International Immunopharmacology 76 (2019) 105839

Contents lists available at ScienceDirect

International Immunopharmacology

journal homepage: www.elsevier.com/locate/intimp

MicroRNA-27a alleviates IL-1β-induced inflammatory response and articular cartilage degradation via TLR4/NF-κB signaling pathway in articular chondrocytes

Wen-Jun Qiu[1], Ming-Ze Xu[1], Xiao-dong Zhu, Yun-Han Ji[*,2]

Department of Orthopedic Surgery Tongren Hospital, Shanghai Jiao Tong University School of Medicine, Shanghai 200336, China

ARTICLE INFO

Keywords:
Osteoarthritis
MicroRNA-27a
Inflammatory response
Articular cartilage degradation
TLR4/NF-κB signaling pathway

ABSTRACT

Osteoarthritis (OA) is a common disease of the articular cartilage, and inflammatory response and articular cartilage degradation have been implicated in the pathogenesis of OA. In recent years, microRNAs (miRNAs) have been potentially involved in the pathogenesis of OA. However, little is known about the role of miRNAs in the inflammatory response and articular cartilage degradation in OA and the underlying molecular mechanism. In the present study, we analyze miRNA profiles in the articular tissues from OA patients using microarray. miR-27a has attracted considerable interest for its suppressive effects on inflammation. Subsequently, the expression levels of miR-27a were validated in the articular tissues of OA patients and IL-1β-stimulated chondrocytes. Using this IL-1β-induced chondrocyte injury model, we found that upregulation of miR-27a suppressed articular cartilage degradation, the reactive oxygen species (ROS) production and inflammatory response as reflected by reductions in pro-inflammatory cytokines, including interleukin (IL)-6 and IL-8 and tumor necrosis factor (TNF)-α. Moreover, toll-like receptor 4 (TLR4), one upstream molecule of NF-κB signaling pathway, was identified as a direct target of miR-27a in chondrocytes. Furthermore, it was demonstrated that overexpression of TLR4 by pcDNA-TLR4 markedly abrogated the inhibitory effects of miR-27a on the inflammatory response and the degeneration of articular cartilage induced by IL-1β. Our findings suggest that miR-27a may be considered as a potential therapeutic target in the treatment of OA.

1. Introduction

Osteoarthritis (OA) is regarded as the most common form of chronic arthritis that often leads to pain and disability and affects approximately 10% of men and 20% of women over the age of 60 in the developed world [1,2]. Although there has been substantial effort to improve OA, such as total joint replacement, the approaches to counteract and avoid OA remain insufficient [3–5]. Thus, further investigation into the pathogenesis of OA is important to optimize therapeutic strategies and develop novel therapeutic drugs for OA patients.

The pathophysiological processes of OA are complicated and inflammation is the most typical pathological change among the various causes of OA, which promotes the reduction of homeostasis and the degradation of extracellular matrix (ECM) [6,7]. Inflammatory cytokines such as interleukin-1 beta (IL-1β) has been widely used to mimic an in vitro model of OA, as it can induce many catabolic factors [12,13]. When chondrocytes are exposed to IL-1β, the production of reactive oxygen species (ROS) and the release of inflammatory mediators are increased [8]. In addition, IL-1β stimulated the expressions of extracellular matrix degradation-related enzyme, such as matrix metalloproteinases (MMPs), which affects the balance of ECM in articular cartilage, thus aggravating the condition of OA [9,10]. Therefore, the IL-1β-induced chondrocytes model of OA was used in the present study for further investigations.

MicroRNAs (miRNAs) are short, non-coding RNAs that are 19 to 25 nucleotides in length that regulate gene expression post-transcriptionally by pairing with complementary nucleotide sequences in the 3′-untranslated regions of specific mRNA targets [11]. Increasing evidence showed that aberrant miRNA expression plays an important role in the development of OA [12]. For example, Xue et al. found that

* Corresponding author at: Department of Orthopedic Surgery Tongren Hospital, Shanghai Jiao Tong University School of Medicine, No.1111 Xianxia Road, Changning District, Shanghai 200336, China.
E-mail address: jyh1715@shtrhospital.com (Y.-H. Ji).
[1] They are the first co-authors.
[2] Equal contributors.

https://doi.org/10.1016/j.intimp.2019.105839
Received 4 June 2019; Received in revised form 30 July 2019; Accepted 19 August 2019
Available online 11 September 2019
1567-5769/

injection of a miR-93-5p-expressing lentivirus alleviated the destruction of articular cartilage in a rat model of OA [13]. Baek et al. showed that inhibition of miR-449a prevented progression of OA in rat OA models through promoting cartilage regeneration [14]. Another group indicated that miR-204-5p ameliorated the OA-like phenotype in rats with surgically induced OA by targeting Runx2 [15]. Ding et al. found that miR-93 protected OA mice from inflammation by targeting TLR4 [16]. Zhao et al. demonstrated that miR-26a alleviated synovitis and cartilage injury by inhibiting the inflammatory response in OA rats [17]. In addition, recent studies reported the alteration of several miRNAs in OA and their potential to serve as diagnostic markers for OA [18]. However, the function of specific miRNAs in inflammatory response and articular cartilage degradation of OA remains unclear.

In this study, we used a miRNA microarray to analyze the miRNA profiles in normal and OA articular tissues and observed the downregulation of miR-27a in OA. Subsequently, the regulatory mechanisms of miR-27a on inflammatory response and articular cartilage degradation were examined in an IL-1β-induced chondrocyte model. Furthermore, it was found that miR-27a targeted TLR4 and inhibited activation of the NF-κB pathway in vitro. These results suggest that miR-27a may serve as a novel therapeutic target in OA treatment.

2. Materials and methods

2.1. Tissue samples and human articular chondrocyte culture

OA cartilage tissues were obtained from 20 patients undergoing hip or knee surgeries (age ranging from 50 to 73 years) at the Department of Orthopedic Surgery Tongren Hospital, Shanghai Jiao Tong University School of Medicine (Shanghai, China). Normal cartilage samples were taken from 10 donors (age between 55 and 70 years) with no previous history of joint pain at the hospital. Written informed consent was obtained from each patient. The study was approved by the Ethics Committee of Shanghai Jiao Tong University School of Medicine.

Primary chondrocyte isolation was performed as described previously [19]. Normal human articular cartilage was minced and digested in 0.25% trypsin for 10 min and 0.1% collagenase II in DMEM at 37 °C for 4 h. The digest was centrifuged at 100*g* for 5 min, cells resuspended in Dulbecco's modified Eagle's medium (DMEM, Thermo Fisher Scientific, Waltham, MA, USA) supplemented with 10% fetal bovine serum (FBS, PAN-Biotech, Aidenbach, Germany) and 1% penicillin-streptomycin (Beyotime, Jiangsu, China) at 37 °C. Cells from the passage 1 and 2 were employed for subsequent studies. IL-1β was used to stimulate chondrocytes for the establishment of OA in vitro model at a final concentration of 10 ng/mL. To ascertain whether the NF-κB pathway participates in IL-1β-induced OA changes in chondrocytes, cells were pretreated with a NF-kB inhibitor, pyrrolidine dithiocarbamate (PDTC; 10 mmol/L, Sigma, MO, USA), for 1 h, followed by 10 ng/mL IL-1β for 24 h.

2.2. miRNA microarray

The miRNA microarray and the bioinformatics analysis were performed at Kanchen Bio-tech Corporation (Shanghai, China). Briefly, total RNA was isolated from 3 OA cartilage tissues and 3 normal cartilage tissues by miRNeasy isolation kit (Qiagen, Milan, Italy). The quantity and integrity of RNA samples was evaluated via NanoDrop ND-1000 spectrophotometry (Thermo Fisher Scientific, Inc., Waltham, MA, USA). Total RNA (200 ng) was labeled with fluorescence dye hy3 or hy5 using the miRCURY Hy3/Hy5 Power Labeling kit and hybridized on the miRCURY™ LNA Array (v.18.0), both obtained from Exiqon; Qiagen, Inc., according to the manufacturer's protocol. Microarray images were taken with Axon GenePix 4000B microarray scanner (Axon Instruments, Foster City, CA, USA). Then the scanning images imported into the GenePix Pro6.0 program (Axon Instruments) for grid alignment and data extraction. The miRNAs with intensities ≥50 were used to calculate a normalization factor in all samples. Normalization was performed using median normalization. The miRNA expression profiles were determined using MEV software (version 4.6; TIGR, Microarray Software Suite4, Boston, United States).

2.3. qRT-PCR

Total RNA was extracted from cartilage tissues and cells with the TRIzol reagent (Invitrogen, USA) according to the manufacturer's protocol. Reverse transcription of miR-27a and TLR4 was synthesized using the miScript II RT kit and the reverse transcription kit (Invitrogen, Carlsbad, CA), respectively. miR-27a and TLR4 expressions were measured using the Exiqon SYBR Green Master Mix (Exiqon, Vedbaek, Denmark) on a Light Cycler instrument (Bio-Rad). The primers for qRT-PCR analysis were as follows: The primers for qRT-PCR analysis were as follows: miR-27a forward: 5′-TTCACAGTGGCTAAG-3′; miR-27a reverse: 5′-GTGCAGGGTCCGAGGT-3′; U6 forward: 5′-TGCGGGTGCTCG CTTCGCAGC-3′; U6 reverse: 5′-CCAGTGCAGGGTCCGAGGT-3′; TLR4 forward: 5′-AGTTGATCTACCAAGCCTTGAGT-3′, TLR4 reverse: 5′-GCT GGTTGTCCCAAAATCACTTT-3′; GAPDH forward: 5′-AGGTCGGTGTGA ACGGATTTG-3′, GAPDH reverse: 5′-TGTAGACCATGTAGTTGAGG TCA-3′. The PCR amplification protocol was as follows: an initial 95 °C for 5 min, followed by 40 cycles of 94 °C for 10 s, 57 °C for 31 s, and 70 °C for 30 s. Relative quantification was determined by normalization to U6 or GAPDH. The qRT-PCR assays were performed in triplicate and the relative expression levels were calculated based on the $2^{-\Delta\Delta}$Ct method [20].

2.4. Cell transfection

When chondrocytes in six-well plate grown to about 80% confluence, 20 nM miR-27a mimics, 20 nM mimics negative control (mimics NC), 20 nM miR-27a inhibitor, and 20 nM inhibitor NC or 2 μg pcDNA-TLR4 were transfected into cells at 37 °C for 24 h, using Lipofectamine® 2000 (Invitrogen). miR-27a mimics, miR-27a inhibitor, the corresponding control vectors, pcDNA-TLR4 and pcDNA vector were purchased from RiboBio Co., Ltd. (Guangzhou, China). Post transfection the cells were stimulated with IL-1β for 24 h and then utilized in subsequent experiments.

2.5. ELISA assay

Cells were transfected with various plasmids, PDTC or miR-27a, and then were exposed to IL-1β (10 ng/ml) for 24 h. Then, the levels of MMP-3 (cat no.ab100607, Abcam, Cambridge, UK), MMP-9 (cat no.ab100610, Abcam, Cambridge, UK), MMP-13 (cat no.ab100605, Abcam, Cambridge, UK), IL-6 (cat no.p1330, Beyotime Biotechnology, Shanghai, China), IL-8 (cat no.p1640, Beyotime Biotechnology, Shanghai, China), and TNF-α (cat no.pt518, Beyotime Biotechnology, Shanghai, China) in the culture supernatants were evaluated using the commercial ELISA kits according to the manufacturer's standard protocols.

2.6. Measurement of reactive oxygen species (ROS)

ROS production in chondrocytes was measured by the kit of ROS (Haimen, Jiangsu, China) according to the kit instructions. After designated treatment, chondrocytes in 6-well plate (5×10^4/well) were stained with 1 μM dichlorodihydrofluorescein diacetate (DCFH-DA) (Beyotime Biotechnology, China) for 30 min at 37 °C. Cells were captured using a fluorescence microscope (IX-81; Olympus Corp., Shinjuku, Tokyo, Japan). The fluorescence intensity was analyzed by using the fluorescence plate reader (Titertek Plus MS 212, ICN, Eschwege, Germany) at Ex/Em = 488/525 nm. The level of fluorescence was used to indicate ROS levels, and data were displayed using the fold change in fluorescence.

2.7. Detection of malonaldehyde, superoxide dismutase, and glutathione peroxidase

After designated treatment, the chondrocytes were lysed using lysis buffer (Beyotime Biotechnology), the lysates were collected for the detection of superoxide dismutase (SOD) and malondialdehyde (MDA) and glutathione peroxidase (GPx). Then, the kits of SOD (cat no.so101), MDA (cat no.so131) and GPx (cat no.s0058) (Beyotime, Shanghai, China) were used to examine the level of SOD, the content of MDA and the activity of GPx according to the manufacturer's instructions.

2.8. MTT assay

Cell viability was detected using the MTT assay. Cells (8×10^3/well) were seeded into 96-well plate for incubation overnight and subjected to designated treatment. After incubation for 48 h, 20 μl of MTT solution (5 mg/mL, Sigma, St. Louis, MO, USA) was added to each well and incubated for 4 h. Absorbance was measured at 560 nm by a microplate reader (Bio-Tek Instruments, Germany).

2.9. Flow cytometry assay

Chondrocytes (1×10^6) were digested with trypsin, harvested after designated treatment, washed in ice-cold PBS, and stained in 500 μL binding buffer with 5 μL PI and 5 μL AnnexinV-FITC (Nanjing KeyGen Biotech Co. Ltd., Nanjing, China) for 20 min in the dark. The stained cells were analyzed with EPICS XL-MCL FACScan (BectoneDickinson, Mountain View, CA, United States).

2.10. NF-κB activity assay

Chondrocytes were plated in 6 well tissue culture plates at a concentration of 1×10^6 cells/well for 24 h. Then, cells were transfected with 2.5 μg of a NF-κB reporter luciferase construct. Six hours later, cells were washed and then treated with miR-27a mimics, PDTC or pcDNA-TLR4 for another 24 h, followed by 10 ng/mL IL-1β for 24 h. Cells were then washed in PBS and harvested in 500 μL 1 × passive lysis buffer. Luciferase was quantified using Promega Luciferase Assay kit on a luminometer. Experimental values were recorded relative to untreated control samples.

2.11. Bioinformatics analysis and dual-luciferase reporter assays

miRNA target prediction tools, including PicTar version 2007 (https://pictar.mdc-berlin.de/) and TargetScan Release 7.0 (http://targetscan.org/) were used to search for the putative targets of miR-27a. For the luciferase reporter assay, HEK 293 T cells were co-transfected with miR-27a mimics, miR-27a inhibitor and the luciferase reporter plasmids using Lipofectamine 2000 (Invitrogen). At 24 h post-transfection, the double luciferase activities were analyzed using the Dual-Luciferase Reporter Assay system (Promega Corporation). Luciferase activity was normalized to Renilla luciferase activity.

2.12. Western blot analysis

Total protein was extracted using radio immunoprecipitation assay (RIPA) lysis buffer (Beyotime Biotechnology, Shanghai, China) supplemented with protease inhibitors (Roche, Guangzhou, China). The protein concentration was determined using a BCA kit (Beyotime Biotechnology, Shanghai, China). The extraction and isolation of nuclear and cytoplasmic protein were performed according to the Nuclear and Cytoplasmic Protein Extraction Kit (Beyotime Biotechnology, Shanghai, China). The nuclear and cytoplasmic proteins were quantified with kit mentioned above (Beyotime Biotechnology, Shanghai, China) according to the recommendation. Next, the proteins in the lysates were separated on SDS-PAGE gels and electrotransferred to PVDF membranes(GE Healthcare, Freiburg, DE), followed by blocking in a 5% skim milk solution for 1 h at room temperature. Primary antibodies against TLR4 (cat. no. 14358; 1:2000), aggrecan (cat. no. ab3778, 1:2000, Abcam, Cambridge, UK), collagen type II (cat. no.ab34712, 1:2000, Abcam, Cambridge, UK), total p65 (cat no.#8242, Cell Signaling Technology, 1:1000 dilution), nuclear p-p65 (cat no.#3033, Cell Signaling Technology, 1:1000 dilution), p-IκB-α (cat no.#2859, Cell Signaling Technology, 1:1000 dilution), IκB-α (cat no.sc-52,900, Santa Cruz Biotechnology, 1:1000 dilution), Histone H3 (cat no.#9728, 1:2000, Cell Signaling Technology, 1:1000 dilution), and β-actin (cat no.#4970, Cell Signaling Technology, 1:2000 dilution) were incubation at 4 °C overnight. Then, membranes were incubated with the corresponding horseradish peroxidase-conjugated goat anti-rabbit or anti-rat secondary antibodies (cat no.ab6721 and 6785, 1:2000, Abcam, Cambridge, UK) for 1 h at room temperature. The bands were detected by enhanced chemiluminescence (ECL) kit (GE Healthcare, Freiburg, DE). The intensity of the bands of interest was analyzed using Bio-Rad Laboratories Quantity One software 3.0 (Bio-Rad Laboratories, Inc.). β-actin protein was used as the inner control of the cytoplasmic proteins; Histone H3 protein was used as the inner control of the nuclear proteins.

2.13. Statistical analysis

The difference between means was analyzed using Student's *t*-test and analysis of variance (ANOVA) using SPSS 13.0 software package (SPSS Inc., Chicago, IL). All data are showed as the mean ± standard deviation (SD). P value < 0.05 was considered statistically significant. Spearman's analysis was used in correlation analysis. $P < 0.05$ was considered to indicate a statistically significant difference.

3. Results

3.1. miR-27a was downregulated in OA cartilage tissues and IL-1β induced chondrocytes

To reveal the role of miRNAs in the progression of OA, microarray analysis was performed to determine miRNA levels in OA cartilage tissues. Of 58 differently expressed miRNAs in the OA group 32 miRNAs were downregulated and 26 miRNAs were upregulated, compared to the normal group (Fig. 1A). Among the differentially expressed miRNAs, miR-27a was one of the most significantly downregulated in the articular cartilages of OA patients based on the microarray expression data. Consistent with our results, miR-27a was also found to be downregulated in human OA chondrocytes, indicating that miR-27a may be involved in the progression of OA [21–23]. In addition, several other studies have shown that miR-27a act as a suppressive regulator of inflammatory response in many types of injury model, including traumatic brain injury (TBI), acute lung injury (ALI) and so on [24–27]. However, whether miR-27a has a protective effect against the inflammatory response and articular cartilage degradation in OA remains unknown. Thus, we chose miR-27a for further investigation. The expression of miR-27a was significantly downregulated in OA cartilage tissues, compared to normal cartilage tissues using qRT-PCR analysis (Fig. 1B). In addition, it was also found that miR-27a expression was markedly downregulated in the IL-1β stimulated chondrocytes that is widely used as the in vitro model of OA [28], and this inhibitory effect was dose-dependent (Fig. 1C). Collectively, these results suggest that miR-27a may be associated with the progression of OA.

3.2. Upregulation of miR-27a inhibited the cartilage degradation in chondrocytes upon IL-1β condition

To further examine the effect of miR-27a on the cartilage degradation, miR-27a mimics were transfected into the cultured chondrocytes, followed by IL-1β stimulation. The qRT-PCR analysis showed

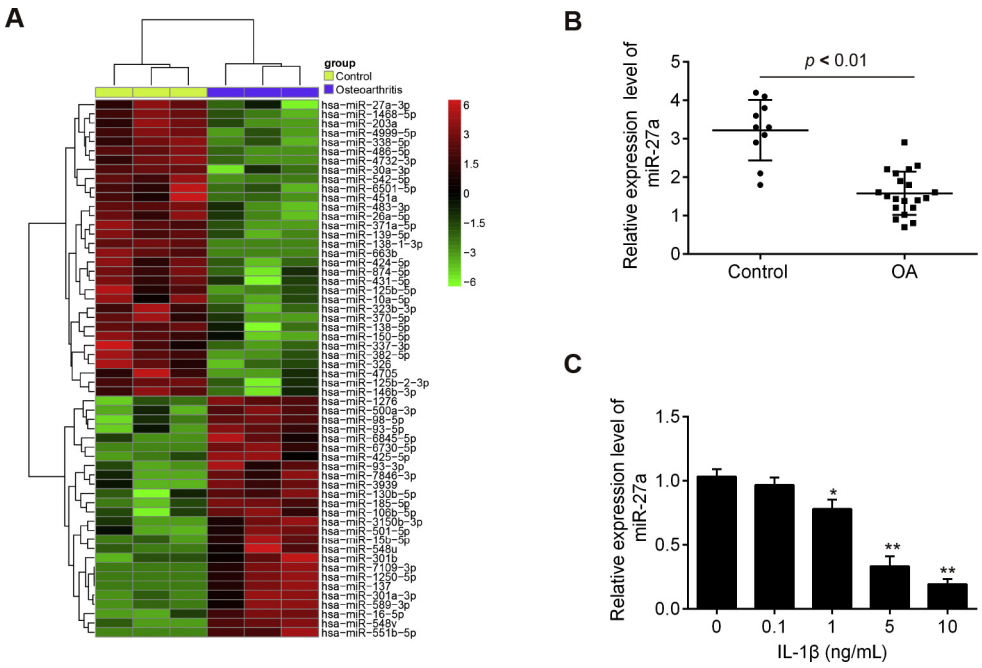

Fig. 1. miR-27a was downregulated in OA cartilage tissues and IL-1β induced chondrocytes. (A) Heat map of miRNA profiles represents the differentially expressed miRNAs between OA cartilage tissues (n = 3) and normal cartilage tissues (n = 3). Green indicates low expression levels; red indicates high expression levels. (B) qRT-PCR analysis was used to assess the expression levels of miR-27a in OA cartilage tissues from 20 patients with OA and normal cartilage samples from 10 donors. p < 0.01 vs. Control group. (C) Different concentrations of IL-1β were used to stimulate chondrocytes and the expression of miR-27a was measured by qRT-PCR. Data were represented as the mean ± SD of three independent experiments. *p < 0.05, **p < 0.01 vs. control group. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

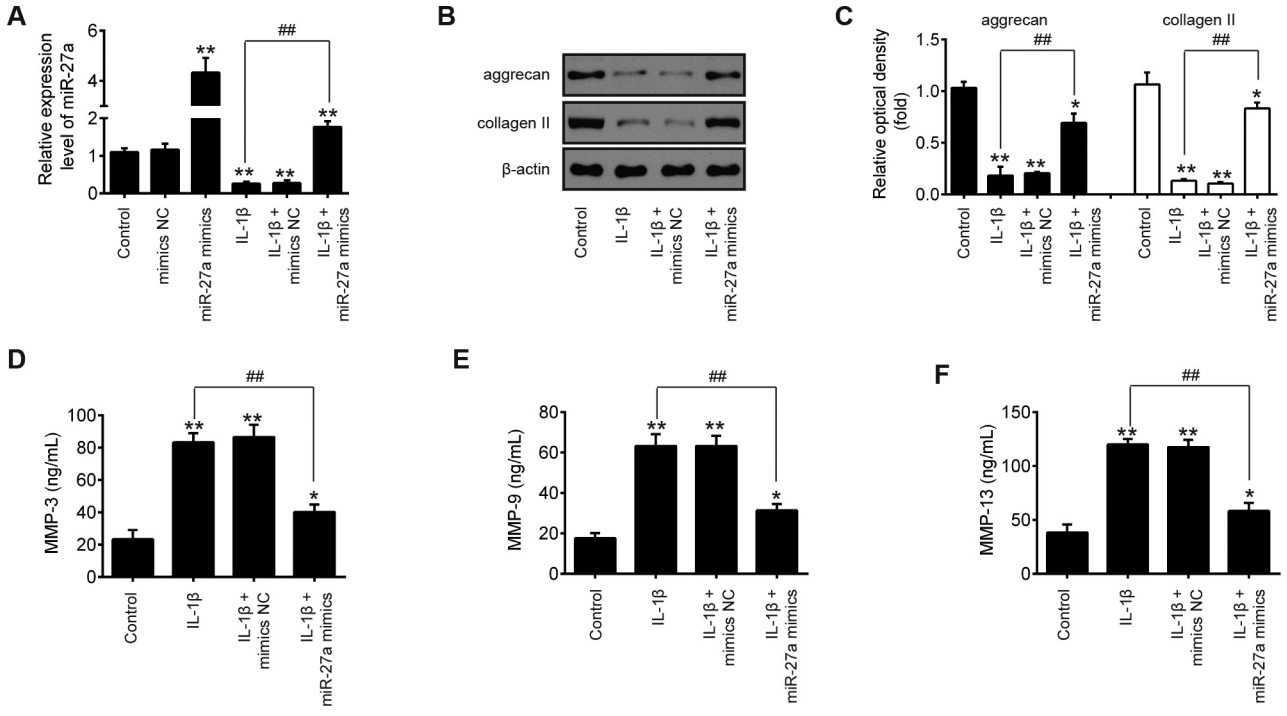

Fig. 2. Upregulation of miR-27a inhibited the cartilage degradation in chondrocytes upon IL-1β condition. (A) The miR-27a level in the chondrocytes transfected with miR-27a mimics or negative control was measured by the qRT-PCR assay. After transfection with miR-27a mimics, cells were exposed to IL-1β. Then the expression of miR-27a was also determined. (B, C) The protein levels of aggrecan and collagen II were analyzed by western blotting. The bands were semi-quantitatively analyzed by using Image J software, normalized to β-actin density. (D-F) The contents of MMP-3, MMP-9 and MMP-13 in supernatants were evaluated by ELISA assay. Data were represented as the mean ± SD of three independent experiments. *p < 0.05, **p < 0.01 vs. Control group; ##p < 0.01 vs. IL-1β group.

the miR-27a was notably increased after transfection of miR-27a mimics (Fig. 2A). It was also observed that the decreased miR-27a expression in IL-1β treated chondrocytes was reversed after miR-27a mimics transfection (Fig. 2A). Subsequently, the roles of miR-27a on the expression of collagen type II, aggrecan and the contents of MMPs (MMP-3, −9 and MMP-13) were then examined in chondrocytes. The results showed that IL-1β significantly decreased the expressions of collagen type II, aggrecan, compared with that in control, whereas these inhibitory effects were attenuated by miR-27a overexpression (Fig. 2B, C). As expected, IL-1β stimulation led to a significant increase of MMP-3, MMP-9 and MMP-13 contents compared to control group, while the increased MMPs contents induced by IL-1β were reversed by miR-27a overexpression (Fig. 2D-F). Taken together, these data suggest that upregulation of miR-27a might alleviate IL-1β-induced cartilage degradation in chondrocytes.

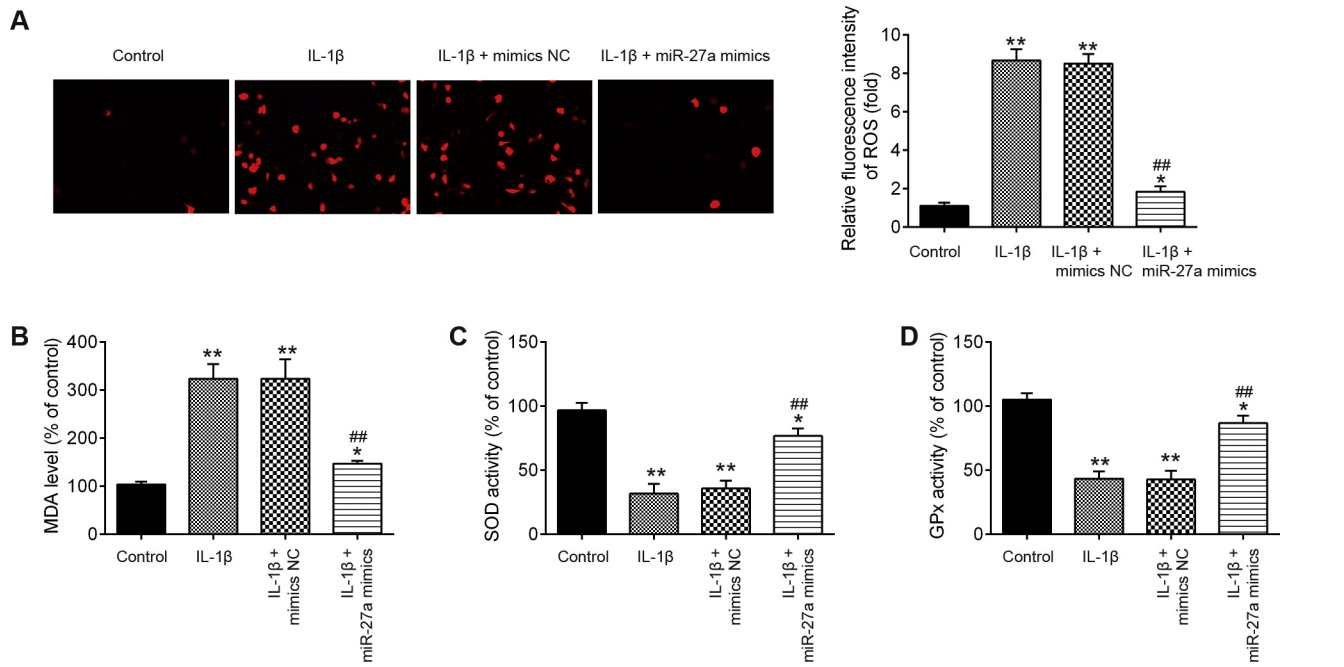

Fig. 3. Upregulation of miR-27a suppressed IL-1β-induced oxidative stress response and ROS production in chondrocytes. Chondrocytes were transfected with miR-27a mimics or negative control for 24 h and then exposed to IL-1β for 24 h, the cells and cell supernatants were collected for further analysis. (A) The ROS production was measured by a commercial kit of ROS. (B-D) The levels of SOD, MDA and GPx in chondrocytes were detected by ELISA kits. Data were represented as the mean ± SD of three independent experiments. *p < 0.05, **p < 0.01 vs. Control group; ##p < 0.01 vs. IL-1β group.

3.3. Upregulation of miR-27a suppressed IL-1β-induced oxidative stress response and ROS production in chondrocytes

As ROS and the resulting oxidative stress, together with MMPs, serve to maintain homeostasis in chondrocytes by facilitating cell turnover [29,30], the present study explored the impact of miR-27a on ROS production using DCF-DA assay in chondrocytes. As shown in Fig. 3A, IL-1β stimulation significantly increase the level of ROS production compared to control group, whereas this enhancement was remarkably reduced by miR-27a overexpression. Furthermore, the changes of oxidative stress markers, SOD, MDA and GPx in chondrocytes were analyzed by ELISA assay. The results showed that the level of MDA was obviously increased, but SOD and GPx levels were significantly reduced after IL-1β stimulation. However, miR-27a upregulation attenuated these effects of IL-1β on SOD, MDA and GPx in chondrocytes (Fig. 3B-D). These data indicated that miR-27a could reduce IL-1β-induced oxidative stress in chondrocytes.

3.4. Upregulation of miR-27a suppressed IL-1β-induced inflammatory response in chondrocytes

Next, the effect of miR-27a on the release of pro-inflammatory cytokines that contribute to the clinical symptoms of OA was further assayed in chondrocytes. First, we assessed the effect of miR-27a on the cell viability of chondrocytes. As shown in Fig. 4A, IL-1β stimulation significantly decreased cell viability compared with control group, but this inhibitory effect was attenuated by miR-27a overexpression. We further analyzed the effect of miR-27a on the apoptosis and the levels of apoptosis-related protein, cleaved caspase-3. The results showed that the apoptosis portion and the levels of cleaved caspase-3 were significantly increased in IL-1β treated chondrocytes compared to control group, while the promoting effect was attenuated when miR-27a was overexpressed (Fig. 4B, C). More importantly, we observed the effects of miR-27a on pro-inflammatory cytokines by ELISA assays and the results showed that the levels of IL-6, IL-8 and TNF-α were significantly increased in IL-1β treated chondrocytes compared to control group, whereas these promoting effects was markedly reduced by miR-27a upregulation (Fig. 4D-F). These data suggest that miR-27a can protect chondrocytes against IL-1β induced inflammatory response.

3.5. TLR4 was a direct target of miR-27a

To gain insights into the molecular mechanism underlying by which miR-27a attenuates IL-1β induced cartilage degradation and inflammatory response in chondrocytes, we relied on TargetScan and PicTar to predict target genes of miR-27a and identified TLR4 as a potential target of miR-27a (Fig. 5A). Subsequently, we measured the expression of TLR4 mRNA in OA cartilage tissues and found that the TLR4 was significantly increased in OA cartilage tissues compared with control group (Fig. 5B). The further correlation analysis indicated that miR-27a expression was negatively associated with TLR4 expression in OA cartilage tissues (Fig. 5C). In addition, to investigate whether TLR4 was regulated by miR-27a, we measured the mRNA and protein expression of TLR4 by qRT-PCR and Western Blot. As shown in Fig. 5D, E, the mRNA and protein levels of TLR4 were markedly reduced by miR-27a overexpression, but were significantly increased by miR-27a knockdown. On the other hand, luciferase reporter assays showed that overexpression of miR-27a inhibited, while miR-27a knockdown promoted the relative luciferase activity in plasmids containing the wild-type (WT) 3′UTR sequence of TLR4-mRNA, but not a mutated control sequence, when compared with the control group (Fig. 5F). Next, we detected the effect of miR-27a on TLR4 expression in IL-1β treated chondrocytes by qRT-PCR. Our results showed that IL-1β stimulation significantly increased the expression of TLR4 compared with control group, but this promoting effect was attenuated by miR-27a overexpression, and enhanced by miR-27a inhibition (Fig. 5G). These results indicate that miR-27a can specifically bind to TLR4 mRNA and downregulate the gene expression in chondrocytes.

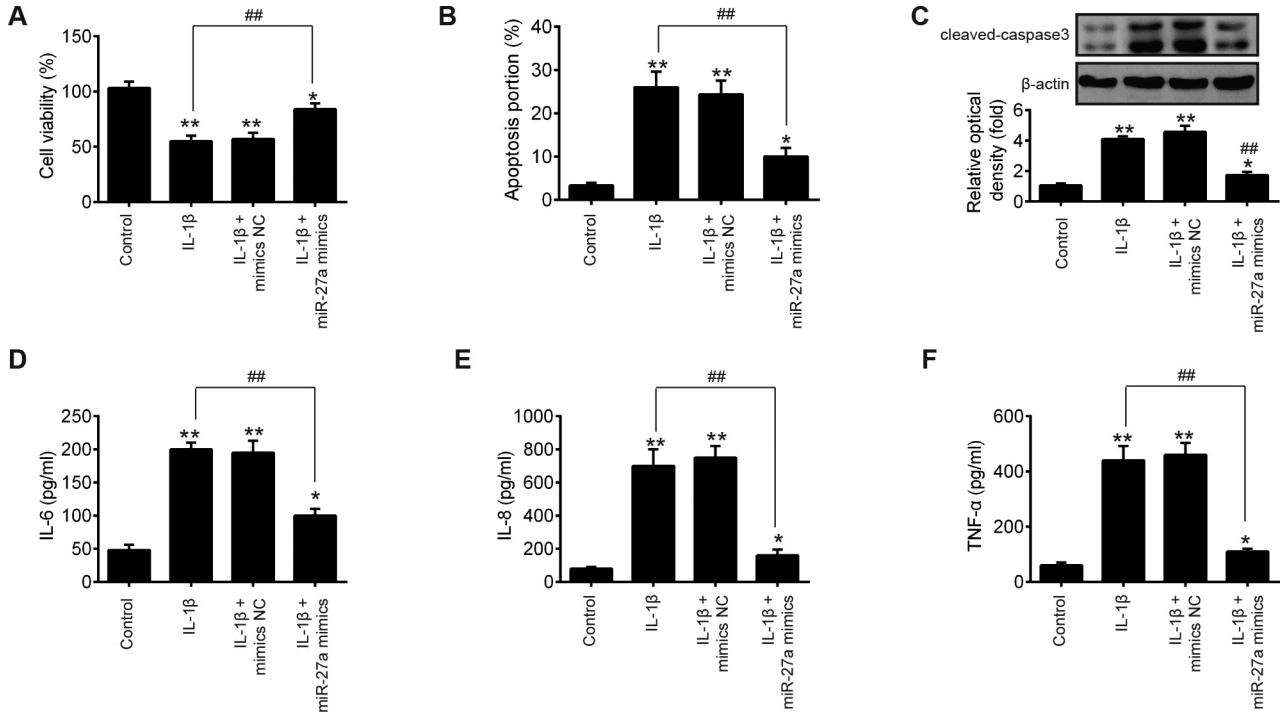

Fig. 4. Upregulation of miR-27a suppressed IL-1β-induced inflammatory response in chondrocytes. Chondrocytes were transfected with miR-27a mimics or negative control for 24 h and then exposed to IL-1β for 24 h, the cells and cell supernatants were used for further analysis. (A) Cell viability was detected by MTT assay. (B) Cell apoptosis was measured by Flow cytometry assay. (C) The protein level of cleaved caspase-3 was analyzed by western blotting. The bands were semi-quantitatively analyzed by using Image J software, normalized to β-actin density. (D–F) The levels of IL-6, IL-8 and TNF-α were measured by ELISA kits. Data were represented as the mean ± SD of three independent experiments. $*p < 0.05$, $**p < 0.01$ vs. Control group; ##p < 0.01 vs. IL-1β group.

3.6. Overexpression of miR-27a inhibited IL-1β induced NF-κB signaling pathway in chondrocytes

Since TLR4 is one of the upstream molecules of NF-κB signaling pathway, which is directly related to the inflammatory response in OA [31,32], further experiments were designed to investigate the effects of miR-27a on the activation of NF-κB pathway in IL-1β-treated chondrocytes. The results of Western blot showed that the level of phosphorylated IκB-α (p-IκB-α) and nuclear p-p65 was obviously increased, while IκB-α levels were significantly reduced after IL-1β stimulation (Fig. 6A, B). However, miR-27a upregulation attenuated the effects of IL-1β on the expression of IκBα, p-IκB-α and nuclear p-p65 in chondrocytes. In addition, we found that the NF-κB activity was markedly increased after IL-1β stimulation compared with control group, whereas this promoting effect was attenuated by miR-27a overexpression (Fig. 6C). All data indicate that miR-27a may suppress IL-1β induced inflammatory response by regulation of NF-κB signaling pathway in chondrocytes.

3.7. Upregulation of miR-27a suppressed IL-1β-induced inflammatory response through TLR4/NF-κB signaling pathway in chondrocytes

To determine whether miR-27a exerts the anti-inflammatory mechanism by targeting TLR4, chondrocytes were transfected with pcDNA-TLR4 or pcDNA-vector. As expected, cells treated with pcDNA-TLR4 showed a significant increase in TLR4 protein expression (Fig. 7A). Subsequently, chondrocytes were co-transfected with miR-27a mimics along with pcDNA-TLR4. As shown in Fig. 7B, C, increased nuclear p-p65 expression and NF-κB activity in response to IL-1β stimulation were significantly reduced by miR-27a overexpression, while TLR4 overexpression attenuated the inhibitory effects of miR-27a overexpression in chondrocytes. It was also observed that miR-27a overexpression alleviated the reduction of cell viability in IL-1β induced chondrocytes, whereas TLR4 overexpression partly abrogated the promoting effect of miR-27a overexpression on cell viability. Subsequently, it was demonstrated that miR-27a overexpression suppressed cell apoptosis and its inhibitory effect were reversed by TLR4 overexpression in IL-1β induced chondrocytes (Fig. 7D, E). In addition, TLR4 overexpression also reversed the inhibitory effects of miR-27a mimics on the levels of IL-6, IL-8 and TNF-α in IL-1β induced chondrocytes (Fig. 7F-H). These data indicate that miR-27a suppressed IL-1β-induced inflammatory response by targeting TLR4.

To investigate whether NF-κB signaling pathway was involved in the suppressive effect of miR-27a on IL-1β-induced inflammatory response, the activity of the NF-κB signaling pathway was blocked by a NF-κB inhibitor, PDTC. As shown in Fig. 7B, C, PDTC also suppressed IL-1β-induced the activation of NF-κB signaling pathway in chondrocytes. Besides, the cell viability, cell apoptosis, and proinflammatory cytokines (IL-6, IL-8 and TNF-α) caused by IL-1β was attenuated by PDTC (Fig. 7D-H), which is similar with the role of miR-27a overexpression. All data indicate that miR-27a suppressed IL-1β-induced inflammatory response through blocking the activation of NF-κB signaling pathway.

3.8. Upregulation of miR-27a suppressed IL-1β-induced the cartilage degradation through TLR4/NF-κB signaling pathway in chondrocytes

Next, to determine whether miR-27a suppressed the cartilage degradation by targeting TLR4, the protein expression levels of aggrecan and type II collagen, as well as the concentrations of MMPs were analyzed again. The results showed that miR-27a overexpression increased aggrecan and type II collagen expression and decreased MMP3, MMP9 and MMP13 concentrations in IL-1β induced chondrocytes, while TLR4 overexpression attenuated the effects of miR-27a overexpression (Fig. 8A-E). Besides, reduction of ROS, increased SOD, MDA expressions and decreased GPx activity in response to miR-27a upregulation were reversed by TLR4 overexpression in IL-1β induced chondrocytes (Fig. 8F-I). These data indicate that miR-27a suppressed IL-1β-induced cartilage degradation by targeting TLR4.

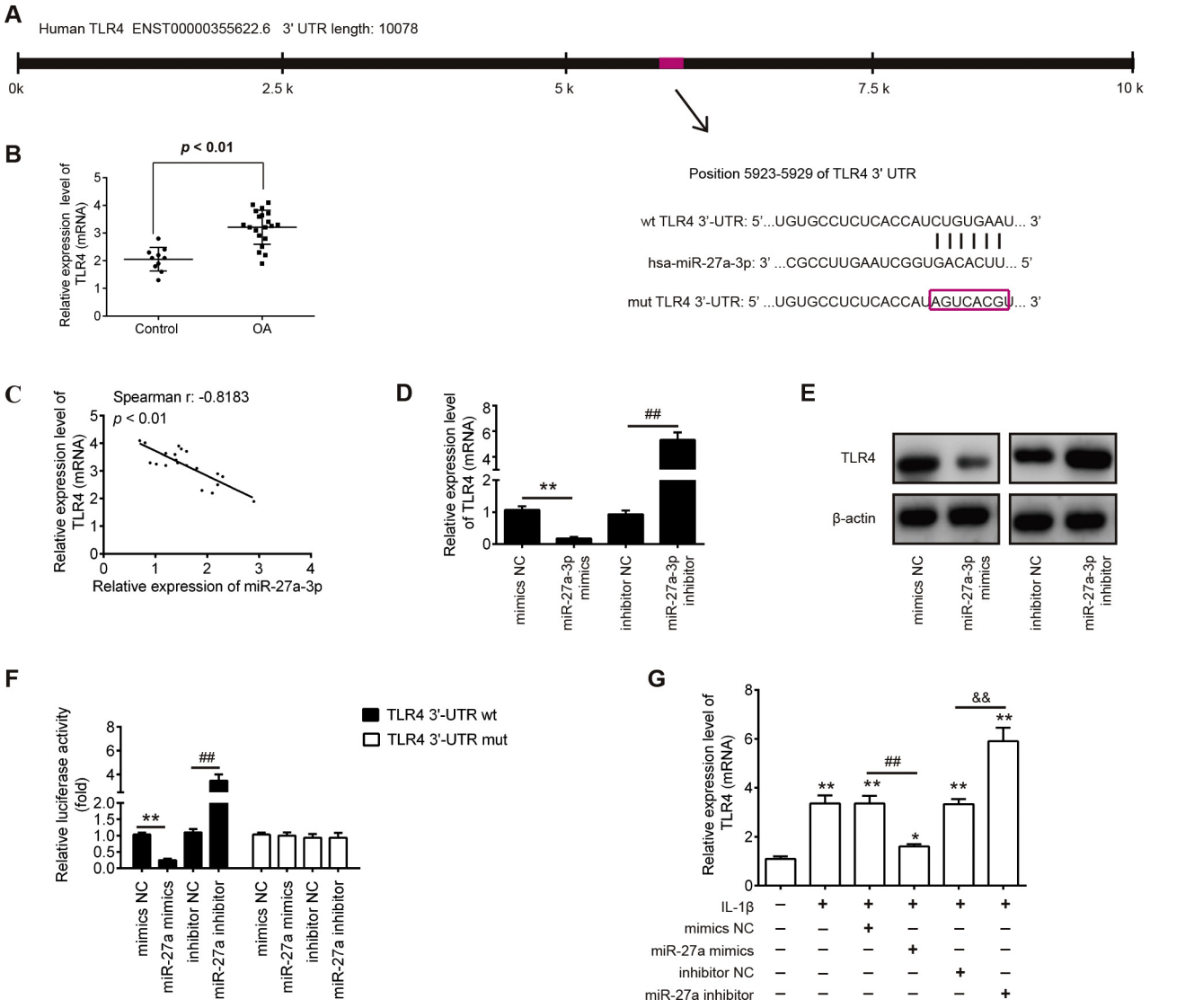

Fig. 5. TLR4 was a direct target of miR-27a. (A) The putative binding site of miR-27a and TLR4 is shown. (B) qRT-PCR analysis was used to assess the expression levels of TLR4 in OA cartilage tissues from 20 patients with OA and normal cartilage samples from 10 donors. $p < 0.01$ vs. Control group. (C) Spearman's correlation was used to assess the correlation of miR-27a and TLR4 expression in OA cartilage tissues (r:-0.8133; $p < 0.01$). (D) Chondrocytes were transfected with miR-27a mimics, miR-27a inhibitor or negative control for 24 h, the cells and cell supernatants were collected for further analysis. The protein level of TLR4 was analyzed by western blotting. (E) Luciferase assay of chondrocytes co-transfected with firefly luciferase constructs containing the TLR4 wild-type or mutated 3′-UTRs and miR-27a mimics, mimics NC, miR-27a inhibitor or inhibitor NC, as indicated (n = 3). (F) Chondrocytes were transfected with miR-27a mimics, miR-27a inhibitor or negative control for 24 h and then exposed to IL-1β for 24 h, the expression of TLR4 was measured by qRT-PCR. Data represent the mean ± SD of three independent experiments. *$p < 0.05$, **$p < 0.01$ vs non-treated group; ## $p < 0.01$ vs IL-1β + mimics NC; && $p < 0.01$ vs IL-1β + inhibitor NC.

To investigate whether NF-κB signaling pathway was involved in the suppressive effect of miR-27a on IL-1β-induced cartilage degradation, the activity of the NF-κB signaling pathway was blocked by PDTC. As shown in Fig. 8A-B, the decreased aggrecan and type II collagen expression induced by IL-1β were reversed by PDTC. The increased MMP3, MMP9 and MMP13 concentrations by IL-1β was attenuated by PDTC (Fig. 8C-E). Besides, the production of ROS caused by IL-1β was also attenuated by PDTC (Fig. 8F). Finally, increased MDA levels, as well as decreased activities of MDA and GPx induced by IL-1β were attenuated by PDTC (Fig. 8G-I). All results are similar with the role of miR-27a overexpression in IL-1β-induced cartilage degradation. Collectively, these data indicate that miR-27a suppressed IL-1β-induced cartilage degradation through blocking the activation of NF-κB signaling pathway.

4. Discussion

In the present study, the miR-27a expressions were significantly downregulated in the OA articular cartilage tissues from OA patients and in IL-1β-stimulated chondrocytes. Moreover, overexpression of miR-27a attenuate IL-1β induced cartilage degradation and inflammatory response through TLR4/NF-κB pathway in chondrocytes. Our data collectively suggest that miR-27a may represent a promising therapeutic target for OA.

It has been widely accepted that inflammation exerts important role in OA by releasing various inflammatory cytokines and mediators [33]. Among these cytokines, IL-1β has been implicated in the development of OA. Upon stimulation with IL-1β, chondrocytes, the only cells in articular cartilage, will decrease the capability to synthesize type II collagen and aggrecan, and to evoke the production of catabolic enzyme MMPs, resulting in cartilage degradation [34,35]. A number studies have reported that inhibition of inflammatory response may be

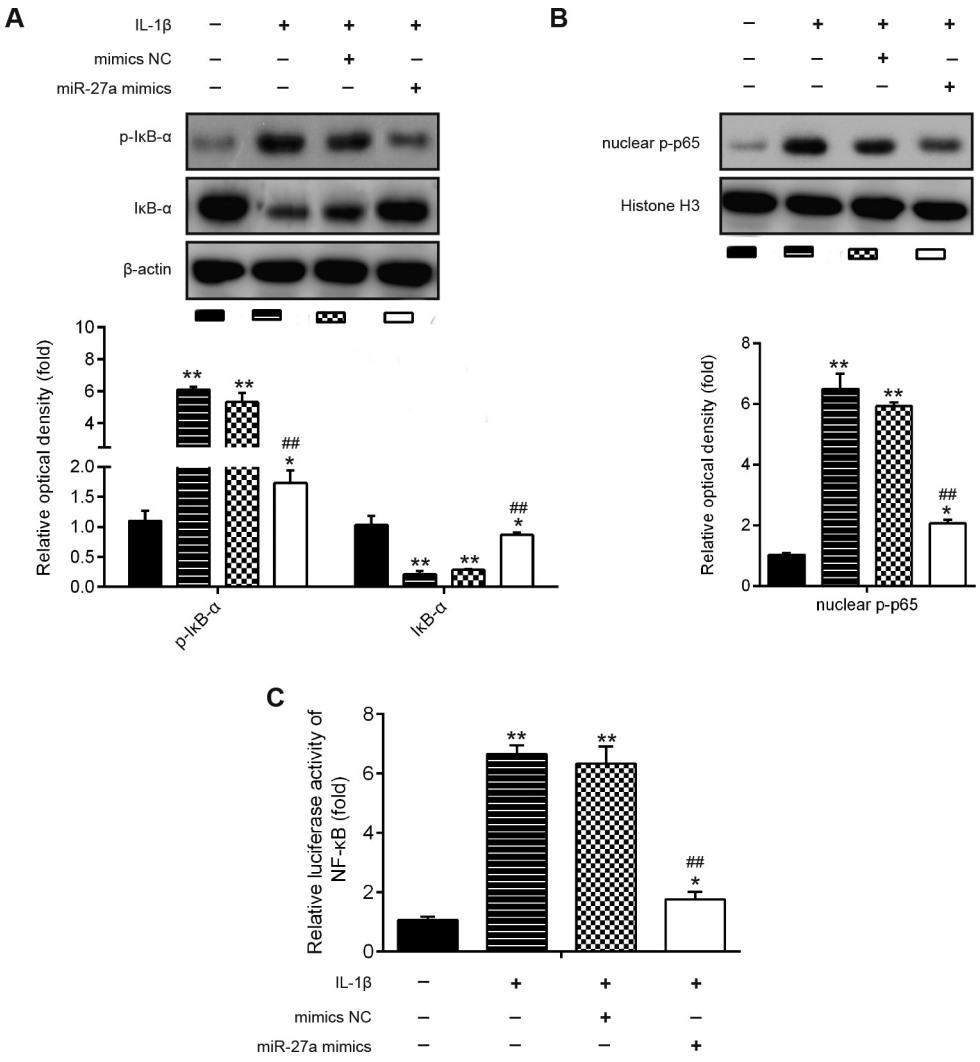

Fig. 6. Overexpression of miR-27a inhibited IL-1β induced NF-κB signaling pathway in chondrocytes. Chondrocytes were transfected with 25 nM miR-27a mimics for 24 h, and then protein was extracted at 24 h after IL-1β stimulation. (A, B) Levels of IκBα, phosphorylated IκB-α (p-IκB-α) and nuclear p-p65 were assessed by performing western blotting. The bands were semi-quantitatively analyzed by using Image J software, normalized to β-actin or Histone H3 density. (C) Relative luciferase activity of NF-κB was quantified using Promega Luciferase Assay kit on a luminometer. Data were represented as the mean ± SD of three independent experiments. *p < 0.05, **p < 0.01 vs. Control group; ##p < 0.01 vs. IL-1β group.

a new beneficial method for treating OA [36–38]. For example, Zheng et al. found that methylene blue act as an inflammatory inhibitor by targeting CILinc02 to regulate the inflammatory response, thereby suppressing the degradation of chondrocyte in OA [39]. Zhao et al. confirmed that downregulation of lncRNA PVT1 ameliorated the progression of OA by alleviating cartilage imbalance toward catabolism and inflammatory response [40]. All results supported a promising therapeutic strategy for OA by attenuating the inflammation.

Increasing evidence has demonstrated that miRNAs are aberrantly expressed in the patients with OA and are involved in several pathophysiological processes, including inflammation and cartilage degradation [13,41]. For example, Dai et al. found that miR-101 is a key modulator of chondrocytes injury in an OA rat model, and inhibition of miR-101 can prevent the IL-1β-induced cartilage degradation in chondrocytes [42]. Yan et al. showed that lentiviral inhibition of miR-34a effectively ameliorated the progression of OA through direct regulation of the SIRT1/p53 signaling pathway [43]. Zheng et al. found that miR-221-3p was downregulated in human cartilage tissues and miR-221-3p upregulation prevented IL-1β-induced cartilage degradation in chondrocytes [44]. Therefore, in the present study, microarray analysis was conducted on clinical samples, and a number of differentially expressed miRNAs were screened, suggesting the potential diagnostic and therapeutic values of miRNAs. More microRNAs that are relevant for the pathogenesis of OA will be studied in the future. In particular, miR-27a was one of the miRNAs exhibiting the most marked down regulation.

It has been generally recognized that miRNAs play different roles in different cells. For example, miR-42-3p promoted the inflammatory response in monocyte-derived DCs (moDCs) [45]. In the contrast, miR-142-3p inhibited inflammation induced by bleomycin through down-regulation of Cox-2 in MLE-12 cells [46]. Another study showed that the overexpression of miR-142-3p inhibited lipopolysaccharide (LSP)-induced inflammation in chondrocytes [47]. For the role of miR-27a in inflammation, miR-27a has been reported to enhance lipopolysaccharide (LPS)-induced inflammation in rat pancreatic acinar cell AR42J [48]. However, miR-27a also found to inhibit inflammatory response of pancreatic acinar cells in acute pancreatitis by targeting PTEN [49]. Although the suppressive role of miR-27a in macrophages has been reported [26], the functions of miR-27a in chondrocytes remain unknown. In the present study, we first demonstrated that miR-27a overexpression suppressed the IL-1β induced inflammatory response and the degeneration of articular cartilage through blocking the activation of TLR4/NF-κB signaling pathway, suggesting the therapeutic values of miR-27a. Intriguingly, in the present study, using an IL-1β-induced chondrocytes injury model, we found that overexpression of miR-27a exerted a protective effect against IL-1β-induced injury, as evidenced by promoting the synthesis of aggrecan, type II collagen, and reducing MMPs (MMP-3, -9 and -13) contents and pro-inflammatory cytokines (IL-6, IL-8 and TNF-α). All results suggest that miR-27a upregulation improved IL-1β-induced chondrocytes injury through suppressing cartilage degradation and inflammatory response.

Previous studies have demonstrated that the essential change in OA is loss of the articular cartilage matrix, which is induced by various

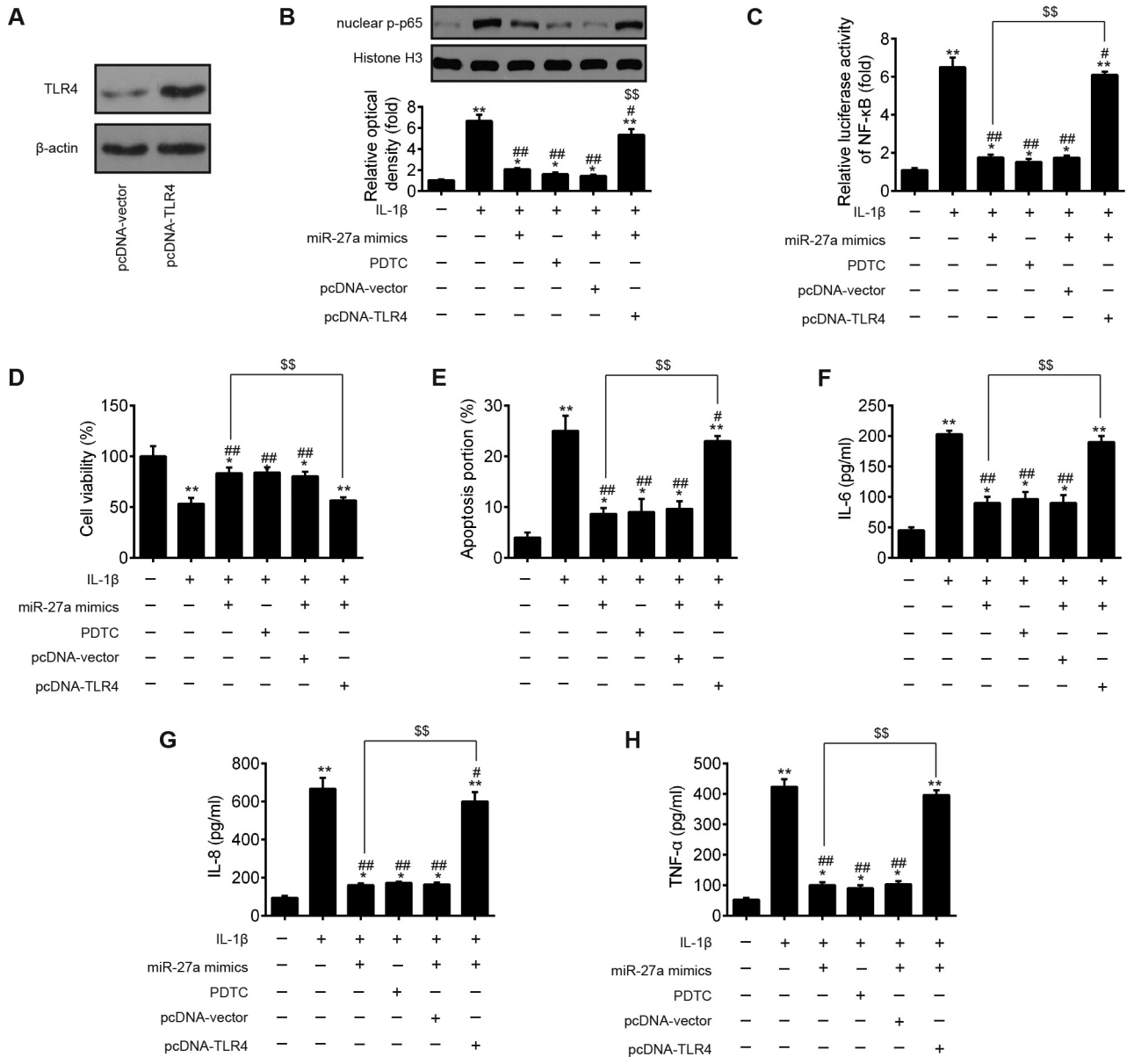

Fig. 7. miR-27a suppressed IL-1β-induced the inflammatory response through TLR4/NF-κB signaling pathway in chondrocytes. (A) Cells were transfected with pcDNA-TLR4 or pcDNA empty vector for 24 h. The protein level of TLR4 was analyzed by western blotting. (B) After co-transfection with pcDNA-TLR4 and miR-27a mimics, cells were exposed to IL-1β. The protein expression of nuclear p-p65 was assessed by performing western blotting. The bands were semi-quantitatively analyzed by using Image J software, normalized to Histone H3 density. (C) Relative luciferase activity of NF-κB was quantified using Promega Luciferase Assay kit on a luminometer. (D) Cell viability was detected by MTT assay. (E) Cell apoptosis was measured by Flow cytometry assay. (F–H) The levels of IL-6, IL-8 and TNF-α were measured by ELISA kits. Data were represented as the mean ± SD of three independent experiments. *p < 0.05, **p < 0.01 vs. Control group; #p < 0.05, ##p < 0.01 vs. IL-1β group; $$p < 0.01 vs. IL-1β + miR-27a mimics group.

factors, especially oxidative stress [50]. It has been generally accepted that overexpression of ROS plays a key role in the degeneration of articular cartilage function [29]. Low concentrations of ROS, such as H_2O_2 can induce the ECM synthesis inhibition, chondrocyte apoptosis, excessive inflammatory response, which further lead to cartilage damage [51,52]. In this study, we firstly evaluated the antioxidant effect of miR-27a against IL-1β-induced chondrocytes reactions and confirmed that miR-27a can suppress the production of ROS.

To elucidate the potential mechanisms by which miR-27a protect chondrocytes against IL-1β-induced injury, bioinformatics analysis was performed to predicate the putative targets of miR-27a, and TLR4 was identified as the potential target of miR-27a. TLR4, an important regulator of the NF-κB signaling pathway, has been shown to regulate the proliferation, apoptosis and inflammation of chondrocytes [16]. Notably, a previous study showed that miR-27a regulated the LPS-induced production of inflammatory cytokines in microglia by targeting TLR4 [53]. Li et al. found that miR-27a upregulation attenuated ischemia reperfusion (IR)-induced inflammatory damage to spinal cord by negatively regulating the TLR4 signaling pathway in rats [54]. However, whether TLR4 is involved in the protection of miR-27a against IL-1β-induced chondrocytes injury remained to be elucidated. In our study, TLR4 was identified as a target of miR-27a in the chondrocytes and negatively regulated by miR-27a. Therefore, we hypothesized that miR-27a protects chondrocytes from IL-1β-induced inflammation and cartilage degradation through targeting TLR4 signaling. As expected, the protective effects of miR-27a overexpression on IL-1β-induced chondrocytes injury were abrogated by the overexpression of TLR4, suggesting that miR-27a protected against IL-1β-

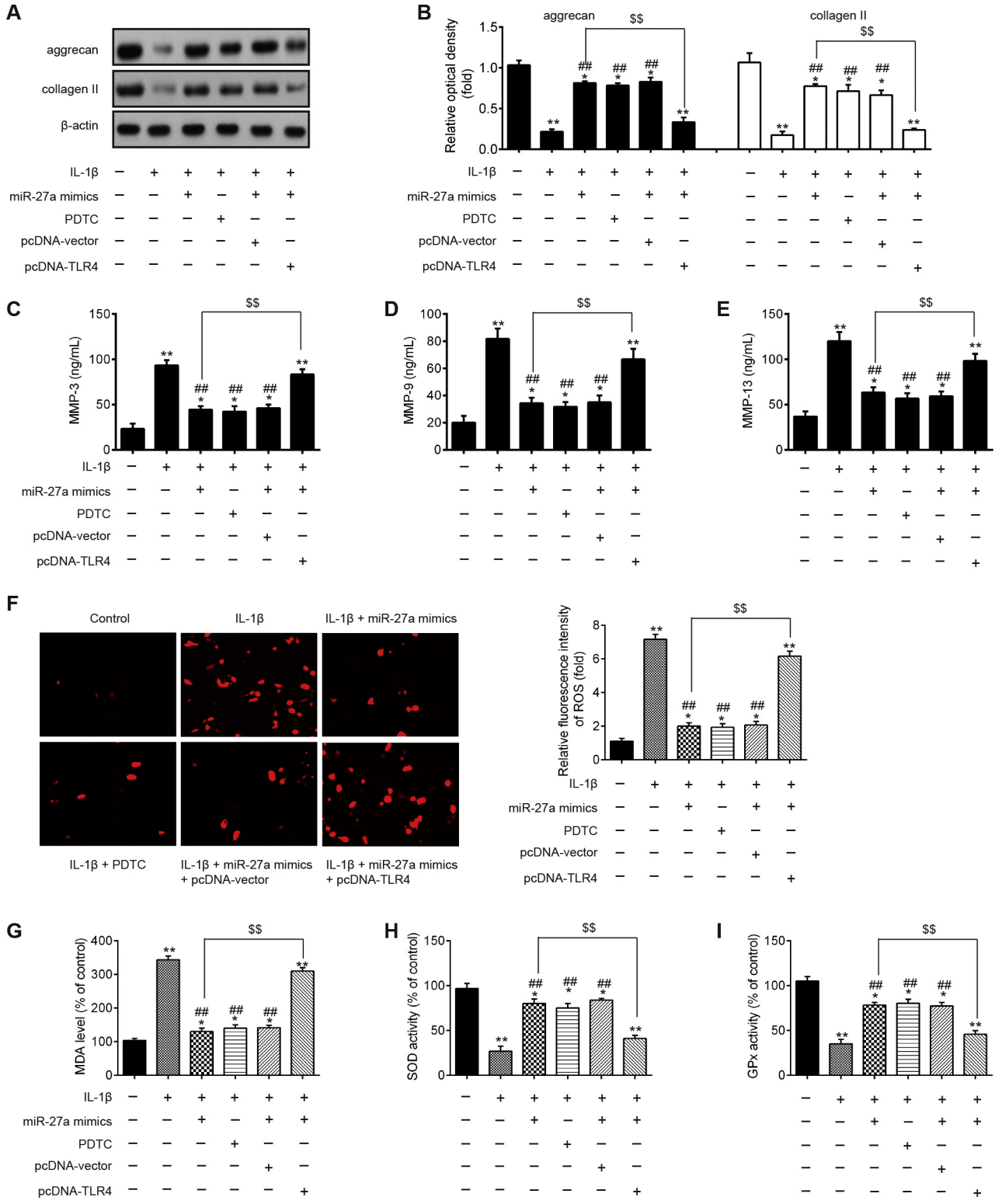

Fig. 8. Upregulation of miR-27a suppressed IL-1β-induced the cartilage degradation through TLR4/NF-κB signaling pathway in chondrocytes. After co-transfection with pcDNA-TLR4 and miR-27a mimics, cells were exposed to IL-1β for 24 h, the cells and cell supernatants were collected for further analysis. (A, B) The protein levels of aggrecan and collagen II were analyzed by western blotting. The bands were semi-quantitatively analyzed by using Image J software, normalized to β-actin density. (C–E) The contents of MMP-3, MMP-9 and MMP-13 in supernatants were evaluated by ELISA assay. (F) The ROS production was measured by a commercial kit of ROS. (G–I) The levels of SOD, MDA and GPx in chondrocytes were detected by ELISA kits. Data were represented as the mean ± SD of three independent experiments. *p < 0.05, **p < 0.01 vs. Control group; #p < 0.05, ##p < 0.01 vs. IL-1β group; $$p < 0.01 vs. IL-1β + miR-27a mimics group.

induced inflammation and cartilage degradation via suppressing the expression of TLR4. However, the possible molecular mechanism requires further investigation.

It is well known that the stimulation of TLR4 can further activate the NF-κB pathway [55]. Once activation, the NF-κB pathway triggers the expression of a variety of inflammation-related mediators inducing degradation of type II collagen and aggrecan, resulting in OA occurrence and progression [56]. Ran et al. found that inhibition of the NF-κB pathway has been demonstrated to mediate the anti-inflammatory activity and therapeutic effects of Schisandrin B in a rat OA model [57]. Wu et al. found that overexpression of sirtuin 6 improved the OA through decreasing NF-κB pathway mediated the inflammatory response of chondrocytes [58]. In addition, Wang et al. showed that thymoquinone, an active ingredient isolated from *Nigella sativa*, repressed the IL-1β-induced inflammation in chondrocytes of OA by suppressing NF-κB signaling pathways [59]. In order to elucidate the mechanisms underlying involved in the miR-27a mediated protection against IL-1β-induced chondrocytes injury, the effects of aberrantly expressed miR-27a on key kinases in the NF-κB signaling pathway were examined. The results showed that the NF-κB signaling pathway was activated in IL-1β-treated chondrocytes, and miR-27a overexpression blocked the activity of this signaling pathway. In addition, it was also found that the suppression of NF-κB pathway by PDTC inhibited IL-1β-induced inflammation in chondrocytes. All these data suggest that the protective effect of miR-27a on IL-1β-induced chondrocytes injury may be mediated by NF-κB signaling pathway.

However, there are some limitations in the present study. For example, only miR-27a was explored, whereas other miRNAs may also be relevant for the pathogenesis of OA. Additionally, this study was mainly done on cell model and the results showed that miR-27a upregulation inhibited IL-1β-induced chondrocytes injury via blocking the activation of the TLR4/NF-κB signaling pathway. Therefore, this study requires further experiments in animals to investigate the role of miR-27a in the improvement of OA. In the future, further systematic and in-depth studies investigating the pathogenesis of OA will be conducted.

5. Conclusion

In conclusion, the present study revealed that miR-27a upregulation inhibited IL-1β-induced chondrocytes injury via blocking the activation of the TLR4/NF-κB signaling pathway in vitro. Our findings indicate a therapeutic potential of miR-27a in OA treatment, however this requires further experiments in animals prior to clinical application.

Author contributions

Planned experiments: Yun-Han Ji; Performed experiments: Wen-Jun Qiu, Ming-Ze Xu and Xiao-dong Zhu; Analyzed data: Wen-Jun Qiu, Ming-Ze Xu and Xiao-dong Zhu; Contributed reagents or other essential material: Yun-Han Ji; Wrote the paper: Yun-Han Ji; Manuscript review: Yun-Han Ji.

Acknowledgments

None.

Declaration of competing interest

We have no conflict of interest to declare.

References

[1] M. Cross, E. Smith, D. Hoy, S. Nolte, I. Ackerman, M. Fransen, L. Bridgett, S. Williams, F. Guillemin, C.L. Hill, L.L. Laslett, G. Jones, F. Cicuttini, R. Osborne, T. Vos, R. Buchbinder, A. Woolf, L. March, The global burden of hip and knee osteoarthritis: estimates from the global burden of disease 2010 study, Ann. Rheum. Dis. 73 (7) (2014) 1323–1330.
[2] X. Li, J.S. Kim, A.J. van Wijnen, H.J. Im, Osteoarthritic tissues modulate functional properties of sensory neurons associated with symptomatic OA pain, Mol. Biol. Rep. 38 (8) (2011) 5335–5339.
[3] R.R. Bannuru, C.H. Schmid, D.M. Kent, E.E. Vaysbrot, J.B. Wong, T.E. McAlindon, Comparative effectiveness of pharmacologic interventions for knee osteoarthritis: a systematic review and network meta-analysis, Ann. Intern. Med. 162 (1) (2015) 46–54.
[4] H. Cho, A. Walker, J. Williams, K.A. Hasty, Study of osteoarthritis treatment with anti-inflammatory drugs: cyclooxygenase-2 inhibitor and steroids, Biomed. Res. Int. 2015 (2015) 595273.
[5] P.M. van der Kraan, Osteoarthritis year 2012 in review: biology, Osteoarthr. Cartil. 20 (12) (2012) 1447–1450.
[6] M. Feldmann, Pathogenesis of arthritis: recent research progress, Nat. Immunol. 2 (9) (2001) 771–773.
[7] Q.F. Wang, Y.F. Ma, Y.S. Yin, A new understanding of the diagnosis and treatment of knee osteoarthritis, Zhongguo gu shang = China journal of orthopaedics and traumatology 29 (9) (2016) 779–781.
[8] R. Ramonda, M. Lorenzin, V. Modesti, C. Campana, A. Ortolan, P. Frallonardo, L. Punzi, Serological markers of erosive hand osteoarthritis, European Journal of Internal Medicine 24 (1) (2013) 11–15.
[9] Q. Liu, X. Zhang, L. Dai, X. Hu, J. Zhu, L. Li, C. Zhou, Y. Ao, Long noncoding RNA related to cartilage injury promotes chondrocyte extracellular matrix degradation in osteoarthritis, Arthritis & Rheumatology 66 (4) (2014) 969–978.
[10] L. Cong, Y. Zhu, G. Tu, A bioinformatic analysis of microRNAs role in osteoarthritis, Osteoarthr. Cartil. 25 (8) (2017) 1362–1371.
[11] V. Ambros, The functions of animal microRNAs, Nature 431 (7006) (2004) 350–355.
[12] S. Diaz-Prado, C. Cicione, E. Muinos-Lopez, T. Hermida-Gomez, N. Oreiro, C. Fernandez-Lopez, F.J. Blanco, Characterization of microRNA expression profiles in normal and osteoarthritic human chondrocytes, BMC Musculoskelet. Disord. 13 (2012) 144.
[13] H. Xue, Y. Tu, T. Ma, T. Wen, T. Yang, L. Xue, M. Cai, F. Wang, M. Guan, miR-93-5p attenuates IL-1beta-induced chondrocyte apoptosis and cartilage degradation in osteoarthritis partially by targeting TCF4, Bone 123 (2019) 129–136.
[14] D. Baek, K.M. Lee, K.W. Park, J.W. Suh, S.M. Choi, K.H. Park, J.W. Lee, S.H. Kim, Inhibition of miR-449a promotes cartilage regeneration and prevents progression of osteoarthritis in in vivo rat models, Molecular therapy. Nucleic acids 13 (2018) 322–333.
[15] J. Cao, X. Han, X. Qi, X. Jin, X. Li, miR2045p inhibits the occurrence and development of osteoarthritis by targeting Runx2, Int. J. Mol. Med. 42 (5) (2018) 2560–2568.
[16] Y. Ding, L. Wang, Q. Zhao, Z. Wu, L. Kong, MicroRNA93 inhibits chondrocyte apoptosis and inflammation in osteoarthritis by targeting the TLR4/NFkappaB signaling pathway, Int. J. Mol. Med. 43 (2) (2018) 779–790.
[17] Z. Zhao, X.S. Dai, Z.Y. Wang, Z.Q. Bao, J.Z. Guan, MicroRNA-26a reduces synovial inflammation and cartilage injury in osteoarthritis of knee joints through impairing the NF-kappaB signaling pathway, Biosci. Rep. 39 (4) (2019).
[18] S. Miyaki, H. Asahara, Macro view of microRNA function in osteoarthritis, Nat. Rev. Rheumatol. 8 (9) (2012) 543–552.
[19] L.A. Vonk, A.H. Kragten, W.J. Dhert, D.B. Saris, L.B. Creemers, Overexpression of hsa-miR-148a promotes cartilage production and inhibits cartilage degradation by osteoarthritic chondrocytes, Osteoarthr. Cartil. 22 (1) (2014) 145–153.
[20] K.J. Livak, T.D. Schmittgen, Analysis of relative gene expression data using real-time quantitative PCR and the 2(−Delta Delta C(T)) method, Methods 25 (4) (2001) 402–408.
[21] G. Tardif, D. Hum, J.P. Pelletier, N. Duval, J. Martel-Pelletier, Regulation of the IGFBP-5 and MMP-13 genes by the microRNAs miR-140 and miR-27a in human osteoarthritic chondrocytes, BMC Musculoskelet. Disord. 10 (2009) 148.
[22] R.C. Billinghurst, L. Dahlberg, M. Ionescu, A. Reiner, R. Bourne, C. Rorabeck, P. Mitchell, J. Hambor, O. Diekmann, H. Tschesche, J. Chen, H. Van Wart, A.R. Poole, Enhanced cleavage of type II collagen by collagenases in osteoarthritic articular cartilage, J. Clin. Invest. 99 (7) (1997) 1534–1545.
[23] A.J. Fosang, K. Last, V. Knauper, G. Murphy, P.J. Neame, Degradation of cartilage aggrecan by collagenase-3 (MMP-13), FEBS Lett. 380 (1–2) (1996) 17–20.
[24] L. Sun, M. Zhao, Y. Wang, A. Liu, M. Lv, Y. Li, X. Yang, Z. Wu, Neuroprotective effects of miR-27a against traumatic brain injury via suppressing FoxO3a-mediated neuronal autophagy, Biochem. Biophys. Res. Commun. 482 (4) (2017) 1141–1147.
[25] Q. Cai, T. Wang, W.J. Yang, X. Fen, Protective mechanisms of microRNA-27a against oxygen-glucose deprivation-induced injuries in hippocampal neurons, Neural Regen. Res. 11 (8) (2016) 1285–1292.
[26] M. Ju, B. Liu, H. He, Z. Gu, Y. Liu, Y. Su, D. Zhu, J. Cang, Z. Luo, MicroRNA-27a alleviates LPS-induced acute lung injury in mice via inhibiting in fl ammation and apoptosis through modulating TLR4/MyD88/NF-kappaB pathway, Cell Cycle 17 (16) (2018) 2001–2018.
[27] S. Cheleschi, A. De Palma, A. Pecorelli, N.A. Pascarelli, G. Valacchi, G. Belmonte, S. Carta, M. Galeazzi, A. Fioravanti, Hydrostatic pressure regulates MicroRNA expression levels in osteoarthritic chondrocyte cultures via the Wnt/beta-catenin pathway, Int. J. Mol. Sci. 18 (1) (2017).
[28] D.J. Blasioli, D.L. Kaplan, The roles of catabolic factors in the development of osteoarthritis, Tissue Eng. B Rev. 20 (4) (2014) 355–363.
[29] K. Yudoh, T. Nguyen v, H. Nakamura, K. Hongo-Masuko, T. Kato, K. Nishioka, Potential involvement of oxidative stress in cartilage senescence and development of osteoarthritis: oxidative stress induces chondrocyte telomere instability and downregulation of chondrocyte function, Arthritis Research & Therapy 7 (2) (2005) R380–R391.

[30] N.M. Khan, A. Haseeb, M.Y. Ansari, T.M. Haqqi, A wogonin-rich-fraction of Scutellaria baicalensis root extract exerts chondroprotective effects by suppressing IL-1beta-induced activation of AP-1 in human OA chondrocytes, Sci. Rep. 7 (2017) 43789.
[31] D.W. Yang, G.B. Qian, M.J. Jiang, P. Wang, K.Z. Wang, Inhibition of microRNA-495 suppresses chondrocyte apoptosis through activation of the NF-kappaB signaling pathway by regulating CCL4 in osteoarthritis, Gene Ther. 26 (6) (2019) 217–229.
[32] H. Liang, H. Wang, L. Luo, S. Fan, L. Zhou, Z. Liu, S. Yao, X. Zhang, K. Zhong, H. Zhao, Z. Zha, Toll-like receptor 4 promotes high glucose-induced catabolic and inflammatory responses in chondrocytes in an NF-kappaB-dependent manner, Life Sci. 228 (2019) 258–265.
[33] W. Zheng, H. Zhang, Y. Jin, Q. Wang, L. Chen, Z. Feng, H. Chen, Y. Wu, Butein inhibits IL-1beta-induced inflammatory response in human osteoarthritis chondrocytes and slows the progression of osteoarthritis in mice, Int. Immunopharmacol. 42 (2017) 1–10.
[34] J. Lorenz, N. Schafer, R. Bauer, Z. Jenei-Lanzl, R.H. Springorum, S. Grassel, Norepinephrine modulates osteoarthritic chondrocyte metabolism and inflammatory responses, Osteoarthr. Cartil. 24 (2) (2016) 325–334.
[35] Q. Tang, G. Zheng, Z. Feng, M. Tong, J. Xu, Z. Hu, P. Shang, Y. Chen, C. Wang, Y. Lou, D. Chen, D. Zhang, M. Nisar, X. Zhang, H. Xu, H. Liu, Wogonoside inhibits IL-1beta induced catabolism and hypertrophy in mouse chondrocyte and ameliorates murine osteoarthritis, Oncotarget 8 (37) (2017) 61440–61456.
[36] Y. Lou, C. Wang, W. Zheng, Q. Tang, Y. Chen, X. Zhang, X. Guo, J. Wang, Salvianolic acid B inhibits IL-1beta-induced inflammatory cytokine production in human osteoarthritis chondrocytes and has a protective effect in a mouse osteoarthritis model, Int. Immunopharmacol. 46 (2017) 31–37.
[37] Q. Tang, Z. Feng, M. Tong, J. Xu, G. Zheng, L. Shen, P. Shang, Y. Zhang, H. Liu, Piceatannol inhibits the IL-1beta-induced inflammatory response in human osteoarthritic chondrocytes and ameliorates osteoarthritis in mice by activating Nrf2, Food Funct. 8 (11) (2017) 3926–3937.
[38] W. Zheng, Z. Feng, S. You, H. Zhang, Z. Tao, Q. Wang, H. Chen, Y. Wu, Fisetin inhibits IL-1beta-induced inflammatory response in human osteoarthritis chondrocytes through activating SIRT1 and attenuates the progression of osteoarthritis in mice, Int. Immunopharmacol. 45 (2017) 135–147.
[39] J. Zheng, Q. Li, Methylene blue regulates inflammatory response in osteoarthritis by noncoding long chain RNA CILinc02, J. Cell. Biochem. 120 (3) (2019) 3331–3338.
[40] Y. Zhao, J. Zhao, X. Guo, J. She, Y. Liu, Long non-coding RNA PVT1, a molecular sponge for miR-149, contributes aberrant metabolic dysfunction and inflammation in IL-1beta-simulated osteoarthritic chondrocytes, Biosci. Rep. 38 (5) (2018).
[41] Y. Ding, L. Wang, Q. Zhao, Z. Wu, L. Kong, MicroRNA93 inhibits chondrocyte apoptosis and inflammation in osteoarthritis by targeting the TLR4/NFkappaB signaling pathway, Int. J. Mol. Med. 43 (2) (2019) 779–790.
[42] L. Dai, X. Zhang, X. Hu, C. Zhou, Y. Ao, Silencing of microRNA-101 prevents IL-1beta-induced extracellular matrix degradation in chondrocytes, Arthritis research & therapy 14 (6) (2012) R268.
[43] S. Yan, M. Wang, J. Zhao, H. Zhang, C. Zhou, L. Jin, Y. Zhang, X. Qiu, B. Ma, Q. Fan, MicroRNA-34a affects chondrocyte apoptosis and proliferation by targeting the SIRT1/p53 signaling pathway during the pathogenesis of osteoarthritis, Int. J. Mol. Med. 38 (1) (2016) 201–209.
[44] X. Zheng, F.C. Zhao, Y. Pang, D.Y. Li, S.C. Yao, S.S. Sun, K.J. Guo, Downregulation of miR-221-3p contributes to IL-1beta-induced cartilage degradation by directly targeting the SDF1/CXCR4 signaling pathway, J. Mol. Med. 95 (6) (2017) 615–627.
[45] Y. Wang, J. Liang, H. Qin, Y. Ge, J. Du, J. Lin, X. Zhu, J. Wang, J. Xu, Elevated expression of miR-142-3p is related to the pro-inflammatory function of monocyte-derived dendritic cells in SLE, Arthritis research & therapy 18 (1) (2016) 263.
[46] F. Guo, S.C. Lin, M.S. Zhao, B. Yu, X.Y. Li, Q. Gao, D.J. Lin, microRNA-142-3p inhibits apoptosis and inflammation induced by bleomycin through down-regulation of Cox-2 in MLE-12 cells, Brazilian journal of medical and biological research = Revista brasileira de pesquisas medicas e biologicas 50 (7) (2017) e5974.
[47] X. Wang, Y. Guo, C. Wang, H. Yu, X. Yu, H. Yu, MicroRNA-142-3p inhibits chondrocyte apoptosis and inflammation in osteoarthritis by targeting HMGB1, Inflammation 39 (5) (2016) 1718–1728.
[48] X. Zhang, T. Gao, Y. Wang, Geniposide alleviates lipopolysaccharide (LPS)-induced inflammation by downregulation of miR-27a in rat pancreatic acinar cell AR42J, Biol. Chem. 400 (8) (2019) 1–21.
[49] L. Kong, Q. Wu, L. Zhao, J. Ye, N. Li, H. Yang, Effect of microRNA-27a-5p on apoptosis and inflammatory response of pancreatic acinar cells in acute pancreatitis by targeting PTEN, J. Cell. Biochem. 120 (9) (2019) 15844–15850.
[50] J.W. Michael, K.U. Schluter-Brust, P. Eysel, The epidemiology, etiology, diagnosis, and treatment of osteoarthritis of the knee, Deutsches Arzteblatt International 107 (9) (2010) 152–162.
[51] P. Lepetsos, A.G. Papavassiliou, ROS/oxidative stress signaling in osteoarthritis, Biochim. Biophys. Acta 1862 (4) (2016) 576–591.
[52] M. Kapoor, J. Martel-Pelletier, D. Lajeunesse, J.P. Pelletier, H. Fahmi, Role of proinflammatory cytokines in the pathophysiology of osteoarthritis, Nat. Rev. Rheumatol. 7 (1) (2011) 33–42.
[53] Y.N. Lv, A.J. Ou-Yang, L.S. Fu, MicroRNA-27a negatively modulates the inflammatory response in lipopolysaccharide-stimulated microglia by targeting TLR4 and IRAK4, Cell. Mol. Neurobiol. 37 (2) (2017) 195–210.
[54] X.Q. Li, H.W. Lv, Z.L. Wang, W.F. Tan, B. Fang, H. Ma, MiR-27a ameliorates inflammatory damage to the blood-spinal cord barrier after spinal cord ischemia: reperfusion injury in rats by downregulating TICAM-2 of the TLR4 signaling pathway, J. Neuroinflammation 12 (2015) 25.
[55] D. Yin, S. Zhou, X. Xu, W. Gao, F. Li, Y. Ma, D. Sun, Y. Wu, Q. Guo, H. Liu, L. Han, Z. Wang, Y. Wang, J. Zhang, Dexmedetomidine attenuated early brain injury in rats with subarachnoid haemorrhage by suppressing the inflammatory response: the TLR4/NF-kappaB pathway and the NLRP3 inflammasome may be involved in the mechanism, Brain Res. 1698 (2018) 1–10.
[56] S. Rigoglou, A.G. Papavassiliou, The NF-kappaB signalling pathway in osteoarthritis, Int. J. Biochem. Cell Biol. 45 (11) (2013) 2580–2584.
[57] J. Ran, C. Ma, K. Xu, L. Xu, Y. He, S.A.A. Moqbel, P. Hu, L. Jiang, W. Chen, J. Bao, Y. Xiong, L. Wu, Schisandrin B ameliorated chondrocytes inflammation and osteoarthritis via suppression of NF-kappaB and MAPK signal pathways, Drug design, development and therapy 12 (2018) 1195–1204.
[58] Y. Wu, L. Chen, Y. Wang, W. Li, Y. Lin, D. Yu, L. Zhang, F. Li, Z. Pan, Overexpression of Sirtuin 6 suppresses cellular senescence and NF-kappaB mediated inflammatory responses in osteoarthritis development, Sci. Rep. 5 (2015) 17602.
[59] D. Wang, J. Qiao, X. Zhao, T. Chen, D. Guan, Thymoquinone inhibits IL-1beta-induced inflammation in human osteoarthritis chondrocytes by suppressing NF-kappaB and MAPKs signaling pathway, Inflammation 38 (6) (2015) 2235–2241.