue sections were routinely deparaffinized, and then the sections were soaked in xylene solution for 10 min. After treatment with the ethanol, the sections were stained with fast-green. After washing, the sample was stained with safranin O solution, the sections were then observed using a light microscope. (B) The effect of AAV-miR22-3p treatment on OA determined by HE staining. (C) Markin score between AAV-miR-22-3p treatment group and operation group.

cause of OA [4]. At present, the incidence of OA is rising sharply, affecting human health, and there is no clinically effective drug to treat OA. Exploring the pathological mechanism of OA is of required to overcome and treat the disease. miRNAs exhibits a variety of important regulatory effects in cell and tissue [12]. Studies have shown that Micro RNA can participate in different cellular processes (e.g. cell apoptosis, proliferation) by regulating multiple target genes, thereby affecting the occurrence and progression of diseases [11]. At the same time, studies have showed that the expression changes of miRNA are closely linked with diseases, such as obesity and OA. In this work, we studied the effect of miR-22-3p on osteoarthritis (OA), the results showed that miR-22-3P exhibits the potential therapeutic effect on OA. Further experiments and bioinformatics analysis demonstrate that inflammasome may be the target of miR-22-3p. In summary, in the current study, we found that Micro-22-3P can be used to alleviate the osteoarthritis (OA).

Recently, intensive studies on Mir-22-3p have been performed, and the corresponding results indicate that Mir-22-3p is involved in a series of physiological and pathological processes. It has been shown that Mir-22-3p is involved in the occurrence and development of different types of tumors (such as lung cancer) [13]. Furthermore, Mir-22-3p also displays the important biological regulatory functions, for example, Mir-22-3p was participated in regulating neuropathic pain and protecting the endothelium against oxidative stress. More interesting is that researchers have found that Mir-22-3p is involved in the process of inflammation regulation, Mir-22-3p exhibited the protective effects against gouty inflammation and retinal pigment epithelial inflammatory damage [13, 14]. However, as of now, the effect of Mirco-22-3p on osteoarthritis has not been reported in the relevant literature. For this, we firstly studied the effect of Mir-22-3p on OA in the current work. We firstly established an inflammatory chondrocyte model *in vitro*, and osteoarthritis (OA) model using LPS (*in vivo*). In this study, we found that miR-22-3P was significantly down-regulated in LPS-induced C28/I2 cell model, suggesting that the miR-22-3P might be related to the occurrence of osteoarthritis (OA). Further work showed that miR-22-3p over-expression remarkably

blocked the expression of NLRP3, IL-1β and Caspase-1. On the contrary, inhibition of miR-22-3p could enhance the expression of NLRP3, IL-1β and Caspase-1. These observation indicated that miR-22-3p up-regulation can inhibit inflammatory responses by targeting NLRP3. In addition to miR-22-3p, previous study showed that miRNA-34a is closed linked with OA, and further studies were performed to explore the mechanism by which miRNA-34a could regulate cell apoptosis in OA, and the results showed that miRNA-34a expression in the articular cartilage of OA patients was up-regulated [16]. Silencing miRNA-34a exhibits an antagonistic effect on OA. Additionally, Hu et al. has studied the effect of miR-22-3p on the NLRP3 inflammasome in light-stimulated retinopathy, who found that miR-22-3p could inhibit the expression of NLRP3-related inflammatory cytokines by targeting to inflammasome, which may be a potential mechanism by which miR-22-3p could protect RPE cells [16].

We further evaluated the effect of miR-22-3p on OA *in vivo*. For this, a lentiviral vector for the over-expression of miR-22-3p was constructed and injected into the joint cavity of OA model mice. Safranin-fast green stain was performed to observe the morphological changes of articular cartilage, and results showed that the cartilage damage was significantly reduced in AAV-miR22-3p treatment group. Furthermore, HE staining of knee joint sections showed that the surface cells of cartilage in the operation group were well-arranged by AAV-miR-22-3p treatment. These results indicated that miR-22-3p could alleviate OA *in vivo*. Further work showed that miR-22-3p exhibits the potential for the treatment of OA by targeting inflammasomes. In addition, we also found that miR-22-3p is involved in the regulation of inflammatory response through NF-κB pathway. It can be found that miR-22-3p could greatly inhibit the activation of p-p65 and p-IκBα. These results indicated that the anti-inflammatory effect of miR-22-3p is closely related to the NF-κB signaling pathway. Of course, an interesting problem is that the action mechanism by which miR-RNAs works is very complicated, because Mir-RNA has many potential targets. The potential binding site of Mir-RNA is located at 3'UTR of the mRNA [20–25]. Therefore, the specific action mechanism of miR-22-3p by which miR-22-3p exhibits potential anti-OA effects still needs to be further determined in future studies.

In short, in the current study, we found that miR-22-3p exhibited an antagonistic effect by targeting to NLRP3 inflammasome. This work suggests that miR-22-3p can be used to inhibit osteoarthritis (OA), which provides a novel possible target for OA treatment.

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Author contributions: Wang Ling xia and Fan Zhi fen the experiments; Wang Ling xia drafted the manuscript. Wang Ling xia edited the manuscript. Wang Ling xia and Fan Zhi fen conceived of the study, and participated in its design and coordination. All authors have accepted responsibility for the entire content of this manuscript and approved its submission.

Competing interests: The authors declare that they have no conflicts of interest.

Informed consent: Informed consent was obtained from all individuals included in this study.

Ethical approval: The local Institutional Review Board deemed the study exempt from review.

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