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MicroRNA-107 regulates autophagy and apoptosis of osteoarthritis chondrocytes by targeting TRAF3

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ABSTRACT

Osteoarthritis (OA) is a degenerative joint disease with characteristics of reduced cartilage cellularity, subchondral sclerosis and synoviti. Ultrasonic diagnosis plays pivotal role in diagnosing OA in the clinical, while biological markers are equal important to the diagnosis of OA. This study aimed to identify and characterize a biomarker, the expression of microRNA-107 (miR-107) in normal and OA chondrocytes, and to explore its effect on OA pathogenesis. Transfection with miR-107 mimic or inhibitor was used to investigate the effect of miR-107 on OA chondrocytes and to identify miR-107 target. Activation of AKT, mTOR and P65 was evaluated by Western blot analysis. Chondrocyte apoptosis was detected by using flow cytometer. Our results showed that the expression level of miR-107 in OA chondrocytes was obviously lower than control chondrocytes. Overexpression of miR-107 inhibited apoptosis and promoted autophagy in OA chondrocytes. Additionally, overexpression of miR-107 inhibited the activation of AKT/mTOR and NF-κB pathway by targeting TRAF3 genes. In vivo analysis revealed that miR-107 was also lowly expressed in rats with OA, and its abnormal expression significantly affected cell apoptosis. In conclusion, miR-107 regulated apoptosis and autophagy of OA chondrocytes by targeting TRAF3, and it might be used as a potential target for OA therapy.

1. Introduction

Osteoarthritis (OA) is a degenerative joint disease characterized by the reduced cartilage cellularity, subchondral sclerosis and synovitis [1], and is often accompanied by pain and disability and affects millions of patients all over the world [2]. It is estimated that OA will become the fourth most disabling disease by the year 2020 [1]. Ultrasonic diagnosis including high-frequency ultrasound and low intensity pulsed ultrasound play certain roles in OA diagnosis [3], however, biological markers also play significant roles in diagnosing OA in recent years [4]. Multiple factors are implicated in the pathogenesis of OA, during which chondrocytes death is postulated as central feature of OA [5]. Chondrocytes, which are mainly responsible for the anabolic-catabolic balance in cartilage, play a pivotal role in the regulation of articular cartilage [6]. Increasing evidences have indicated that in osteoarthritic cartilage, chondrocyte death is associated with apoptosis or apoptosis-like programmed cell death [7–10]. It has been reported that chondrocytes play a critical role in the pathology of OA through chondrocyte apoptosis and cartilage matrix degradation [8,11].

MicroRNAs (miRNAs) are a class of small non-coding RNAs that regulate gene expression by binding the 3′ untranslated region (UTR) of their target messenger RNAs (mRNAs). MiRNAs are critical in numerous diseases, such as neurological diseases, tumors, inflammatory diseases [12]. MiR-107 plays an important role in various tissues via different regulatory mechanisms, such as Alzheimer's disease, non-small cell lung cancer [13,14]. Chen and his colleagues found that miR-107 was down-regulated in glioma and p53 could regulate the expression of miR-107 [15]. Up-regulation of miR-107 suppressed cell proliferation, migration, and invasion of glioma [16]. It has been reported that different expression of 7 human miRNAs are identified in normal and OA cartilage [17]. Vonk, L. A. and his colleagues discovered that overexpression of hsa-miR-148a promoted cartilage production and inhibited cartilage degradation in OA chondrocytes [18]. These studies indicated that miRNA had implication in OA pathogenesis. The identification of the effect of miRNA on chondrocyte metabolism will be a potential therapy for OA.

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2. Materials and methods

2.1. Preparation of cartilage

Human cartilage samples were obtained from 42 OA patients (12 men and 30 women) aged 42–72 years who were diagnosed by high-frequency ultrasound as gonitis and underwent total knee replacement surgery. The OA patients were diagnosed according to China Medical University of Rheumatology criteria, and had not taken non-steroidal anti-inflammatory drugs or steroids for at least 2 weeks prior to surgery or had not any intra-articular injection for at least 1 month prior to surgery. 34 trauma patients (9 men and 25 women) aged 40–71 years with femoral neck fracture with no known history of OA or RA who underwent total hip replacement surgery according to the previous study were enrolled in this study, as the control (normal cartilage samples). The patients were stratified according to the Kellgren/Lawrence (K.L.) Image Criterion. The study was approved by the institutional ethics committee of China Medical University. All samples were processed after receiving all patient consent and methods were carried out in accordance with the approved guidelines and regulations.

2.2. Isolation and cultivation of chondrocytes

As previously described [19], human articular cartilage was minced to digestion with 0.2% collagenase II in Dulbecco Modified Eagle Medium (DMEM). Chondrocytes were maintained in DMEM containing 10% fetal bovine serum (FBS, Gibco, USA) for 24 h at 37 °C. The cells were filtered through a 0.075 mm cell strainer and washed before cultivation or miRNA isolation with sterile phosphate buffered saline (PBS). First passage chondrocytes were obtained after 2 weeks. All the experiments were done within 3 days of passage 1 culture. During the culture period, cells were incubated at 37 °C in a humidified atmosphere of 5% CO_2 and 95% air, and the medium was changed every 2 days.

2.3. Animal experiments

Totally, 48 female SD rats (200–250 g body weight), which obtained from Shanghai Animal Center, Chinese Academy of Sciences (China), was cultured in the animal research facility in accordance with the animal approval protocol. The total rats were randomly separated into 2 groups, control group (n = 8) and OA group (n = 40), with the following detailed treatment. 1) Control group: SD rats were operated by the sham operation; 2) OA group, rats were treated to establish OA model under general anesthesia using the modified Hulth method [20]. Briefly, a medial parapatellar was incised, followed by opening knee joints, transecting anterior cruciate ligaments, and excising medical meniscus. All surgery was performed in the left knees. The medial lateral stress experiment and the anterior drawer test were used to verify the success of surgery. Rats in control group were operated with sham surgery, with medial parapatellar incision but no changes on cartilage articularis. Five weeks after the surgery, rats in control group were sacrificed for cartilage sample tissues collection. As for OA group, the total 40 model rats were then divided into 5 groups (n = 8), including OA, OA + antagomir-107 mut, OA + antagomir-107, OA + agomir-107 mut and OA + agomir-107 groups, thereinto, the 32 OA rats in the 4 groups were specially treated with the following details. Briefly, after 3 weeks of model surgery, rats in each group were respectively intra-articular injected with antagomiR-107 mut/antagomiR-107/agomir-107 mut/agomir-107 at 60 mg/kg body weight continuously for 3 days. Two weeks after the final injection, rats were sacrificed for cartilage sample tissues collection. The sequence information for miRNAs was shown in Stable 1.

2.4. Transfection

Human chondrocytes were transfected with miR-107 mimic or miR-107 inhibitor (Acon, Hangzhou, China), using Lipofectamine 3000 according to the manufacturer's instructions (Invitrogen, CA, USA). Briefly, cells were cultured in a 96-well plate with a density of 2×10^4/well. Then lipofectamine 3000 was diluted in Opti-MEM medium (5 μL: 0.15 μL) with 2 tubes, and the transfected DNA was diluted in Opti-MEM medium (10 μL: 0.2 μL). The diluted DNA was mixed with 0.4 μL of P3000 ™ and then cultured for 5 min at room temperature. Subsequently, the diluted lipofectamine 3000 was mixed with the cultured transfected DNA with volume 1:1 to produce the DNA-lipid complex. Finally, 10 μL of the produced complex added into the cells and then cultured for 4 days at 37 °C for further analysis. MiR-107 mimic control or inhibitor control (Acon, Hangzhou, China) was used as controls. The overexpression vector of *TRAF3* gene was constructed by ligating the complete open reading frame of *TRAF3* to pcDNA3.1 vector, and was transfected to chondrocytes. Blank vector was transfected as a negative control. To downregulated TRAF3 level, its specific siRNA (si-TRAF3) or control (si-control) synthesized by Acon were transfected to cells. After transfection for 48–96 h, the cells were used for the following experiments.

2.5. Flow cytometery

Detection of apoptosis was conducted using the annexin V-FITC/PI apoptosis detection kit according to manufacturer's protocol. Briefly, cells plated to a density of 3×10^5 per well in six-well plate and then were transfected for 48 h. Floating cells as well as residual attached cells were collected, washed with PBS twice, stained for 15 min at room temperature with Annexin-V-FITC and PI, and examined using flow cytometer. Analysis was performed by the software supplied in the instrument. Annexin V binds to phosphatidyl serine that becomes exposed on the plasma membrane of cells undergoing apoptosis. This allows the discrimination of living cells (un-stained with either fluorochrome) from early apoptotic cells (stained only with annexin V) and late apoptotic cells (stained with both annexin V and PI).

2.6. TUNEL staining assay

The apoptotic cells of cartilage tissue were analyzed using TUNEL staining following to the manufacturer's protocol (Invitrogen). Briefly, tissue samples were equilibrated for 10 min at room temperature, then incubated with DNaseI (300 U/mL in 50 mM Tris-HCl, pH 7.5) for 10 min at room temperature to induce DNA strand breaks prior to labeling procedures. The mixture was added to the slides and incubated at 37 °C for 2 h in a humidified atmosphere in the dark, followed by washed with PBS 3 times for 5 min each. Finally, the apoptotic cells were quantified for estimating cells apoptosis.

2.7. Dual-luciferase reporter assay

TargetScan (http://www.targetscan.org) was used to predict the miR-107 targets. The miR-107 target region of *TRAF3* 3′UTR was inserted into the pmiR-RB-Report vector and named TRAF3-wt (RiboBio, Guangzhou, China). As a control, the plasmid containing the mutant sequence of the miR-107 target region of the *TRAF3* 3′UTR was also inserted into the pmiR-RB-Report vector and named TRAF3-mut. Chondrocytes were transiently co-transfected with the 100 ng TRAF3-wt or pmiR-TRAF3-mut and 30 nM miR-107 mimic or its negative control using Lipofectamine 3000 (Invitrogen, USA) in 24-well plates, respectively. Each experiment was repeated 3 times. Luciferase activity was determined with Dual-Luciferase® Assay System according to manufacturer's instructions (Promega, WI, USA) 48 h after transfection. Luminescence was measured using Modulus™ II (Turner Biosystems, USA).

2.8. Western blot analysis

After treatments, chondrocytes were harvested, washed with cold PBS and lysed in ice-cold protein lysis buffer (Roche) as previously described [1]. Lysates were centrifuged (15,000*g* for 15 min at 4 °C) and the supernatant was used for detection of protein. Equivalent amounts of lysate protein (20 μg) were resolved by 12% SDS–PAGE and transferred to a PVDF membrane (Bio-Rad, USA) and the blots were incubated with diluted anti-LC3 (1:1000), anti-Beclin-1 (1:1000), anti-Bax (1:1000), anti-Bcl-2 (1:1000) or anti-GAPDH (1:2000) primary antibodies in TBST overnight at 4 °C. Blots were then incubated with horse radish peroxidase-conjugated secondary antibody and the antibody reactive proteins were visualized and analyzed using the Syngene Pxi imager and the Syngene Image analysis software respectively.

2.9. Reverse transcription-quantitative polymerase chain reaction (RT-qPCR)

Total RNAs were isolated from cultured chondrocytes using Trizol reagent (Invitrogen Life Technologies). MiRNA was extracted from cultured chondrocytes using TaKaRa RNAiso reagent. RNA samples were reverse-transcribed into cDNA using the reverse transcription system (Toyobo Co., Ltd., Osaka, Japan). Real-time PCR was performed with gene-specific primers, the SYBR Premix Ex Taq II, and the SYBR PrimeScript miRNA RT-PCR Kit (TAKARA, JAPAN), using the ABI StepOnePlus Sequence Detection System v2.1 (Applied Biosystems, Singapore). The following primers were used: 5′-GGACAAGTACAAGG CTGAGAAGGCCCACCTGGTGCTGT-3′ (forward) and 5′-ACAGCACCAG GTGGGCCTTCTCAGCCTTGTACTTGTCC-3′ (reverse) for *TRAF3*, 5′-CAAGTTCAACGGCACGTCAA-3′ (forward) and 5′-TGGTGAAGACGC CAGAGACTC-3′ (reverse) for GAPDH. The expression of *TRAF3* mRNA was quantified with *GAPDH* mRNA expression as endogenous control. The expression of miR-107 was quantified with *RNU6B* control. Quantification of the relative levels of *TRAF3* and miR-107 were determined by the $2^{-\Delta\Delta Ct}$ method according to previous studies.

2.10. Statistical analysis

Statistical analyses were performed using SPSS 17.0 software. The values are presented as the Means ± SD and the statistically significant difference between the experimental groups and controls was determined using Student's *t*-test. Each experiment was repeated three times using three independent samples. A value of $P < 0.05$ was considered to be statistically significant.

3. Results

3.1. MiR-107 inhibits apoptosis and promotes autophagy in OA chondrocytes

Quantitative real-time PCR (qPCR) was used to detect the mRNA level of miR-107 in OA and control chondrocytes. The results showed that miR-107 in OA chondrocytes was obviously lower than control chondrocytes (Fig. 1A, $^{**}P < 0.01$). In order to explore the function of miR-107, control chondrocytes were transfected with inhibitor control or miR-107 inhibitor, and OA chondrocytes were transfected with mimic control or miR-107 mimic. Transfection effect was detected with qPCR (Fig. 1B, $^{***}P < 0.001$). The results of flow cytometry showed that miR-107 inhibitor dramatically increased cell apoptosis, while miR-107 mimic obviously inhibited apoptosis (Fig. 1C, $^{***}P < 0.001$). The protein level of Bax and Bcl-2 had similar tendency with apoptosis (Fig. 1D, $^{***}P < 0.001$). Autophagy was decreased in control cells transfected with miR-107 inhibitor, while it was increased in OA transfected with miR-107 mimic. Meanwhile the expression level of Beclin-1 and LC3-II/I was similar to the variation trend of autophagy (Fig. 1E).

3.2. MiR-107 targets TRAF3 in human OA chondrocytes

TargetScanHuman analyzed that TNF Receptor Associated Factor 3 (*TRAF3*) was the target gene of miR-107. Western blot and qPCR analyses showed higher mRNA and protein levels of *TRAF3* in OA chondrocytes compared with control chondrocytes (Fig. 2A and B, $^{**}P < 0.01$). The results of dual luciferase report system showed that 3′UTR of *TRAF3* had the action sites with miR-107 (Fig. 2C, $^{**}P < 0.01$). In addition, miR-107 inhibitor promoted the mRNA level of *TRAF3* in control chondrocytes, while miR-107 mimic suppressed the mRNA level of *TRAF3* in OA chondrocytes (Fig. 2D, $^{***}P < 0.001$). As shown in Fig. 2E, the protein level of *TRAF3* had similar trend with mRNA level. The results show that miR-107 might suppress the expression of *TRAF3* by means of reducing the stability of mRNA and inhibiting translation.

3.3. MiR-107 regulates apoptosis and autophagy via TRAF3

In order to determine the effect of *TRAF3* on apoptosis and autophagy, control chondrocytes were transfected with blank vector or pc-TRAF3 and OA chondrocytes were transfected with si-control or si-TRAF3. Western blot was used to detect transfection efficiency (Fig. 3A, $^{***}P < 0.001$), apoptosis and autophagy. The results showed that overexpression of *TRAF3* in control chondrocytes promoted apoptosis and the ratio of BAX/BCL2. However, down-regulation of *TRAF3* in OA chondrocytes inhibited apoptosis and the ratio of BAX/BCL2 (Fig. 3B and C, $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$). Additionally, overexpression of TRAF3 in control chondrocytes suppressed the expression of Beclin-1 and LC3-II/I, while down-regulation of *TRAF3* in OA chondrocytes promoted the expression of Beclin-1 and LC3-II/I (Fig. 3D). The results indicated that miR-107 might regulate apoptosis and autophagy via TRAF3.

3.4. The effect of miR-107 on AKT/mTOR and NF-κB pathway in human OA chondrocytes

AKT/mTOR pathway is well known to regulate autophagy. To investigate the effect of miR-107 on AKT/mTOR and NF-κB pathway, cells were transfected with miR-107 inhibitor, miR-107 mimic or their negative control in OA chondrocytes, respectively. As shown in Fig. 4, the level of p-AKT, p-p65, p-mTOR were increased in control chondrocytes transfected with miR-107 inhibitor. However, OA chondrocytes transfected with miR-107 mimic down-regulated the level of p-AKT, p-p65 and p-mTOR. These results suggested that miR-107 inhibited the activation of AKT/mTOR and NF-κB pathway. Meanwhile, increased level of p-AKT, p-p65 and p-mTOR were discovered in OA chondrocytes transfected with miR-107 mimic and pc-TRAF3, indicating that overexpression of TRAF3 promoted the activation of AKT/mTOR and NF-κB pathway.

3.5. In vivo animal analysis of miR-107 on chondrocytes apoptosis

To facilitate the possible effects of miR-107 in chondrocytes of OA in vivo, we construct the OA model in SD rats and transfected several kinds of vectors to assess the apoptotic cells in cartilage tissues. The results showed that mRNA level of miR-107 was lower in rats with OA compared with that in sham control ($P < 0.01$, Fig. 5A), but its level was significantly decreased or increased by the transfection of antagomiR-107 or agomiR-107 ($P < 0.01$, $P < 0.05$, respectively, Fig. 5A). These findings revealed that miR-107 was lowly expressed in animal OA model. Subsequently, the apoptotic cells from OA rats cartilage tissues were assessed using TUNEL assay, and the results showed that the percentage of cell apoptosis was very high in OA rats compared to that in the sham control ($P < 0.001$, Fig. 5B), and also, the apoptotic cells was dramatically increased or decreased by the antagomiR-107 or agomiR-107 transfection ($P < 0.001$, $P < 0.01$, respectively, Fig. 5B).

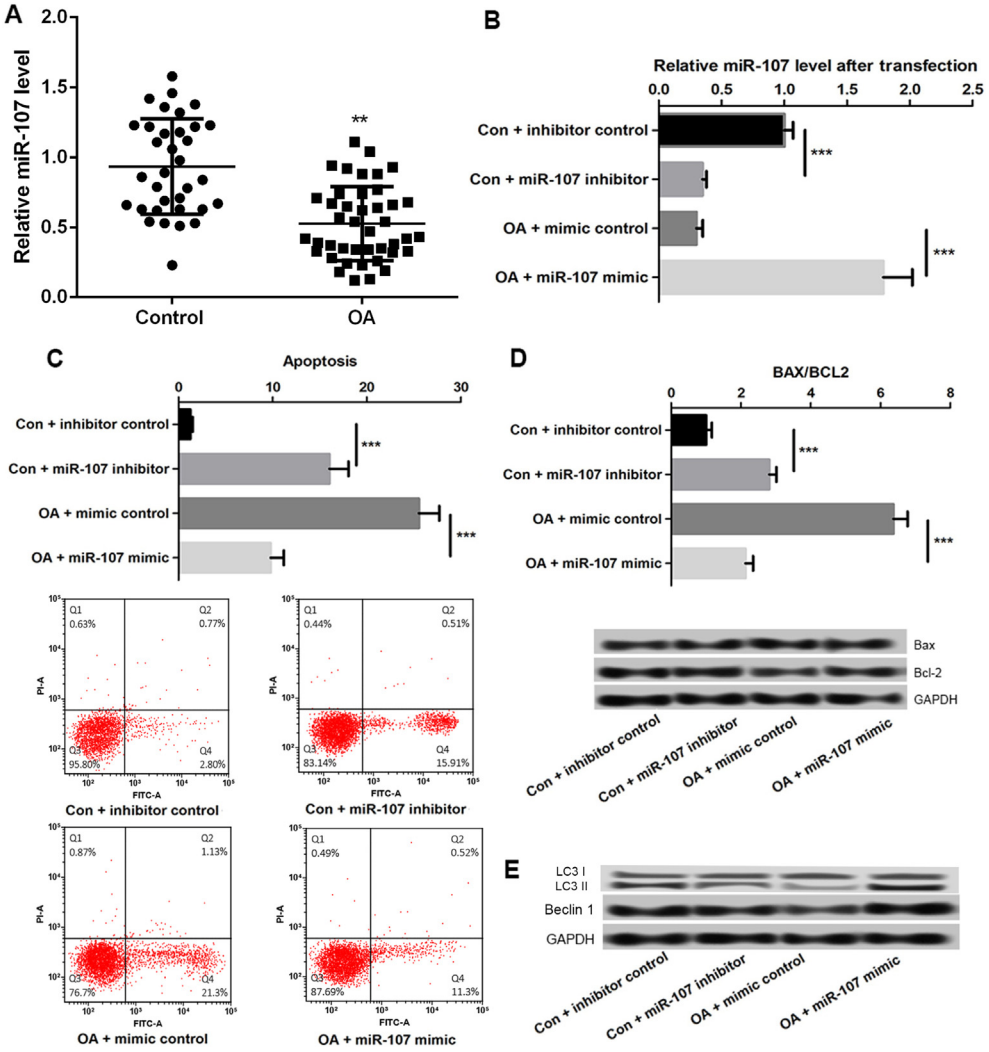

Fig. 1. Effects of miR-107 on apoptosis and autophagy in OA chondrocytes. (A) Expression of miR-107 in normal and OA chondrocytes. (B) Cells were transfected with miR-107 mimic or miR-107 inhibitor and their negative control. (C) Cell apoptosis was determined by flow cytometer. (D) Western blotting analysis of protein levels of BAX and BCL2. (E) The expression level of Beclin-1 and LC3-II/I. The values represent the mean ± SD of three independent experiments (**P < 0.01, ***P < 0.001). 1: Control + inhibitor control; 2: Control + miR-107 inhibitor; 3: OA + mimic control; 4: OA + miR-107 mimic.

In addition, western blot assay revealed that the apoptotic proteins of Bax/Bcl-2 ratio was high in OA rats, but was significantly decreased/increased in antagomiR-107/agomiR-107 transfected OA groups (Fig. 5C). Meanwhile, the protein levels of cell apoptosis related LCI/II and Beclin 1 also performed the same change tendency in OA rats group and in the antagomiR-107/agomiR-107 transfected OA groups (Fig. 5D). All of these data revealed that miR-107 played pivotal roles in regulating cell apoptosis in animal.

4. Discussion

Osteoarthritis (OA) is a pathological condition induced by the destruction or degeneration of the articular cartilage. However, the effect of chondrocytes on OA needs further exploration. Chondrocytes play critical roles in regulating the process of matrix anabolism and catabolism, and then balance the metabolism of cartilage matrix. Injured chondrocytes lose the degradation ability of the damaged matrix in chondrocytes and, thus, induce the irreversible pathological process of osteoarthritis [6]. Some studies raised the close relationship between articular chondrocytes apoptosis and the severity of cartilage degeneration [21]. Increasing evidences found that chondrocytes apoptosis greatly promoted the progression of osteoarthritis [22–24]. It has been reported that miRNA is differentially expressed in normal and OA chondrocytes [17,25]. In the present study, miR-107 was down-regulated in OA chondrocytes compared with control chondrocytes. Meanwhile, miR-107 inhibited apoptosis in OA chondrocytes. Autophagy is a cellular homeostasis mechanism which plays an important role in cell metabolism such as the removing of dysfunctional or damaged macromolecules and organelles in diseases [26]. Lotz MK and his colleagues discovered that the activation of autophagy had inhibiting effect on the progression of OA [27]. In addition, pharmacologic activation of autophagy had been proved to have protective effect on cartilage homeostasis or matrix degradation in OA rats [28]. In our study, miR-107 promoted autophagy in OA chondrocytes, indicating the protecting effect of miR-107 on OA chondrocytes.

During the target gene analysis of miR-107, we obtained lots of targets for miR-107. However, we chose the closely OA/bone disease correlated genes for miR-107, and TRAF3 is among them. Also, Scholars revealed that TRAF3 is closely correlated to the process of OA via complete mechanisms [29,30]. TNF receptor-associated factor 3 (TRAF3) is responsible for encoding TNF receptor associated factor (TRAF) proteins [31]. TRAF protein is found to have the function of inducing NF-kB activation and cell death initiated by LTbeta ligation. TRAF3 is part of the TLR and RLH pathways and plays a critical role in IRF3 activation [32]. Additionally, TRAF3 has contributions to some signaling pathways [33]. Our results showed that miR-107 targeted

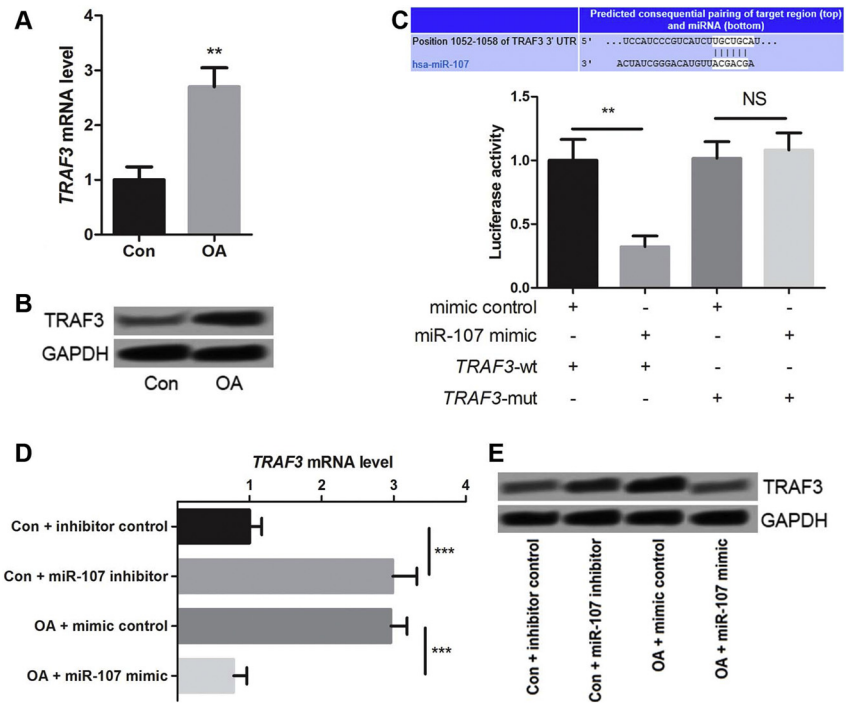

Fig. 2. TRAF3 was the target gene of miR-107. (A, B) The mRNA and protein level of TRAF3. (C) The results of dual luciferase report system showed that 3′UTR of TRAF3 had the action sites with miR-107. (D) The mRNA level of *TRAF3* in different groups. (E) The protein level of *TRAF3* in different groups. The values represent the mean ± SD of three independent experiments (NS: no statistical difference, $^{**}P < 0.01$, $^{***}P < 0.001$). 1: Control + inhibitor control; 2: Control + miR-107 inhibitor; 3: OA + mimic control; 4: OA + miR-107 mimic.

TRAF3 genes and suppressed the expression of TRAF3. We speculated that miR-107 might suppress the expression of TRAF3 by means of reducing the stability of mRNA and inhibiting translation. Scholars revealed that the overexpressed miR-107 could significantly reduce the cell apoptosis of hepatocellular carcinoma [34]. Besides, Wang et al. proved that the abnormally expressed miR-107 regulated epidermis cell autophagy in rats [35]. In this study, our data revealed that the overexpressed miR-107 could reduce the cell apoptosis but enhance cell autophagy via down-regulating TRAF3, which is in accordance with the previous evidences. In addition, we found that TRAF3 participated in cell apoptosis and autophagy, indicating that miR-107 might regulate apoptosis and autophagy via TRAF3. Previous evidence showed that miR-107 is up-regulated in obese mice and functions as an important regulator in OA by targeting TRAF3 [30]. Except for the correlation between OA and obesity in mice, many factors have been reported to prove that OA and obesity is also associated with each other in human person by the following complete aspects [36], for instance, obesity may associated with OA by affecting the burden of joint [37], systemic metabolism including abnormal glucose and tolerance, and abnormal lipids [38], dietary structure, and the changes of posture, gait and exercise habits, especially in diabetic patients [39,40].

It has been reported that mTOR plays an important role in various signaling cascades and biological effects such as protein synthesis, autophagy pathway and cell proliferation [41]. Our results revealed that

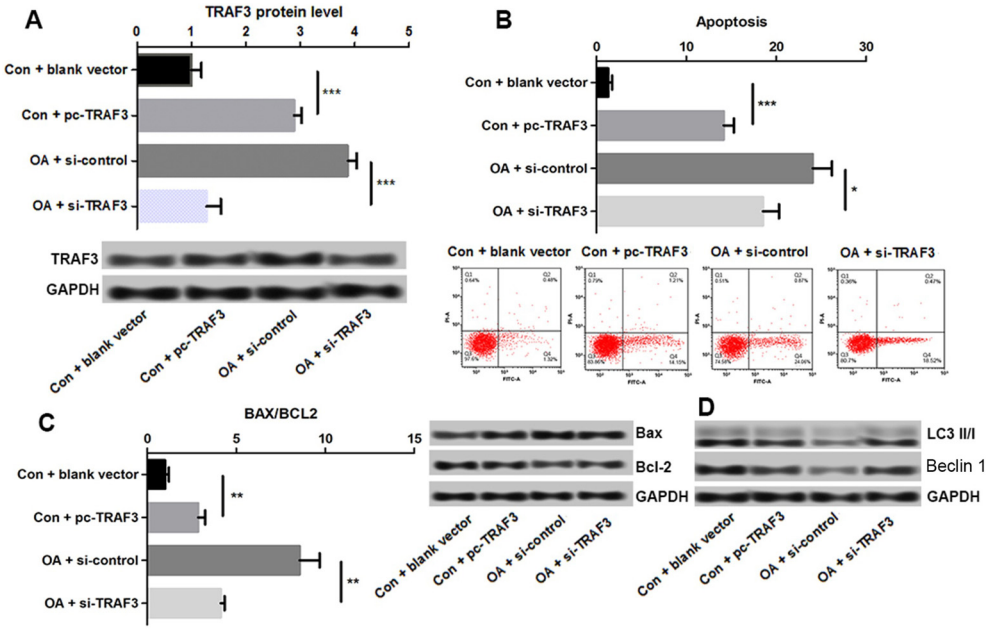

Fig. 3. MiR-107 regulated apoptosis and autophagy via TRAF3. (A) Western blotting analysis of transfection efficiency. (B, C) Apoptosis and the ratio of Bax/Bcl-2. (D) The expression of Beclin-1 and LC3-II/I. The values represent the mean ± SD of three independent experiments ($^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$).

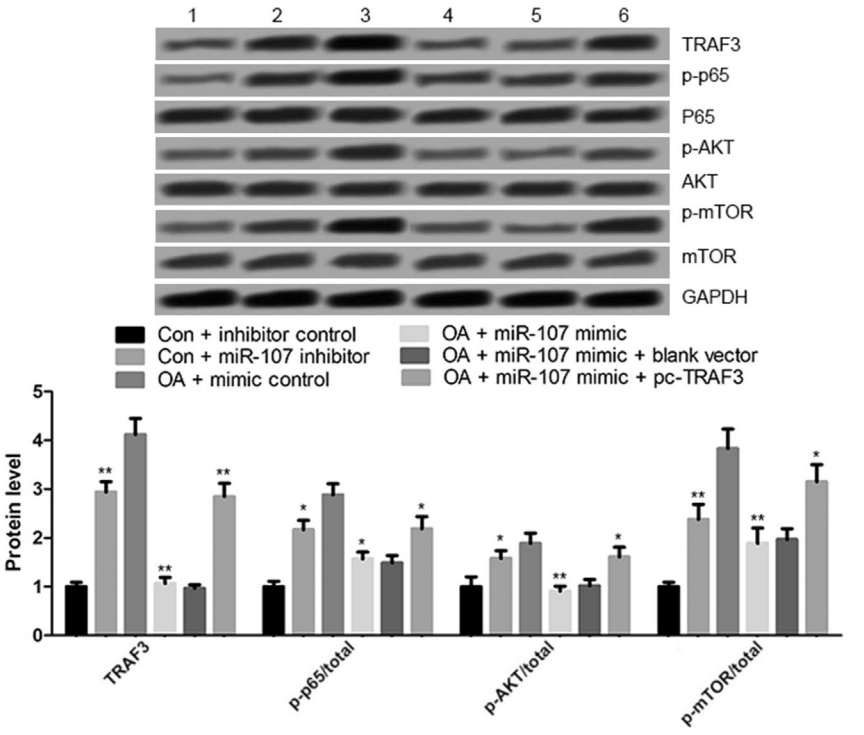

Fig. 4. Western blotting analysis of the level of p-AKT, p-p65 and p-mTOR. The values represent the mean ± SD of three independent experiments. 1: Control + inhibitor control; 2: Control + miR-107 inhibitor; 3: OA + mimic control; 4: OA + miR-107 mimic; 5: OA + miR-107 mimic + blank vector; 6: OA + miR-107 mimic + pc-TRAF3. The values represent the mean ± SD of three independent experiments ($^{*}P < 0.05$, $^{**}P < 0.01$).

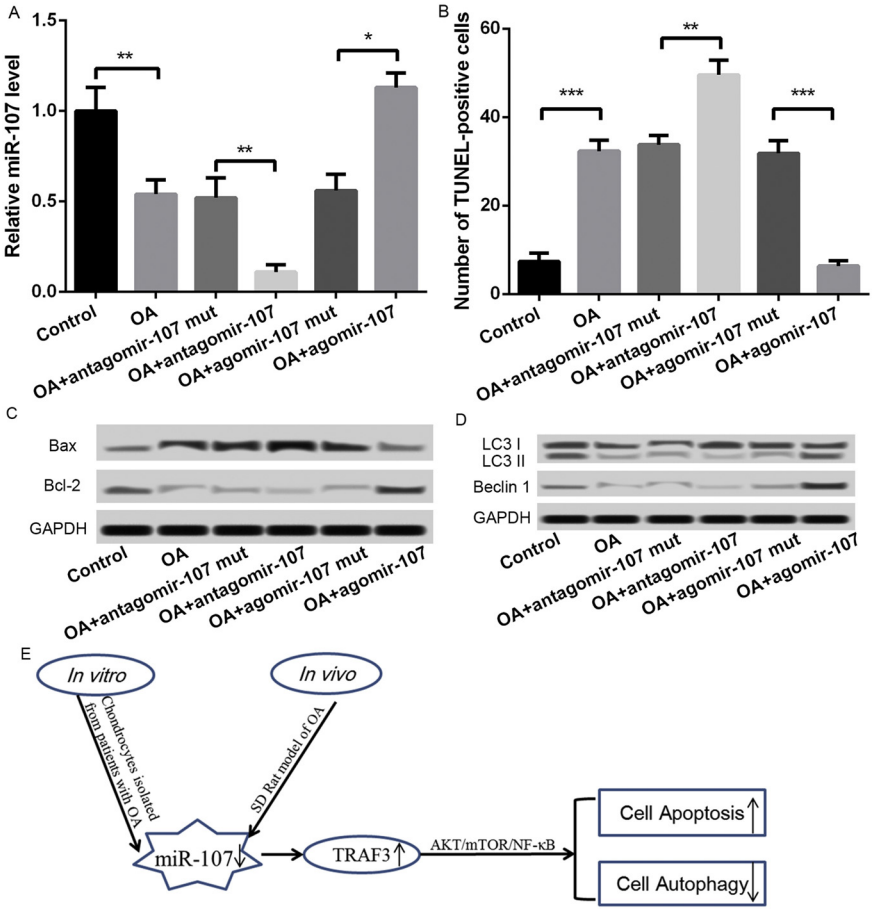

Fig. 5. In vivo analysis of miR-107 expression on cell apoptosis in SD rats. (A) mRNA level of miR-107 in sham control, OA model rats. (B) Effects of miR-107 expression on cell apoptosis in each group of SD rats. (C) Influences of miR-107 expression on the cell apoptosis related protein of Bax and Bcl-2 in each group of SD rats. (D) Influences of miR-107 expression on the cell apoptosis related protein of LC3 I, LC3 II and Beclin 1 in each group of SD rats. (E) Regulatory mechanism of miR-107 in OA. The values represent the mean ± SD of three independent experiments ($^{*}P < 0.05$, $^{**}P < 0.01$, and $^{***}P < 0.001$).

the overexpression of miR-107 suppressed the phosphorylation of mTOR, indicating that mTOR signaling pathway was a major target of miR-107 in OA chondrocytes. AKT is the leading kinase that activates mTORC1 through phosphorylation of tuberous sclerosis complex 2 (TSC2) and then results in the activation of mTORC1 and inhibition of autophagy [42]. Accumulating evidence has shown that PI3K/AKT pathway is involved in the regulation of cell differentiation, proliferation and OA pathological pathogenesis [43,44]. Our data showed that overexpression of miR-107 inhibited the phosphorylation of AKT indicated that miR-107 exerted an inhibitory effect on the PI3K/AKT pathway by targeting TRAF3 genes.

Moreover, we analyzed the possible expression level of miR-107 in OA animal and also assessed the influences of miR-107 on cell apoptosis in OA animal. The data showed (Fig. 5) showed that miR-107 was lowly expressed in OA rats comarped with the sham control rats, and the percentage of apoptotic cells and the cell apoptosis related proteins including Bax/Bcl-2, LC3I/LC3 II and Beclin 1 were all accordingly changed by antagomiR-107/agomiR-107 transfection in OA rats. These findings reflected that miR-107 was indeed affecting the chondrocytes apoptosis in OA in vivo.

5. Conclusion

Our results demonstrated that miR-107 inhibited cell apoptosis and promoted autophagy in OA chondrocytes. Furthermore, overexpression of miR-107 inhibited the activation of AKT/mTOR and NF-κB pathway by targeting TRAF3 genes.

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