European Review for Medical and Pharmacological Sciences 2020; 24: 8263-8272

MiR-145 targeting BNIP3 reduces apoptosis of chondrocytes in osteoarthritis through Notch signaling pathway

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Abstract. – OBJECTIVE: The purpose of this study was to explore the effect of micro ribonucleic acid (miR)-145 on the apoptosis of chondrocytes in osteoarthritis (OA), and to research the association between its targeting on B-cell lymphoma-2 (Bcl-2)/adenovirus E1B 19 kDa interacting protein 3 (BNIP3) and Notch signaling pathway and chondrocyte apoptosis.

MATERIALS AND METHODS: The mouse model of OA was established *via* surgery, and chondrocytes were isolated and cultured *in vitro*. Then, the chondrocytes were transfected with miR-145 inhibitor, miR-145 mimics, miR-negative control (NC), BNIP3-siRNA and BNIP3-vector, respectively, with those normally cultured as the control. After that, the expression levels of miR-145 and BNIP3 in cells were detected *via* quantitative Reverse Transcription-Polymerase Chain Reaction (qRT-PCR), the apoptosis rate was detected *via* flow cytometry, and the apoptosis level was detected using terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling (TUNEL) assay. Moreover, the target gene sequences were predicted and compared using the software, and the BNIP3 Luciferase reporter vectors containing predicted target sites for miR-145 were constructed. Finally, the protein expressions of BNIP3, Notch1, and P21 were determined through Western blotting.

RESULTS: The results of qRT-PCR showed that in OA chondrocytes, the expression of miR-145 was lower than that in normal chondrocytes ($p<0.05$), while the mRNA and protein expressions of BNIP3 were higher than those in normal chondrocytes ($p<0.05$). According to flow cytometry, the apoptosis rate was (4.4±0.6)% in normal cartilage tissues and (29.2±2.1)% in OA cartilage tissues. Overexpression of miR-145 significantly reduced chondrocyte apoptosis ($p<0.05$), while overexpression of BNIP3 markedly increased chondrocyte apoptosis ($p<0.05$). In addition, the Luciferase reporter system showed that miR-145 mimics evidently inhibited BNIP3 ($p<0.05$) and suppressed the Notch signaling pathway ($p<0.05$), while BNIP3 enhanced the expression of Notch signaling pathway ($p<0.05$).

CONCLUSIONS: MiR-145 can reduce OA-induced chondrocyte apoptosis through targeted inhibition on BNIP3 and regulation on Notch signaling pathway.

Key Words:
Osteoarthritis, MiR-145, BNIP3, Notch signaling pathway, Chondrocytes, Apoptosis.

Introduction

Osteoarthritis (OA) is a common joint disease around the world, and the patients account for about 10% of men aged above 60 years old and up to 18% of women[1]. The pathogenesis of OA is complex and involves multiple factors, such as genetics, biology, and biomechanics. In addition, the disease also has joint-specific causes, and joint pain and even loss of function can lead to weakness of human body[2]. In developed countries, OA has caused enormous economic burden on the society, and the expenses on the disease account for 1.0-2.5% of Gross Domestic Product (GDP) according to statistics[3]. The treatment methods for advanced OA include pain management and joint replacement, but they are not suitable for early OA[4,5]. Therefore, better understanding the pathogenesis of OA is of great significance for the prevention and treatment of early OA.

Micro ribonucleic acids (miRNAs) are a class of conserved small non-coding RNAs with 18-25 nucleotides in length, which can regulate the post-transcriptional gene expression. Once binding to the argonaute protein family member, miRNAs will act as a guide for the core silencing complex called the miRNA-induced silencing complex (miRISC), and bind to the complementa-

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ry sequences in the mRNA 3′-untranslated region (3′-UTR), thereby inhibiting translation or causing degradation of mRNAs to regulate the gene expression[6,7]. In the last two decades, miRNAs have been explored in a variety of physiological and pathological development, and several studies have indicated that miR-145 plays an important role in various physiological and pathological processes. For example, miR-145 can regulate OCT4, SOX2, and KLF4, and inhibit the pluripotency of human embryonic stem cells[8]. MiR-145 suppresses cell invasion and metastasis through directly targeting mucin-1[9]. MiR-145 targeted therapy can reduce atherosclerosis[10]. Moreover, miR-145 alleviates TNF-α-driven cartilage matrix degradation in OA *via* directly inhibiting MKK4[11].

Bcl-2/adenovirus E1B 19 kDa interacting protein 3 (BNIP3) and BNIP3-like (BNIP3L) (also known as NIX) are homologous proteins to Bcl-2 in the BH3 domain, which can induce cell death and autophagy. Although BNIP3 or NIX may not induce cell death or autophagy more efficiently than other proteins, their roles make them powerful factors for exploring cell fate[12]. The combined effects of miR-145 and BNIP3 in the pathogenesis of OA are rarely explored. In this study, therefore, the expression of miR-145 in OA tissues and chondrocytes, and the effect of its targeting on BNIP3 on chondrocyte apoptosis in OA were explored, so as to better understand the roles of miR-145, BNIP3, and Notch signaling pathway in the pathogenesis of OA.

Materials and Methods

Main Materials

SYBR Green and reverse transcription (RT) Master Mix Kit (TaKaRa, Otsu, Shiga, Japan), Roswell Park Memorial Institute-1640 (RPMI-1640) medium, α-minimum Eagle's medium (MEM), fetal bovine serum (FBS), L-glutamine, nonessential amino acids, 2.5% trypsin + 0.02% EDTA (ethylenediaminetetraacetic acid) and phosphate-buffered solution (PBS; Gibco, Rockville, MD, USA), Lipofectamine 2000 and TRIzol (Invitrogen, Carlsbad, CA, USA), BNIP3, Notch1, P21 and β-actin antibodies (Abcam, Cambridge, MA, USA), miRNA RT kit (Applied Biosystems, Foster City, CA, USA), Luciferase reporter assay system (Promega, Madison, WI, USA), bioluminescent plate reader (Modulus™), and 0.22 μm pinhole filter (Millipore, Billerica, MA, USA).

Experimental Methods

Establishment of Mouse Model of OA

The mouse model of OA was established according to the method of Kamekura et al[13]. After the mice were anesthetized *via* intraperitoneal injection of 10% chloral hydrate (0.33 mL/100 g of body mass), the right knee joint was disinfected for skin preparation, and the medial skin of tibia was carefully cut open centering on the knee joint. After that, the medial collateral ligament was cut off, the knee joint cavity was opened horizontally, the medial meniscus was dissociated and cut off in the middle, the anterior cruciate ligament deep in the joint cavity was cut off, and then the joint cavity was carefully closed with wound suture. In sham operation group, the medial skin of the right knee joint was cut and the joint was exposed, followed by wound suture. After surgery, the mice were fed with regular diets.

Isolation and Culture of Chondrocytes

The cartilage tissues of mice were isolated under aseptic conditions, washed with PBS for 3 times, cut into 1 mm^3 pieces with scissors, and digested with appropriate amount of 0.25% trypsin + 0.02% EDTA for 30 min and 0.1% collagenase II at 37°C for 20 h in an incubator. Next, the supernatant was taken and centrifuged for 10 min, and the precipitate was collected. Then, the cells were resuspended in DMEM + 10% FBS, inoculated into the culture dish, and cultured in the incubator with 5% CO_2 at 37°C, followed by passage when about 90% of cells were fused. After the cells were washed once with PBS, they were digested with appropriate amount of 0.25% trypsin + 0.02% EDTA at 37°C for 2-3 min in the incubator. Finally, the digestion was terminated using the medium, and the cells were carefully blown over, followed by subculture at 1:3.

Cell Transfection

The well-grown cells were inoculated into a 24-well plate (2×10^4 cells/well), and transfected with the miR-145 inhibitor, miR-145 mimics and miR-negative control (NC), respectively, when they covered 90% of the plate bottom. Specifically, 5 μL of miR-145 inhibitor, miR-145 mimics and miR-NC with the mother liquor concentration of 20 μM was added and mixed evenly in 4 portions of 250 μL of α-MEM, followed by incubation at room temperature for 5 min. Then, the mixed transfection solution was added into the 24-well plate and shaken evenly, followed by incubation in the incubator with 5% CO_2 at 37°C. At

48 h after transfection, the abundance of miR-145 in each group was measured.

BNIP3 MRNA Interference

The well-grown cells were inoculated into a 6-well plate (2×10^5 cells/well), and 5 µL of 20 µM siRNA stock solution was added into 83 µL of serum-free OPTI-MEM on the next day. Then the above mixture was added and mixed evenly with 12 µL of HiPerFect transfection reagent, and incubated at room temperature for 10 min. Thereafter, the transfection complex was added into the 6-well plate and mixed evenly, followed by incubation in the incubator with 5% CO_2 at 37°C. After 48 h, the gene expression was detected.

Total RNA Extraction and QRT-PCR

The cartilage tissues were ground in liquid nitrogen and mixed evenly with TRIzol for 5 min. After that, the tissues were added with 200 µL of chloroform and centrifuged at 4°C and 12000 rpm for 10 min. Then the supernatant was taken, added with an equal volume of isopropanol, and placed at room temperature for 10 min, followed by centrifugation at 4°C and 12000 rpm for 15 min. Thereafter, the cell precipitate was washed twice with freshly prepared 75% ethanol and dissolved with diethylpyrocarbonate (DEPC)-treated water, and the concentration was measured using the NanoDrop spectrophotometer. The primers were designed using Primer3Plus and synthesized by Sangon (Shanghai, China), and the primer sequences of miR-145, BNIP3 and glyceraldehyde 3-phosphate dehydrogenase (GAPDH) are shown in Table I. Subsequently, the first-strand complementary deoxyribose nucleic acid (cDNA) of miR-145 was synthesized using the qScript microRNA cDNA kit, while the total RNA was synthesized into cDNA of BNIP3 and GAPDH using random primers in the RT Master Mix Kit. Then qRT-PCR was performed using the SYBR-Green Real-Time PCR Master Mix and ABI 7500 sequence detection system according to the manufacturer's program.

Table I. Primer sequences of miR-145, BNIP3, and GAPDH.

Gene	Sequences
miR-145 F	5'-GTCCAGTTTTCCCAGGAATCCCT-3'
miR-145 R	5'-CAGTGCGTGTCGTGGAGT-3'
BNIP3 F	5'-AAACAGCACTCTGTCTGAGGA-3'
BNIP3 R	5'-GAGAGTAGCTGTGCGCTTCG-3'
GAPDH F	5'-AGTGCCAGCCTCGTCTCATA-3'
GAPDH R	5'-GGTAACCAGGCGTCCGATAC-3'

Finally, the level of transcription was evaluated using the cycle threshold (Ct) value, and the gene expression normalized to the endogenous reference was obtained using the $2^{-\Delta\Delta Ct}$ method.

Flow Cytometry

Flow cytometry was performed for chondrocytes in cartilage tissues isolated and digested *in vitro* or well-grown chondrocytes cultured *in vitro*. Next, the cells were digested with 0.25% trypsin into single cell suspension and washed with PBS for 3 times. After the cell density was adjusted to 3×10^5 cells/mL, the cells were incubated with Annexin V and propidium iodide (PI) at 4°C for 30 min in the dark, and washed with PBS for 3 times, followed by detection using C6 flow cytometer. At last, the data were analyzed using CFlow Plus software.

One-Step Terminal Deoxynucleotidyl Transferase-Mediated dUTP Nick End Labeling (TUNEL) Assay

The chondrocytes in cartilage tissues isolated or well-grown chondrocytes cultured *in vitro* were washed once with PBS, fixed with an appropriate amount of 4% paraformaldehyde for 15 min, washed again with PBS for 3 times, and transparentized with an appropriate amount of 0.1% Triton X-100 for 2 min. After washing with PBS for 3 times, the samples were added with 50 µL of TUNEL assay buffer, incubated at room temperature for 1 h, washed again with PBS for 3 times, added with an appropriate amount of anti-fluorescence quencher and sealed. Finally, the samples were observed and photographed under a fluorescence microscope.

Luciferase Assay

The chondrocytes cultured *in vitro* were transfected with miR-145 mimics and miR-NC, and wild-type BNIP3 plasmid (BNIP3-WT) and mutant BNIP3 plasmid (BNIP3-MUT) for 48 h, respectively. Then, the transfected cells were collected, and the Luciferase activity was detected using the Luciferase reporter assay system and bioluminescent plate reader. Each experiment was repeated independently for 3 times, with 3 parallel controls.

Western Blotting

The chondrocytes in cartilage tissues isolated or well-grown chondrocytes cultured *in vitro* were lysed with appropriate amount of cell lysis buffer at 4°C overnight, and the adherent cells were collected using a cell scraper and centrifuged at

13,000 rpm to extract the total protein. Later, the protein concentration was determined using the bicinchoninic acid (BCA) method (Pierce, Rockford, IL, USA). After separation *via* 8% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), the protein was transferred onto a polyvinylidene difluoride (PVDF) membrane, sealed with 5% skim milk powder and 0.1% Tween-20 in Tris-buffered saline, and incubated with BNIP3, Notch1, P21, and β-actin primary antibodies at 4°C overnight and then with horseradish peroxidase (HRP)-labeled secondary antibodies. Finally, the protein was exposed and detected using the enhanced chemiluminescence (ECL) reagent.

Statistical Analysis

Statistical Product and Service Solutions (SPSS) 20.0 software (IBM, Armonk, NY, USA) was used for data processing. Data in each group were expressed as mean ± standard deviation (χ±s), and independent-samples *t*-test was adopted for intergroup comparison. $p<0.05$ suggested a statistically significant difference.

Results

Expression Levels of MiR-145 and BNIP3 In Tissue Samples

The results of qRT-PCR revealed that normal cartilage tissues had a significantly higher expression level of miR-145 ($p<0.05$) and a significantly lower expression level of BNIP3 than OA cartilage tissues ($p<0.05$; Figure 1A, B). The results of Western blotting showed that the protein expression of BNIP3 in normal cartilage tissues was significantly lower than that in OA cartilage tissues ($p<0.05$; Figure 1C).

Apoptosis Level of Tissue Samples

The results of flow cytometry manifested that the apoptosis rate of OA cartilage tissues [(29.2±2.1)%] was significantly higher than that of normal cartilage tissues [(4.4±0.6)%] (Figure 2).

Gene Expression in Chondrocytes Cultured In Vitro

The expressions of miR-145 and BNIP3 mRNA in chondrocytes cultured *in vitro* were determined using qRT-PCR. It was found that in normal chondrocytes, the expression of miR-145 was evidently higher ($p<0.05$), while that of BNIP3 mRNA was evidently lower than those in OA chondrocytes ($p<0.05$; Figure 3).

Effect of MiR-145 on OA Chondrocytes

To further verify the effect of miR-145 on OA chondrocytes, OA chondrocytes cultured *in vitro* were transfected with the miR-145 inhibitor, miR-145 mimics, and miR-NC. The results of qRT-PCR showed that transfection with miR-145 inhibitor significantly reduced the expression of miR-145 in OA chondrocytes ($p<0.05$), while transfection with miR-145 mimics markedly raised the expression of miR-145 in OA chondrocytes ($p<0.05$), but miR-NC had no significant effect on such expression ($p>0.05$; Figure 4A). Besides, transfection with miR-145 inhibitor evidently raised the expression of BNIP3 mRNA in OA chondrocytes ($p<0.05$), while transfection with miR-145 mimics evidently lowered the expression of BNIP3 mRNA in OA chondrocytes ($p<0.05$), but miR-NC had no significant effect on such expression ($p>0.05$; Figure 4B). Furthermore, it was found *via* one-step TUNEL assay that OA chondrocyte apoptosis was promoted by

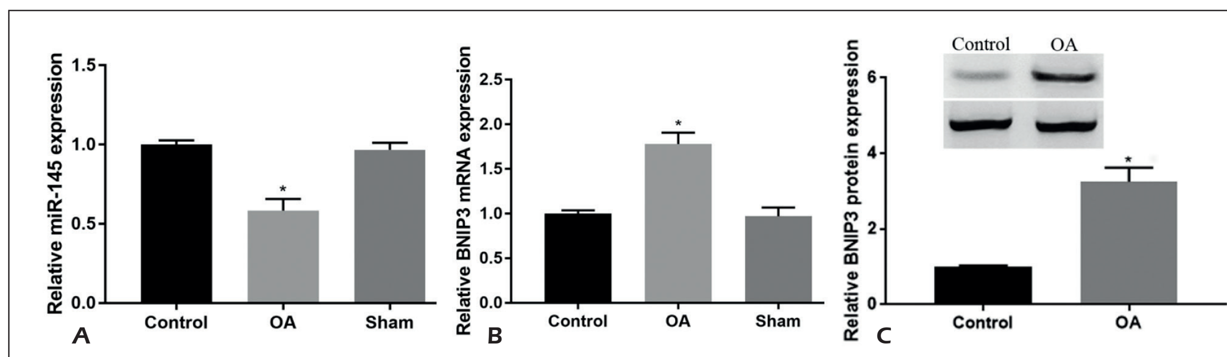

Figure 1. Expression levels of miR-145 and BNIP3 in normal cartilage tissues and OA cartilage tissues. **A**, Expression of miR-145 is detected *via* qRT-PCR. **B**, mRNA expression of BNIP3 is detected *via* qRT-PCR. **C**, Protein expression of BNIP3 is detected *via* Western blotting. Control: normal cartilage tissue samples, OA: OA cartilage tissue samples, Sham: cartilage tissue samples in sham operation group. *: Significant differences compared with other groups.

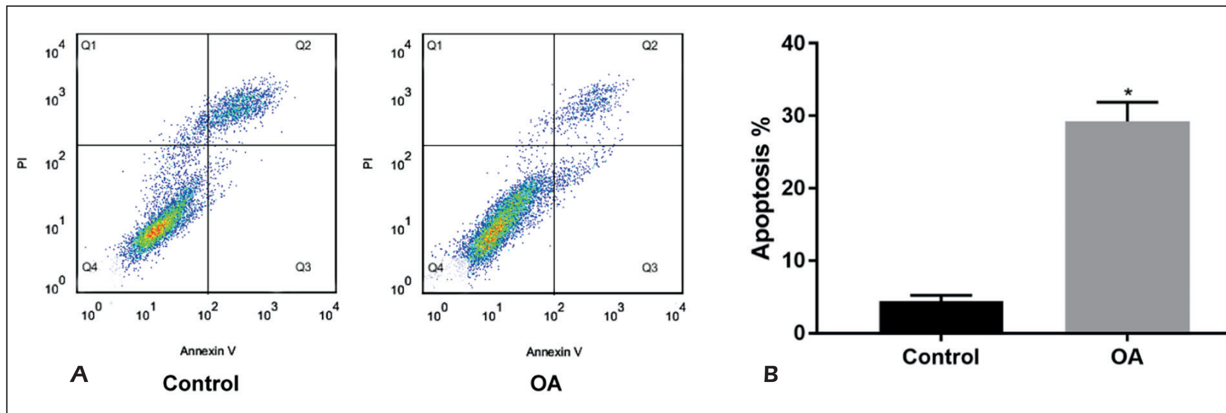

Figure 2. Apoptosis detected using flow cytometry. **A**, Flow cytometry scatter diagram. **B**, Apoptosis rate of OA cartilage tissues is obviously higher than that of normal cartilage tissues ($p<0.05$). Control: normal cartilage tissue samples, OA: OA cartilage tissue samples. *: There is a significant difference ($p<0.05$).

miR-145 inhibitor ($p<0.05$) but inhibited by miR-145 mimics ($p<0.05$; Figure 4C, 4D).

Targeting Relation Between MiR-145 and BNIP3

The BNIP3 luciferase reporter vectors containing the predicted target sites for miR-145 were constructed based on the software prediction results, and the target gene sequences are shown in Figure 5A. OA chondrocytes were transfected with BNIP3-WT and BNIP3-MUT, respectively. The results showed that miR-145 mimics could suppress the BNIP3-WT Luciferase activity ($p<0.05$), while it had no significant effect on the BNIP3-MUT Luciferase activity ($p>0.05$; Figure 5B).

Effect of BNIP3 on Chondrocyte Apoptosis

To further detect the effect of BNIP3 on apoptosis of OA chondrocytes, OA chondrocytes were transfected with BNIP3 siRNA and BNIP3-pEX-2 overexpression vector (BNIP3-vector), respectively. It was observed that mRNA and protein expression levels of BNIP3 were remarkably inhibited by BNIP3 siRNA ($p<0.05$), while they were remarkably promoted by BNIP3-vector ($p<0.05$; Figure 6A, 6B). Furthermore, according to TUNEL assay, BNIP3 siRNA remarkably inhibited apoptosis ($p<0.05$), while BNIP3-vector remarkably enhanced apoptosis ($p<0.05$; Figure 6C, 6D).

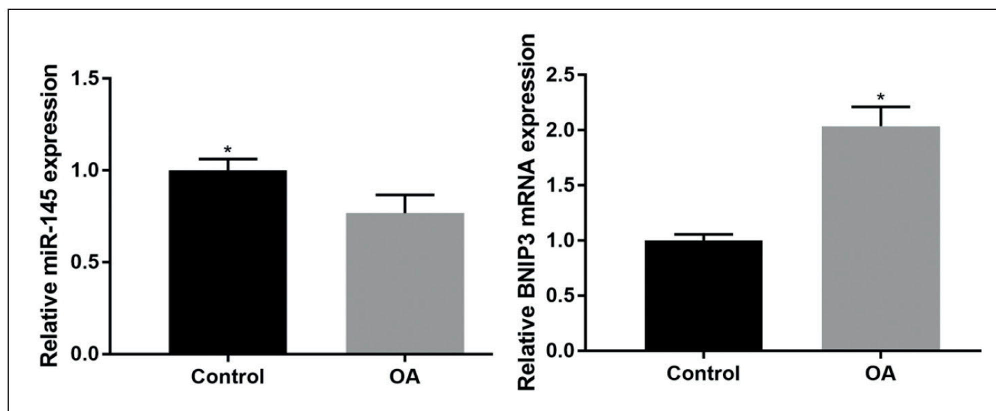

Figure 3. According to qRT-PCR, the expression of miR-145 in normal chondrocytes cultured *in vitro* is evidently higher ($p<0.05$), while that of BNIP3 mRNA is evidently lower than those in OA chondrocytes (p<0.05). Control: chondrocytes in cartilage tissues isolated and digested *in vitro*, OA: chondrocytes in OA cartilage tissues isolated and cultured *in vitro*. *: There is a significant difference ($p<0.05$).

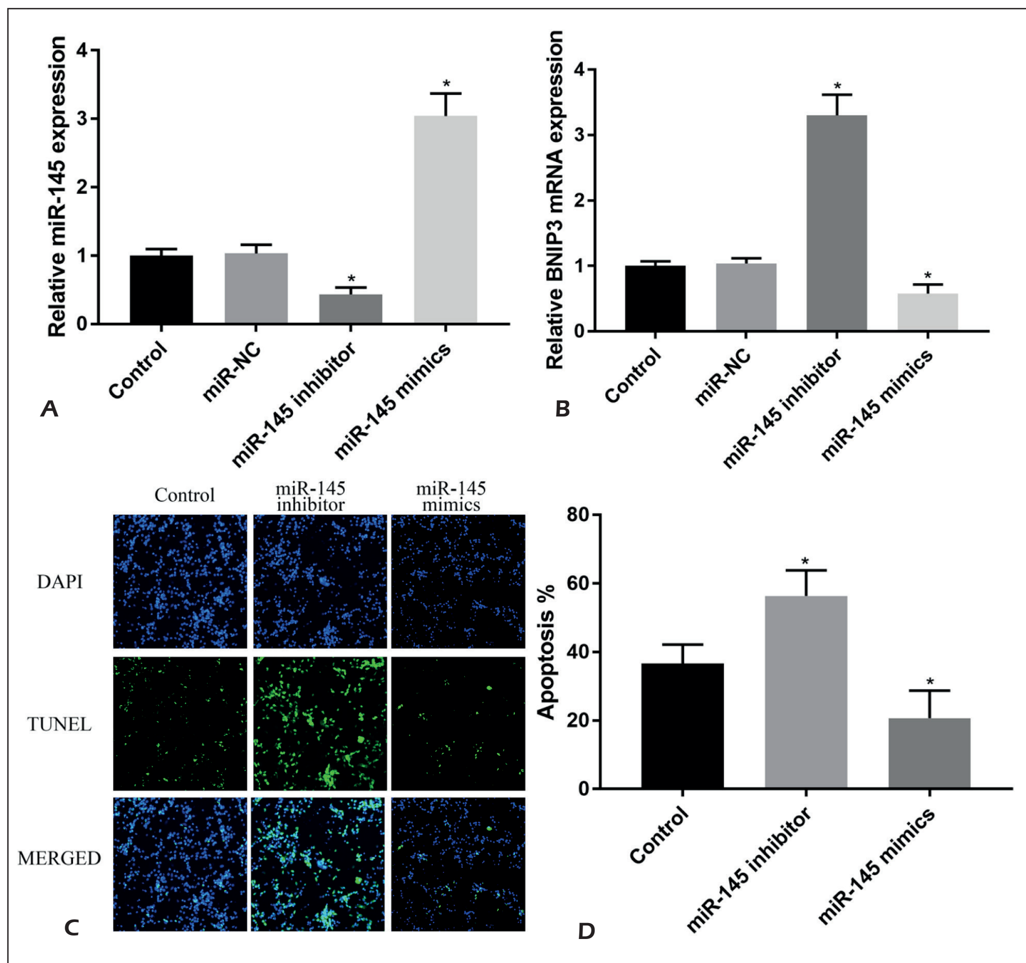

Figure 4. Effects of miR-145 on gene expression and apoptosis of OA chondrocytes. **A**, Abundance of miR-145 is detected after OA chondrocytes are transfected with miR-145 inhibitor, miR-145 mimics and miR-NC, respectively. **B**, Expression of BNIP3 mRNA is detected after OA chondrocytes are transfected with miR-145 inhibitor, miR-145 mimics and miR-NC, respectively. **C**, Apoptosis fluorescence diagram in one-step TUNEL assay (magnification: 400×) **D**, Percentage of cell apoptosis. Control: OA chondrocytes isolated and cultured *in vitro*. MiR-NC: OA chondrocytes isolated and cultured *in vitro* and transfected with miR-NC. MiR-145 inhibitor: OA chondrocytes isolated and cultured *in vitro* and transfected with miR-145 inhibitor. MiR-145 mimics: OA chondrocytes isolated and cultured *in vitro* and transfected with miR-145 mimics. *: significant differences compared with other groups ($p<0.05$).

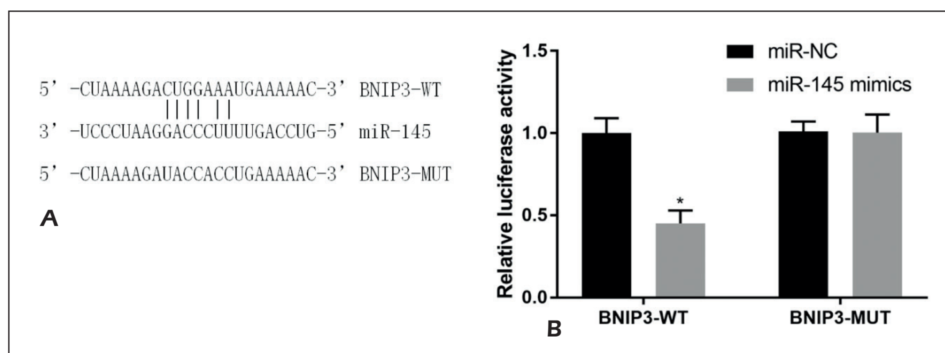

Figure 5. Relation between BNIP3-WT and BNIP3-MUT under the action of miR-145 mimics and miR-NC verified using luciferase reporter system. **A**, Comparison of target gene sequences of miR-145 and BNIP3. **B**, Relative Luciferase activity. *: significant differences compared with other groups ($p<0.05$).

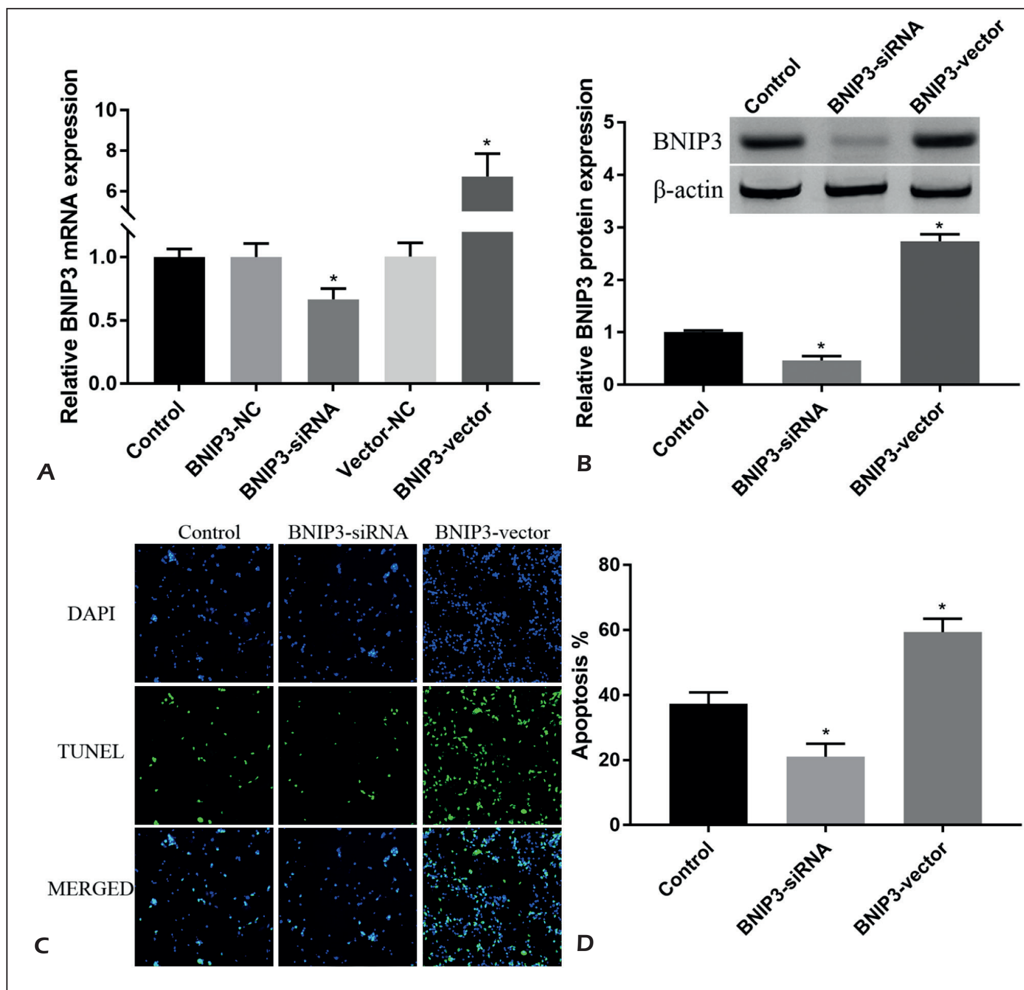

Figure 6. Effect of BNIP3 on apoptosis of OA chondrocytes. **A**, Abundance of BNIP3 mRNA is detected *via* qRT-PCR. **B**, Protein expression of BNIP3 is detected *via* Western blotting. **C**, Apoptosis fluorescence diagram in one-step TUNEL assay (magnification: 400×) **D**, Apoptosis rate. Control: OA chondrocytes isolated and cultured *in vitro*. BNIP3-NC: OA chondrocytes isolated and cultured *in vitro* and transfected with BNIP3-NC. BNIP3-siRNA: OA chondrocytes isolated and cultured *in vitro* and transfected with BNIP3-siRNA. Vector-NC: OA chondrocytes isolated and cultured *in vitro* and transfected with empty vector. BNIP3-vector: OA chondrocytes isolated and cultured *in vitro* and transfected with BNIP3-vector. *: significant differences compared with other groups ($p<0.05$).

Associations of MiR-145 and BNIP3 With Notch Signaling Pathway in Chondrocytes

The associations of miR-145 and BNIP3 with Notch signaling pathway in OA chondrocytes were further verified. The OA chondrocytes were transfected with the miR-145 mimics, BNIP3 siRNA and BNIP3-vector, respectively. The results of Western blotting revealed that BNIP3-vector significantly increased the protein expressions of Notch1 and P21 ($p<0.05$), while the protein expressions of Notch1 and P21 declined significantly in other groups ($p<0.05$; Figure 7).

Discussion

OA is a disease with a high morbidity rate in the world, and miRNAs can regulate many cellular biochemical processes, including cell differentiation, cell cycle progression, and apoptosis. The role of miRNAs during progression of OA has been explored in Nugent[14]. Several miRNAs involved in the pathogenesis of OA have been reported. So, cartilage-specific miR-140 regulates the expression of ADAMTS-5 in chondrocytes[15], miR-140$^{-/-}$ mice display the OA-like phenotype[16], miR-27a affects the expressions of MMP-13 and

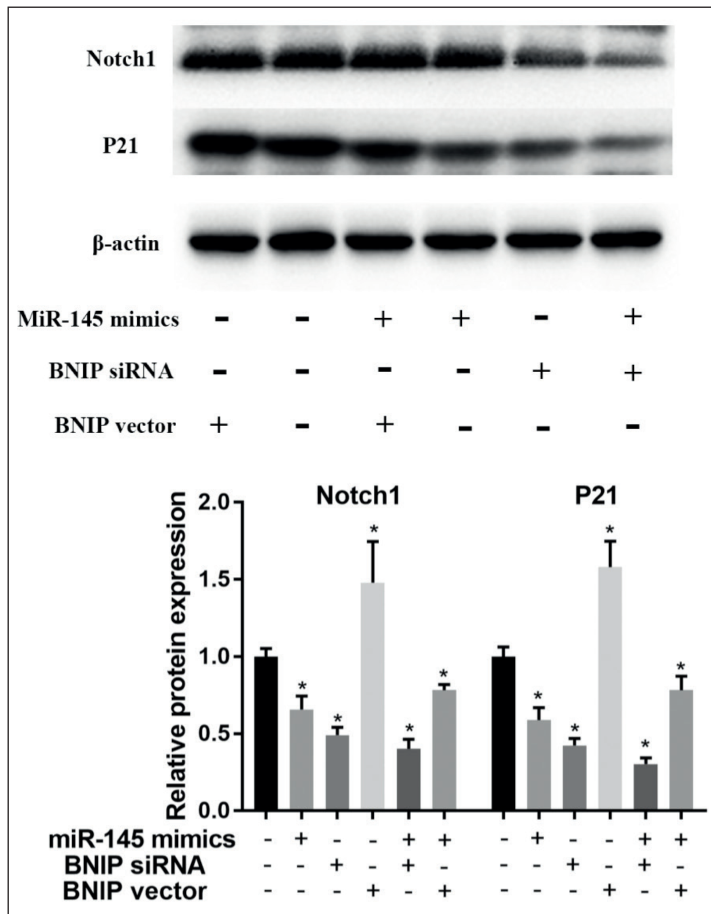

Figure 7. Associations of miR-145 and BNIP3 with Notch signaling pathway in chondrocytes determined using Western blotting. BNIP3-vector can significantly increase the protein expressions of Notch1 and P21 ($p<0.05$), while the protein expressions of Notch1 and P21 decline significantly in other groups ($p<0.05$). MiR-145 mimics: OA chondrocytes isolated and cultured *in vitro* and transfected with miR-145 mimics. BNIP3-siRNA: OA chondrocytes isolated and cultured *in vitro* and transfected with BNIP3-siRNA. BNIP3-vector: OA chondrocytes isolated and cultured *in vitro* and transfected with BNIP3-vector. *: significant differences compared with other groups ($p<0.05$).

IGFBP-5[17], miR-93 regulates the loss of collagen *via* targeting MMP-3[18], and miR-125b regulates the expression of ADAMTS-4 in human chondrocytes[19]. Jones et al[20] compared the expressions of 157 miRNAs in cartilage samples between patients receiving knee replacement and those with joint pain, and analyzed the expression profile of miRNA. They found that compared with normal tissues, lesion tissues have 30 miRNAs with more than 4 folds of differential expression. Also, Hu et al[11] found that miR-145 is a TNF-α-reactive miRNA in chondrocytes, which is characterized by the rapid decline in its expression after *in vitro* stimulation of TNF-α and has a negative correlation with the secretion of TNF-α during experimental OA *in vivo*. In this investigation, similarly to the above findings, it was confirmed in the analysis of miR-145 in OA cartilage tissues and chondrocytes that the miR-145 expression was down-regulated in OA cartilage tissues and chondrocytes. BNIP was originally identified as the Bcl-2/adenovirus E1B 19kDa interacting protein in yeast two-hybrid screening[21], which is a pro-apoptotic protein inhibited by E1B 19kDa protein or Bcl-2[12]. BNIP3 possesses the activity of inducing death in cell lines, and its overexpression induces death of 293T cells[22,23]. In this work, the gene and protein expressions of BNIP3 were determined in OA cartilage tissues and chondrocytes, and the up-regulated expressions of BNIP3 were observed. Then, the apoptosis level of chondrocytes in OA cartilage tissues was further detected, and the enhanced apoptosis was found, suggesting that there is a positive correlation between BNIP3 and OA chondrocyte apoptosis. The reason may be that the apoptosis is induced by up-regulation of BNIP3 due to OA. The prediction results using miRWalk database showed that miR-145 had interaction with BNIP3 and combined with the finding that the expression of miR-145 is negatively correlated with BNIP3, it is speculated that miR-145 may target BNIP3 easily in mice. The gene sequences of miR-145 and BNIP3 were analyzed and compared using the software, and the corresponding Luciferase reporter genes were constructed. The

results of Luciferase reporter assay manifested that the BNIP3 Luciferase activity of chondrocytes transfected with miR-145 plasmid declined by more than 50%. In the case of miR-145 overexpression, the protein level of BNIP3 declined, and the apoptosis was reduced, confirming that miR-145 down-regulates the BNIP3 expression to reduce apoptosis.

In mammals, the Notch signaling pathway is composed of several molecules, including Notch ligand, Notch receptors (Notch 1-4), and recombination signal binding protein for immunoglobulin kappa J region (RBPJ)[24]. When the Notch ligand on the cell surface binds to the Notch receptor on adjacent cells, the Notch signal will be initiated, the Notch receptor will be cleaved, and then the Notch-intracellular domain (ICD) will translocate to the nucleus and bind to RBPJ, forming the transcriptional activator that induces the Hes/Hey family. It has been reported that the Notch signaling pathway is activated in articular chondrocytes to be involved in the development of OA[25,26]. Notch 1 and 2 receptors located on the cell surface of normal mouse and human articular cartilage are highly expressed in articular chondrocytes, but they translocate to the nucleus in denatured cartilage. After bone development, knockout of RBPJ in chondrocytes can inhibit the Notch signal transduction, thereby suppressing the development of OA in the mouse model. Injecting the γ-secretase inhibitor DAPT into the knee joint of the wild-type OA model mouse will produce a similar protective effect. In primary chondrocyte cell lines in mice, MMP13 is increased by overexpression of Notch-ICD but down-regulated in RBPJ-knockout chondrocytes[25]. Articular chondrocytes have loose contact with each other, so the ligand-independent activation of Notch signal may be associated with the onset of OA[27]. In this study, the results showed that the expression of Notch in chondrocytes of OA mice was up-regulated. Overexpression of miR-145 and inhibition on BNIP3 expression could decrease the expression of Notch, while overexpression of BNIP3 significantly promoted the expression of Notch. The importance and innovation of this study mainly lies in the fact that the targeted regulation of miR-145 and BNIP3 gene expression in osteoarthritis chondrocytes has been demonstrated and miR-145 and BNIP3 expressions have been verified to affect apoptosis. Besides, miR-145 participates in the regulation of apoptosis by regulating the Notch signaling pathway. Therefore, this research can provide potential theoretical basis and therapeutic targets for the treatment of chondrocyte apoptosis caused by osteoarthritis.

Conclusions

Briefly, miR-145 can reduce OA-induced chondrocyte apoptosis through targeted inhibition on BNIP3 and regulation on Notch signaling pathway.

Funding Acknowledgements

National Natural Science Foundation (81760875).

Conflict of Interests

The authors declare that they have no conflict of interests.

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