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Research paper

MiR-502-5p inhibits IL-1β-induced chondrocyte injury by targeting TRAF2

Guoliang Zhang [a,b,1], Yanhong Sun [c,1], Yuewen Wang [b], Rui Liu [b], Yimin Bao [b], Qi Li [a,*]

[a] Orthopedical Center of Zhujiang Hospital, Southern Medical University, Guangzhou 510282, China
[b] Department of Orthopedics, Affiliated Hospital of Inner Mongolia Medical University, Hohhot 010050, China
[c] Department of Physiology, Inner Mongolia Medical University, Hohhot 010110, China

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ABSTRACT

Osteoarthritis (OA) is characterized by articular cartilage degradation and joint inflammation. MicroRNAs have been proven to play an important role in the regulation of chondrogenesis. The aim of the present study was to investigate the effect of miR-502-5p in OA. The results showed that miR-502-5p levels were significantly down-regulated in OA articular tissues and IL-1β-induced chondrocytes compared with control groups. MiR-502-5p overexpression inhibited IL-1β-induced reduction in cell viability and increase in cell apoptosis, and alleviated IL-1β-induced extracellular matrix (ECM) metabolic imbalance and pro-inflammatory cytokine production. MiR-502-5p targeted the 3′-untranslated region (UTR) of TRAF2 to inhibit its expression. The IL-1β-induced activation of NF-κB signaling pathway was inhibited by PDTC, an inhibitor of NF-κB, which was also suppressed by the miR-502-5p mimic and TRAF2 siRNA transfection. In conclusion, miR-502-5p may exhibit a protective effect on IL-1β-induced chondrocyte injury by targeting TRAF2 and inhibiting NF-κB signaling pathway.

1. Introduction

Osteoarthritis (OA) is a highly prevalent degenerative joint disease, grossly characterized by articular cartilage degradation and joint inflammation [1]. Chondrocytes are the only cells in articular cartilage, which play an important role in maintaining matrix integrity and tissue homeostasis [2]. As the OA progresses, the chondrocytes become metabolically active and disrupt the metabolic homeostasis of extracellular matrix (ECM), including reducing the synthesis of ECM proteins and enhancing the cartilage catabolic enzyme activity, resulting in the degradation of ECM [3]. Therefore, targeting the chondrocyte injury is of great significance in developing effective therapies to treat OA.

IL-1β is one of the main cytokines involved in OA pathogenesis [2]. It has been reported that IL-1β can induce cell proliferation inhibition, decrease aggrecan and collagen type II synthesis [4] and enhance matrix metalloproteinases-13 (MMP-13) expression in chondrocytes [5]. Moreover, IL-1β can also promote pro-inflammatory factor production [6]. It has been suggested that IL-1β up-regulates the expression of tumor necrosis factor alpha (TNFα) [7] and TNF receptor (TNFR) in chondrocytes [8]. The combined action of TNF and TNFR then activates TNF receptor-associated factor 2 (TRAF2) [2,9]. Several studies have indicated that TRAF2 is a critical mediator of the NF-κB pathway [10–12], and that TRAF2 overexpression can activate NF-κB in the absence of extracellular stimuli [13,14].

MicroRNAs (miRNAs) are endogenous non-coding RNAs containing 20–22-nucleotide [15–17], which bind to the target mRNAs and interfere in the translation process [18]. MiRNAs are evolutionarily conserved and have been found in various organisms [19]. By binding to the complementary sequences predominantly found in the 3′-UTR of the target mRNAs, miRNAs block the translation or decrease the stability of the mRNAs [20,21]. Base pairing between at least six consecutive nucleotides with the target site on the mRNA is reported to be a minimum requirement for the miRNA–mRNA interaction [22]. MiRNAs are involved in a variety of biological processes including cell proliferation, differentiation and apoptosis [23–25]. Moreover, it participates in the initiation and progression of cancer [26,27]. Several miRNAs exhibit tissue- or

Abbreviations: OA, osteoarthritis; miR-502-5p, microRNA-502-5p; IL-1β, interleukin -1 beta; TNFα, tumor necrosis factor alpha; TNFR, tumor necrosis factor receptor; TRAF2, TNF receptor-associated factor 2; ECM, extracellular matrix; MMP-13, matrix metalloproteinase-13; UTR, untranslated region; siRNA, small interfering RNA; PDTC, pyrimidine dithiocarbamic acid.

* Corresponding author at: Orthopedical Center of Zhujiang Hospital, No. 253 Mid., Gongye Avenue, Guangzhou 510282, China.
E-mail address: qilizj@163.com (Q. Li).

[1] Guoliang Zhang and Yanhong Sun contributed equally to this work.

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developmental stage-specific expression pattern and are associated with conditions like heart disease, diabetes and rheumatoid arthritis [28–32]. Recently, miRNAs have been proven to significantly influence chondrogenesis and OA [33].

In this study, we predicted that miR-502-5p was a potential regulator of TRAF2. The regulatory function of miR-502-5p in OA is still poorly understood. Thus, our present study evaluated the effect of miR-502-5p on the IL-1β-induced chondrocyte injury in vitro and investigated the underlying mechanisms.

2. Materials and methods

2.1. Tissue samples

Human OA articular cartilage samples was obtained from 20 OA patients undergoing hip or knee surgeries (age ranging from 50 to 73 years), while normal cartilage samples were taken from 20 post-mortem donors (age between 55 and 70 years) with no previous history of joint pain. Full ethical consent was obtained from all the donors and families. Articular cartilage samples were divided into two parts: one part was used for the RNA isolation, and the other for chondrocyte culture. For polymerase chain reaction (PCR) analysis, the total RNA was isolated from cartilage that had been homogenized on ice with TRIzol reagent (Invitrogen, Tokyo, Japan). For the primary cell culture, we used only chondrocytes from the normal articular cartilage harvested from the femoral condyles and tibial plateaus of the tissue donors.

2.2. Total RNA isolation

Articular cartilage samples from the OA and normal donors were powdered using a Freezer Mill (Glen Creston, London, UK) and the total RNA (including the microRNA fraction) was extracted by homogenization using the TRIzol reagent (Invitrogen, UK). The total RNA was also extracted from the primary chondrocytes by adding the TRIzol reagent directly to the chondrocyte cells grown on tissue culture plates. RNA was resuspended using RNase-free water (Promega UK, Southampton, UK) and quantified using a spectrophotometer (Nanodrop) and an Agilent Bioanalyzer to confirm the quality.

2.3. Reverse transcription; real-time polymerase chain reaction

The cDNA synthesis was performed by the M-MLV Reverse Transcriptase kit (Promega). For quantification of the miRNA expression, reverse transcription was performed using the TaqMan MicroRNA Reverse Transcription Kit and the RT stem-loop primers (Applied Biosystems, Warrington, UK). The miR-502-5p, miR-138, miR-205 and miR-146a expressions were then determined using the TaqMan microRNA assay kit (Applied Biosystems, Foster City, CA, USA) with specific primers. RUN48 was used as the internal control. Then SYBR Green Gene Expression Assay (Qiagen, Valencia, CA) was used for TNF-α, IL-6, MMP-13, aggregan, collagen type II, TRAF2 and internal control β-actin expression with gene-specific primers. The 7500 Real-Time PCR machine (Applied Biosystems, Warrington, UK) was used for the RT-PCR. Fluorescent signals from each sample were collected at the endpoint of every cycle, and the degree of gene and miRNAs expressions was calculated using the $2^{-\Delta\Delta Ct}$ method based on the internal controls.

2.4. Cell culture

To isolate the primary chondrocytes, normal articular cartilage slices were chopped finely with a scalpel blade. Cartilage tissue was predigested with trypsin for 10 min at 37 °C. After removing the trypsin solution, the tissue slices were treated overnight with type IV Clostridium collagenase in Dulbecco's modified Eagle's medium (DMEM) with 5% fetal calf serum (FCS). Then the sample was filtered to remove the undigested cartilage and the chondrocyte cells were pelleted at 2000 g for 5 min before being resuspended. After the initial isolation, the cells were maintained in high-density cultures in DMEM (high glucose) supplemented with 10% FCS, L-glutamine, and antibiotics. After the cells had matured to confluence, they were split once and cultivated to confluence again, for experimental use. IL-1β were used in a final concentration of 5 ng/ml (PeproTech, Rocky Hill, NJ). To evaluate the role of NF-κB pathway during the IL-1β-induced inflammation in this study, the cells were pretreated for one hour with pyrimidine dithiocarbamate (PDTC) (10 μmol/l, Sigma, MO, USA), an NF-κB inhibitor. The cells were then stimulated with IL-1β (5 ng/ml) for 24 h.

2.5. Transfection

MiR-502-5p mimic, negative control and TRAF2 small interfering RNA (siRNA) were obtained from Shanghai GenePharma Co., Ltd. (Shanghai, China). For transfection, 0.4 nmol mimic or siRNA were mixed with 15 μl GenePORTER 2 Transfection Reagent (GTS, San Diego) and then transfected into the chondrocyte cells. After 6 h, the supernatant was replaced with fresh medium and cultured for another 24 h. Post transfection the cells were stimulated with IL-1β for 24 h and then utilized in subsequent experiments.

2.6. MTT assay

The cell viability of the chondrocytes was assessed by MTT assay (MTT kit; Sigma, MO, USA). The cells were grown on 96-well plates at a density of 2×10^5 cells per square centimeter. Then MTT (0.5 mg/ml) was added to the cells and incubated for 4 h at 37 °C. The supernatant was removed, and the crystals were

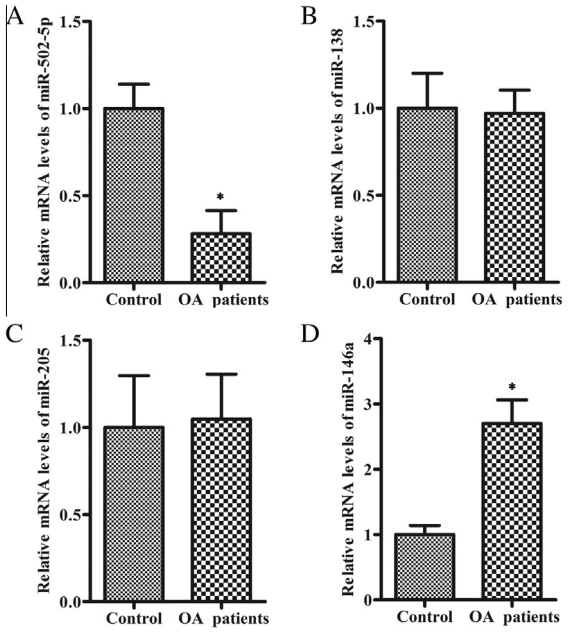

Fig. 1. The expression of the potential target miRNAs in the normal and OA articular cartilage tissues. The expression levels of the miRNAs were detected by RT-PCR assay and normalized to the internal control RNU48. (A) miR-502-5p mRNA level, (B) miR-138 mRNA level, (C) miR-205 mRNA level and (D) miR-146a mRNA level in the OA articular cartilage tissues ($n = 20$) compared with the control group ($n = 20$). The experiments were repeated three times. Data are presented as means ± s.d. $^*P < 0.05$ versus the control group.