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RESEARCH PAPER

miR-296-5p inhibits IL-1β-induced apoptosis and cartilage degradation in human chondrocytes by directly targeting TGF-β1/CTGF/p38MAPK pathway

Zhilin Cao[a], Wenguang Liu[b], Xiaoyi Qu[c], Haiyong Bi[a], Xiujiang Sun[a], Qian Yu[d], and Gong Cheng[a]

[a]Department of Orthopedics, Yantaishan Hospital, Yantai, Shandong Province, China; [b]Department of Joint Surgery, The Second Hospital of Shandong University, Jinan, Shandong Province, China; [c]Department of Nursing, Nurse School of Yantai City of Shandong Province, China; [d]Department of Hospital Surgary, Yantaishan Hospital, Yantai, Shandong Province, China

ABSTRACT

Osteoarthritis (OA) is characterized by apoptosis of chondrocytes and an imbalance of extracellular matrix (ECM) synthesis and catabolism. Emerging evidence has demonstrated that miRNAs are involved in OA pathologies, but the role of miR-296-5p in OA remains unclear. The present study proposes to reveal the functions and mechanisms of miR-296-5p in a cell model of OA. In this study, human chondrocytes were treated with 5 ml interleukin-1 beta (IL-1β) to induce apoptosis and cartilage degradation. Our results showed that miR-296-5p was downregulated in chondrocytes stimulated with IL-1β. Overexpressed miR-296-5p enhanced cell proliferation and inhibited apoptosis and matrix degrading enzyme expression in response to IL-1β stimulation, and knockdown of miR-296-5p showed the opposite effect. Further, we found that miR-296-5p directly targeted the 3′-untranslated region (3′-UTR) of TGF-β1 mRNA, and miR-296-5p inactivated the TGF-β1/CTGF/p38MAPK signaling pathway. Overexpression of TGF-β1 alleviated the inhibition of miR-296-5p on chondrocyte apoptosis and cartilage degradation. In conclusion, miR-296-5p inhibited the progression of OA through the CTGF/p38MAPK pathway by directly targeting TGF-β1.

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Introduction

Osteoarthritis (OA) is one of the most prevalent degenerative joint diseases, which seriously affects the health of the elderly and is characterized by articular cartilage degradation [1,2]. Articular cartilage contains two components, articular cartilage chondrocytes and extracellular matrix (ECM) [3]. Normally, chondrocytes can regulate the synthesis and degradation of type II collagen, aggrecan, and glycoproteins, to maintain the dynamic balance of cartilage ECM and exert its biological functions [4,5]. However, during the onset of OA, chondrocytes activate several proinflammatory cytokines, such as interleukin-1β (IL-1β), which can stimulate the expression of collagenase (matrix metalloproteinases, MMPs) and aggrecanase (a disintegrin-like and metalloprotease with a thrombospondin type 1 motif, ADAMTS) [6]. These matrix degradation-related proteolytic enzymes contribute to the degradation of ECM and destruction of cartilage, and finally aggravate the pathologies of OA [5,7,8]. Therefore, IL-1β-induced chondrocytes are often used as a cell model of OA *in vitro*.

MicroRNAs (miRNAs), a family of endogenous noncoding RNA molecules containing about 17 to 25 nucleotides, are highly conserved and prevalent in eukaryotic cells [9]. miRNAs play important roles in post-transcriptional negative regulation of their target genes through base pairing [10]. Accumulating studies indicate that multiple miRNAs are aberrantly regulated in cartilage degradation, and several miRNAs have been proven to act in vital regulatory roles in OA progression [3,11]. For instance, miR-93-5p, miR-204-5p and miR-221-3p are downregulated in OA tissues, and they can ameliorate OA progression via suppression of chondrocyte apoptosis and cartilage degradation [3,12,13]. miR-296-5p, a member of the miR-296 family, is located on the chromosome 20q13.32 genomic locus and regarded as a tumor angiogenesis-related miRNA [14,15]. A recent study demonstrated that miR-296-5p is downregulated in OA patients [16], but its function remains unclear.

CONTACT Xiujiang Sun xriversun@163.com

Zhili Cao and Wenguang Liu contribute equally to this study and regard as co-first author.

Transforming growth factor β1 (TGF-β1) plays a key role in the formation of articular cartilage during growth and development, and emerging studies indicate that its overexpression causes the degradation of proteoglycans, thereby leading to OA [17]. Connective tissue growth factor (CTGF/CCN2) is a 36 – 38 kDa cysteine-rich secreted protein that function as a downstream mediator of TGF-β1 in multiple tissues [18,19]. Upregulation of CTGF is frequently observed in arthritis, and an increasing number of articles have demonstrated that CTGF, mediated by TGF-β1, participates in the development of rheumatoid arthritis (RA), and inhibition of CTGF attenuates inflammatory responses and MMP levels in an IL-1β-stimulated knee OA model [20,21]. It has been reported that CTGF activates the p38MAPK signaling pathway in dilated cardiomyopathy, and CTGF induces matrix protein expression via the p38MAPK signaling pathway [22,23]. The P38MAPK pathway acts as a very important part of cell gene expression, proliferation, differentiation and adaptation to environmental stress, and inhibition of p38MAPK signaling ameliorates chondrocyte apoptosis and cartilage degradation in OA [24–26]. However, the molecular mechanism underlying the association between TGF-β1, CTGF and the p38MAPK pathway is still unclear.

This study aimed to explore the effects and underlying mechanism of miR-296-5p on an IL-1β-induced cell model of OA. In this study, we found that miR-296-5p was downregulated in IL-1β-evoked chondrocytes. Moreover, overexpression of miR-296-5p alleviated IL-1β-induced chondrocyte apoptosis and cartilage matrix degradation through targeting of TGF-β1 and inactivation of the connective CTGF/p38MAPK signaling pathway. These results indicated that miR-296-5p is a potential target for clinical prevention and therapeutic treatment of OA.

Material & methods

Cell culture and treatment

Normal human knee articular chondrocytes (NHAC-kn) were obtained from Lonza Bioscience (Walkersville, USA), and cultured in Dulbecco's modified Eagle's medium (DMEM, Invitrogen, Carlsbad, USA) containing 10% Fetal Bovine Serum (FBS, Thermo Fisher Scientific, USA) at 37°C with 5% CO_2 in a humidified incubator. NHAC-kn cells were treated with IL-1β (PeproTech, NJ, USA) at a series of concentrations (1, 2, 5 and 10 μg/ml) for 12 h.

Cell transfection

MiR-295-5p mimics and mimic controls, miR-295-5p inhibitor and inhibitor control, small interfering RNA against TGF-β1 (TGF-β1 siRNA) and siRNA control, were synthesized and obtained from Gene Pharma (Shanghai, China). The TGF-β1 overexpression plasmid (pcDNA-TGF-β1) was constructed and obtained from Generay Biotech (Shanghai, China). IL-1β-stimulated NHAC-kn cells were transfected with Lipofectamine 3000 (Invitrogen, Carlsbad, USA) for 48 h.

Cell viability assay

The Cell Counting Kit-8 (CCK-8, Dojindo, Japan) was used to determine cell viability in this study. Briefly, NHAC-kn cells were seeded at a density of 2×10^3 cells per well in 96-well plates. After culturing for 48 h, 10 μl CCK-8 solution was added to each well and then incubated at 37°C for 2 h, and the optical density (OD) was measured using a 96-well microplate reader at 450 nm.

Cell apoptosis assay

The apoptotic rate of NHAC-kn cells was analyzed with an annexin V-fluorescein isothiocyanate (FITC) conjugate combined with the propidium iodide (PI) assay (Dojindo, Japan) and evaluated by fluorescence-activated cell sorting (BD Biosciences, San Diego, USA).

RNA extraction and quantitative reverse-transcription PCR (qRT-PCR)

The total RNA of treated or non-treated NHAC-kn cells was extracted with TRIzol Reagent (Invitrogen, Carlsbad, USA). The TaqMan MicroRNA Reverse Transcription Kit and TaqMan miRNA assay (Qiagen, China) were used to quantify miR-296-5p expression. U6 was used as the internal control. The PrimeScript RT reagent kit (Takara) was used to

synthesize cDNA of the target mRNA, and SYBR Premix Ex Taq (Takara) was used for the analysis according to the manufacturer's instructions, with β-actin as an internal control. The qRT-PCR procedure was as follows: 1 min at 95°C, 20 s at 95°C and 10 s at 56°C and 15 s at 72°C for 35 cycles then held at 4°C. Finally, we used the $2^{-\Delta\Delta CT}$ method to quantify the abundance of miRNAs or mRNA. The primers used were miR-296-5p: forward, 5′- GTATCCAGTGCA GGGTCCGA-3′, reverse, 5′- CGACGAGGGCCC CCCCT-3′; U6: forward, 5′-CGCTTCGGCA GCACATATAC-3′, reverse, 5′-AAATATGGAACG CTTCACGA-3′; MMP-13: forward, 5′-GACAGTGGAGGTGGCCTTAC-3′, reverse, 5′-ACAAGTGGGTAGATAAACAAGGT-3′; ADAMT S-5: forward, 5′-CTTTAGAGGGAGAAAATTC TGG-3′, reverse, 5′-AAAGATTTACCATTG GGTGG-3′; collagen II: forward, 5′-CTCAAGTCGCTGAACAACCA-3′, reverse, 5′-GTCTCCGCTCTTCCACTCTG-3′; aggrecan: forward, 5′-ACCCCAACACCTACAAGCACA-3′, reverse, 5′-AAAGCGACAAGAAGACACCA-3′; β-actin: forward, 5′-CCTCTATGCCAACACAGT-3′, reverse, 5′-AGCCACCAATCCACACAG-3′.

Western blot

NHAC-kn cells were harvested and lysed in radioimmunoprecipitation assay (RIPA) buffer (Thermo Scientific) with a protease inhibitor cocktail (Millipore, MA, USA). The protein extraction was resolved by sodium dodecyl sulfate polyacrylamide gel electrophoresis (10% resolving gel with a 5% stacking gel), and subsequently transferred onto a PVDF membrane (Millipore, MA, USA). After blocking in 5% nonfat milk for 1 h at room temperature, the membrane was incubated with primary antibody at 4°C overnight. After washing with PBS three times, the membranes were then treated with corresponding secondary antibodies conjugated to horseradish peroxidase for 2 h at room temperature and incubated with enhanced chemiluminescence solution (Bio-Rad, Hercules, USA). Finally, the densitometer technology Quantity One software (Bio-Rad, Hercules, USA) was used for signal visualization and quantification. The antibodies used in western blotting included anti-CDK2 (ab32147); anti-Cyclin D1 (ab16663); anti-Bcl-2 (ab32370); anti-Bax (ab32503); anti-cleaved-Caspase 9 (ab2324); anti-TGF-β1 (ab92486); anti-CTGF (ab231824); anti-p-p38 (ab133581); anti-p-ERK (ab 201015); anti-pJNK (ab207477); anti-GAPDH (ab181602), which were all purchased from Abcam (USA).

Enzyme-linked immunosorbent assay (ELISA)

Samples were centrifuged, and the culture supernatant was collected for analysis. The concentrations of MMP-13 and ADAMTS-5 in NHAC-kn cells were determined by ELISA Kits (R&D Systems, Minneapolis, USA) according to the manufacturer's instructions.

Luciferase activity assay

The wild-type pGL3-TGF-β1-3′-UTR (WT-TGF-β1) and mutant type pGL3-TGF-β1-3′-UTR (MUT-TGF-β1) were constructed and purchased from Agilent Technologies (Santa Clara, USA). The plasmids were transfected into cells using Lipofectamine 3000 (Invitrogen, Carlsbad, USA) for 48 h. Luciferase activities were consecutively measured according to the Dual-Luciferase Reporter Assay System manufacturer's protocol (Promega, Madison, USA).

Statistical analysis

All experiments were repeated in triplicate. Statistical analysis was performed with SPSS 22.0 software (SPSS Inc., USA). The data are all indicated as the mean ± SD. The resulting value of $^{*}p < 0.05$, $^{**}p < 0.01$, $^{\#}p < 0.05$ or $^{\&}p < 0.05$ was considered statistically significant.

Results

miR-296-5p is downregulated in IL-1β-treated NHAC-kn cells

As shown in Figure 1(a,b), cell viability and miR-296-5p expression in NHAC-kn cells were both decreased in a dose-dependent manner after treatment with IL-1β in a concentration gradient (1, 2, 5 and 10 μg/ml) for 12 h ($^{*}p < 0.05$, $^{**}p < 0.01$).

miR-296-5p inhibits apoptosis in IL-1β-treated NHAC-kn cells

Chondrocyte apoptosis and cartilage degradation are the main pathological features of OA [3,27]. Because miR-296-5p was downregulated in IL-1β-induced NHAC-kn cells, we speculated that miR-296-5p played a role in OA pathologies. The effects of miR-296-5p mimics and inhibitors on chondrocyte proliferation and apoptosis were determined using CCK-8, Annexin V FITC/PI and western blot assays. The results showed that miR-296-5p mimics and inhibitor were significantly elevated and reduced miR-296-5p levels, respectively (Figure 2(a), $p < 0.05$). After IL-1β stimulation, miR-296-5p mimics significantly improved cell viability and suppressed cell apoptosis rate, and miR-296-5p inhibitor remarkably reduced cell viability and increased the cell apoptosis rate (Figure 2(b,c), $p < 0.05$). With respect to the western blot data, miR-296-5p mimics facilitated the expression of cell cycle makers, including CDK2 and Cyclin D1, and attenuated the expression of apoptotic effector Bax/Bcl-2 and cleaved Caspase 9; moreover, miR-296-5p inhibitor showed the opposite effect (Figure 2(d–h), $p < 0.05$). These results indicated that miR-296-5p inhibited IL-1β-induced NHAC-kn apoptosis.

miR-296-5p alleviates cartilage degradation in IL-1β-treated NHAC-kn cells

To determine the effect of miR-296-5p on IL-1β-induced cartilage degradation, we analyzed the expression of major cartilage degradation-related genes, including MMP-13 and ADAMTS-5, collagen II and aggrecan, which are responsible for the balance of ECM catabolism and synthesis in NHAC-kn cells. The results showed that the mRNA and protein expression levels of MMP-13 and ADAMTS-5 were increased remarkably, whereas the mRNA and protein expression levels of collagen II and aggrecan were decreased remarkably by IL-1β stimulation. Further, miR-296-5p mimics reduced MMP-13 and ADAMTS-5 expression and enhanced collagen II and aggrecan expression, while miR-296-5p inhibitor improved MMP-13 and ADAMTS-5 expression and attenuated collagen II and aggrecan expression in NHAC-kn cells (Figure 3, $p < 0.05$). The results suggested that miR-296-5p inhibited cartilage matrix degradation in IL-1β-stimulated NHAC-kn cells.

miR-296-5p regulates the CTGF/p38MAPK axis via targeting of TGF-β1

Next, we attempted to understand the mechanisms of miR-296-5p in OA. Based on the bioinformatics software Starbase 2.0., we found that the 3′-untranslated region (3′-UTR) of TGF-β1 mRNA possesses a potential miR-296-5p binding site (Figure 4(a)). To determine the direct binding between miR-296-5p and TGF-β1, a luciferase reporter assay containing WT-TGF-β1 or MUT-TGF-β1 was constructed and transfected into NHAC-kn cells. The result showed that miR-296-5p mimics significantly attenuated

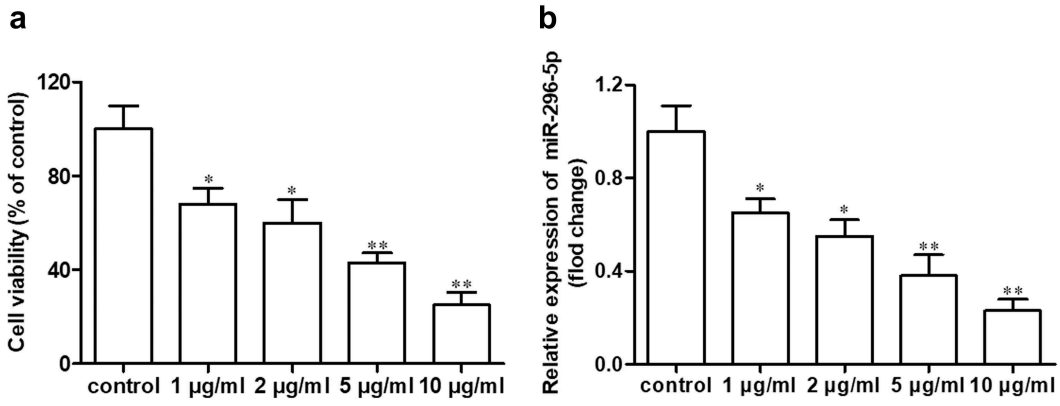

Figure 1. miR-296-5p is downregulated in IL-1β-treated NHAC-kn cells.

NHAC-kn cells were treated with a concentration gradient (1, 2, 5 and 10 μg/ml) of IL-1β for 12 h. (a) Cell viability was evaluated by CCK-8 assay. (b) The relative expression of miR-296-5p was analyzed by using qRT-PCR. IL-1β, interleukin-1β; NHAC-kn, normal human knee articular chondrocytes; qRT-PCR, quantitative reverse-transcription PCR. *$p < 0.05$ vs control, **$p < 0.01$ vs control.

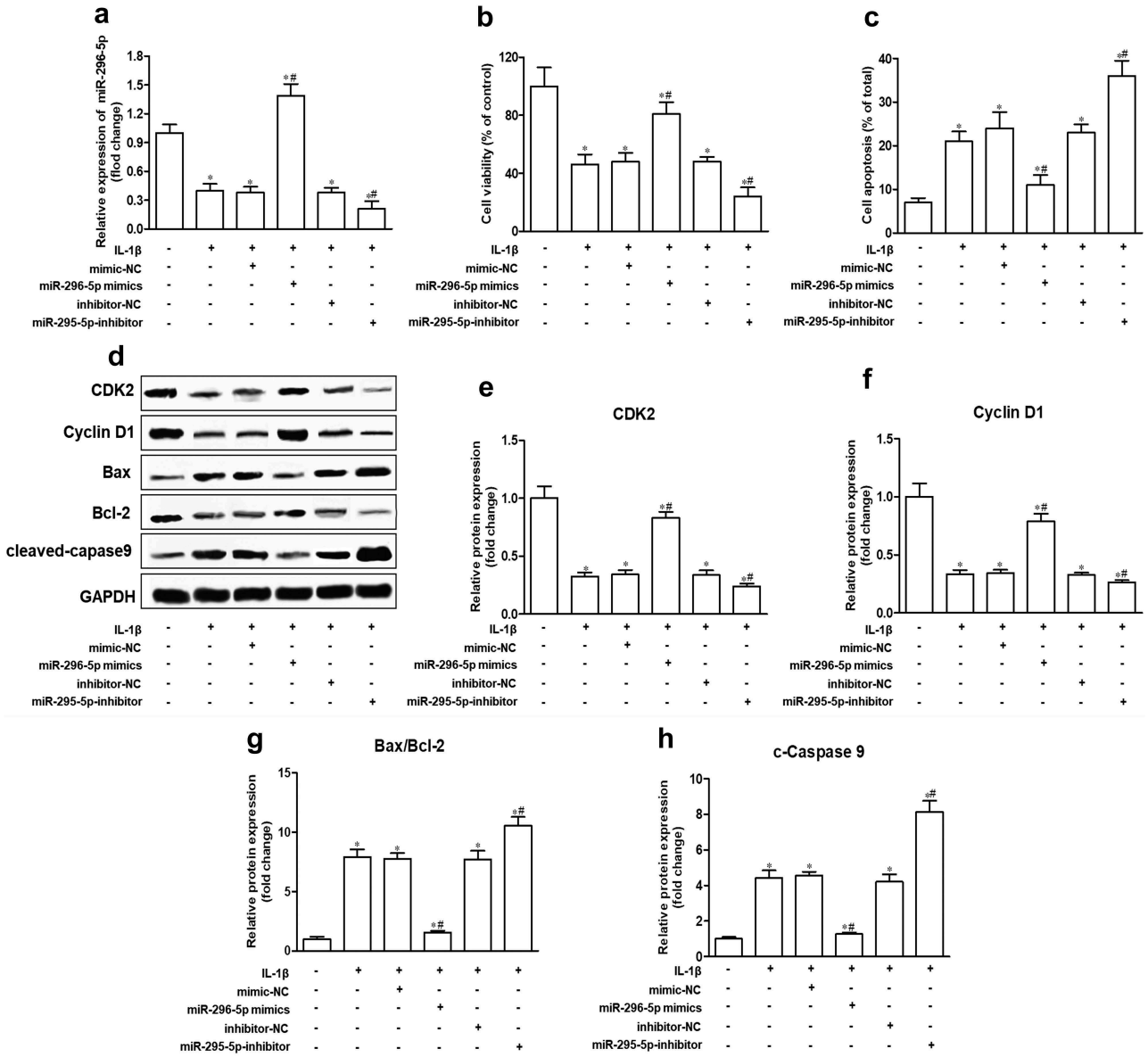

Figure 2. miR-296-5p inhibits apoptosis in IL-1β-treated NHAC-kn cells.

NHAC-kn cells were transfected with constructed miR-296-5p mimics (10 nM), miR-296-5p inhibitor (10 nM), or their negative control (mimic-NC, inhibitor-NC) for 48 h and then treated with 5 μg/ml IL-1β for 12 h. (a) The relative expression of miR-296-5p was measured though qRT-PCR. (b) Cell viability was examined by CCK-8 assay. (c) Apoptosis was analyzed by Annexin V FITC/PI assay. (d–h) Protein expression and relative densitometric analysis of cell cycle makers, including CDK2 and Cyclin D1, and apoptotic effectors, including Bax, Bcl-2 and cleaved-Caspase 9 (c-Caspase 9). CDK2, cyclin-dependent kinase 2. Bcl-2, B-cell lymphoma-2; Bax, Bcl2-associated X protein; Caspase 9, Cysteine aspartic acid specific protease 9. *$p < 0.05$ vs control, #$p < 0.05$ vs IL-1β-treated group.

the luciferase activity of WT-TGF-β1, rather than MUT-TGF-β1 (Figure 4(b), $p < 0.05$). Moreover, miR-296-5p mimics conspicuously attenuated the expression of TGF-β1 mRNA and protein in NHAC-kn cells (Figure 4(c,d), $p < 0.05$). In the present study, we found that small interfering RNA against TGF-β1 (TGF-β1 siRNA) ameliorated the promotion of miR-296-5p inhibitor on TGF-β1, CTGF, p-p38, p-ERK and p-JNK expression levels, and TGF-β1 over-expression vector (pcDNA-TGF-β1) counteracted the suppression of miR-296-5p mimics on the expression of these genes (Figure 4(e–j), $p < 0.05$). These data revealed that miR-296-5p was able to blunt the activation of the TGF-β1/CTGF/p38MAPK pathway in NHAC-kn cells.

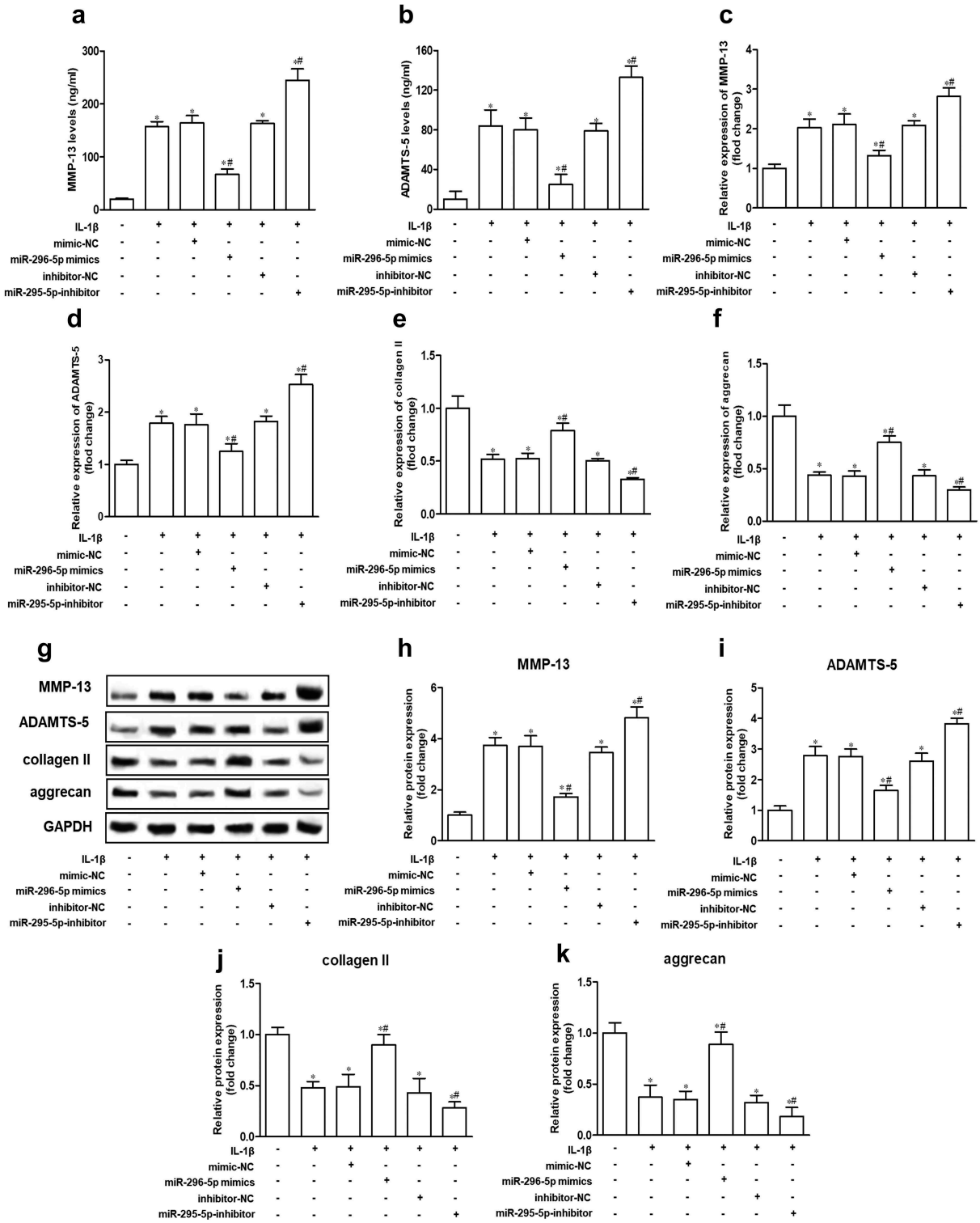

Figure 3. miR-296-5p alleviates cartilage matrix degradation in IL-1β-treated NHAC-kn cells.

NHAC-kn cells were transfected with miR-296-5p mimics, miR-296-5p inhibitor, or a negative control (mimic-NC, inhibitor-NC) before IL-1β stimulation. The levels of MMP-13 (a) and ADAMTS-5 (b) in the culture medium were tested by ELISA. (c–f) The mRNA expression of MMP-13, ADAMTS-5, collagen II and aggrecan. (g–k) The protein expression and relative densitometric analysis of MMP-13, ADAMTS-5, collagen II and aggrecan. MMP-13: matrix metallopeptidase, member 13, ADAMTS-5: a disintegrin and metalloproteinase with a thrombospondin type 1 motif, member 5. *$p < 0.05$ vs control. #$p < 0.05$ vs IL-1β-treated group.

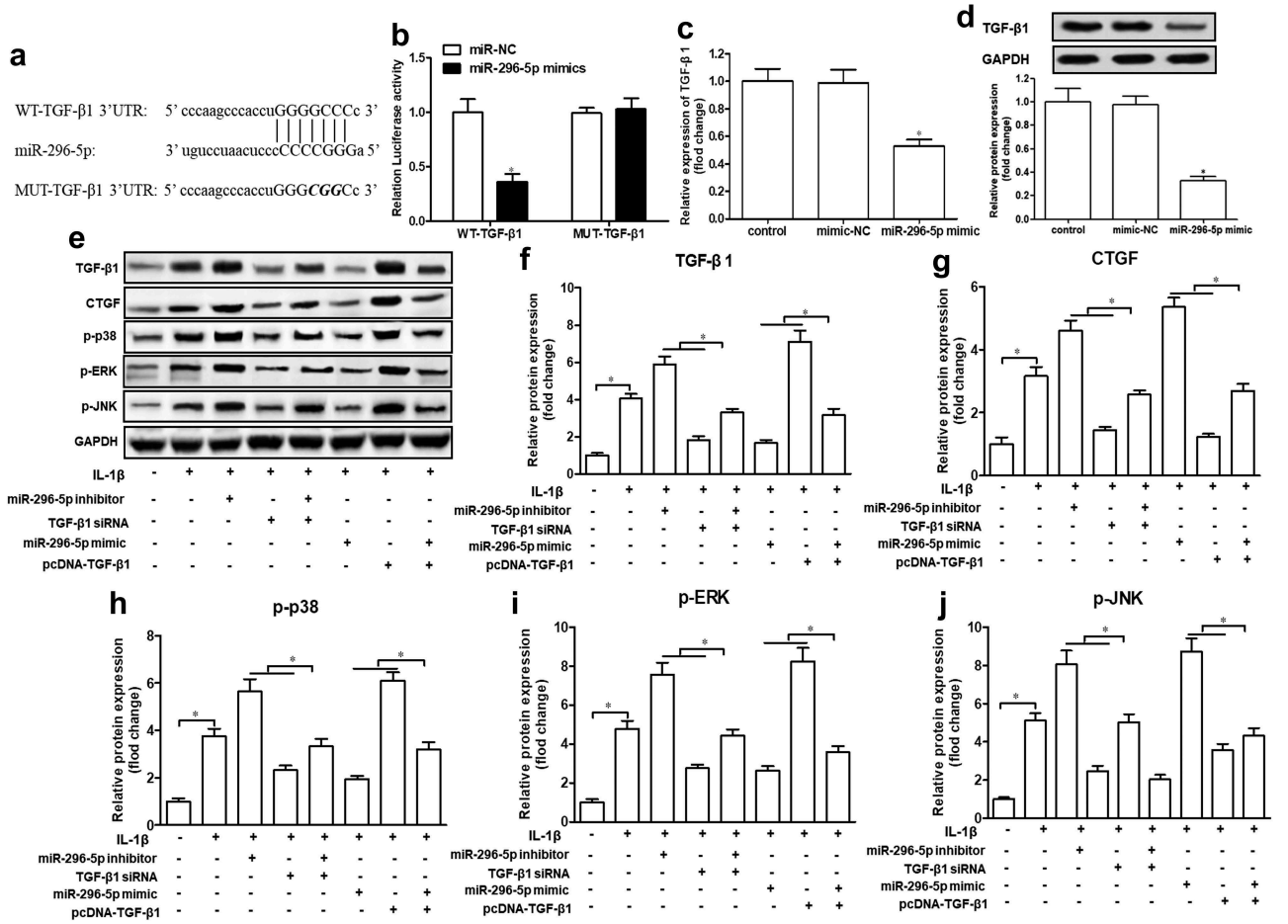

Figure 4. miR-296-5p regulates TGF-β1/CTGF/p38MAPK axis via targeting of TGF-β1.

(a). Schematic diagram shows the sequence alignment of miR-296-5p and the 3′-UTR of TGF-β1 mRNA. Italics indicate the mutated seed region. (b) NHAC-kn cells were transfected with miR-296-5p mimics or mimic-NC, along with WT-TGF-β1-3′-UTR or MUT-TGF-β1-3′-UTR reporter constructs, and the relative luciferase activity was measured. (c–d) The mRNA and protein expression of TGF-β1 in NHAC-kn cells after transfected with miR-296-5p mimics or mimic-NC. (e–j) NHAC-kn cells were treated with IL-1β after miR-296-5p inhibitor, small interfering RNA against TGF-β1 (TGF-β1 siRNA), miR-296-5p mimics, or/and TGF-β1 overexpression vector (pcDNA-TGF-β1) transfection. The protein expression of TGF-β1, CTGF, p-p38, p-ERK and p-JNK were analyzed by western blot. TGF-β1: transforming growth factor beta 1, CTGF: connective tissue growth factor. *p < 0.05 vs control.

TGF-β1 partly attenuates the suppressive effect of miR-296-5p on IL-1β-induced chondrocyte apoptosis and cartilage degradation

Lastly, we explored the role of TGF-β1 in the function of miR-296-5p on IL-1β-induced chondrocyte apoptosis and cartilage degradation. Our data demonstrated that TGF-β1 siRNA significantly abrogated the effect of miR-296-5p on cell viability, the apoptosis rate and MMP-13 and ADAMTS-5 levels, while pcDNA-TGF-β1 significantly impaired the function of miR-296-5p mimics on cell viability, the apoptosis rate and MMP-13 and ADAMTS-5 levels, in IL-1β-treated NHAC-kn cells (Figure 5(a–d), $p < 0.05$). Hence, our data suggested that miR-296-5p inhibited IL-1β-induced chondrocyte apoptosis and cartilage degradation via blocking activation of the TGF-β1/CTGF/p38MAPK pathway.

Discussion

The involvement of miRNAs in osteoarthritis (OA) pathogenesis has received much attention in recent years [3,11]. In this study, we aimed to characterize the potential function and mechanism of miR-296-5p in a cell model of OA. Our data demonstrated that miR-296-5p was significantly downregulated in IL-1β-induced chondrocytes, which is consistent with a study by Song et al.,

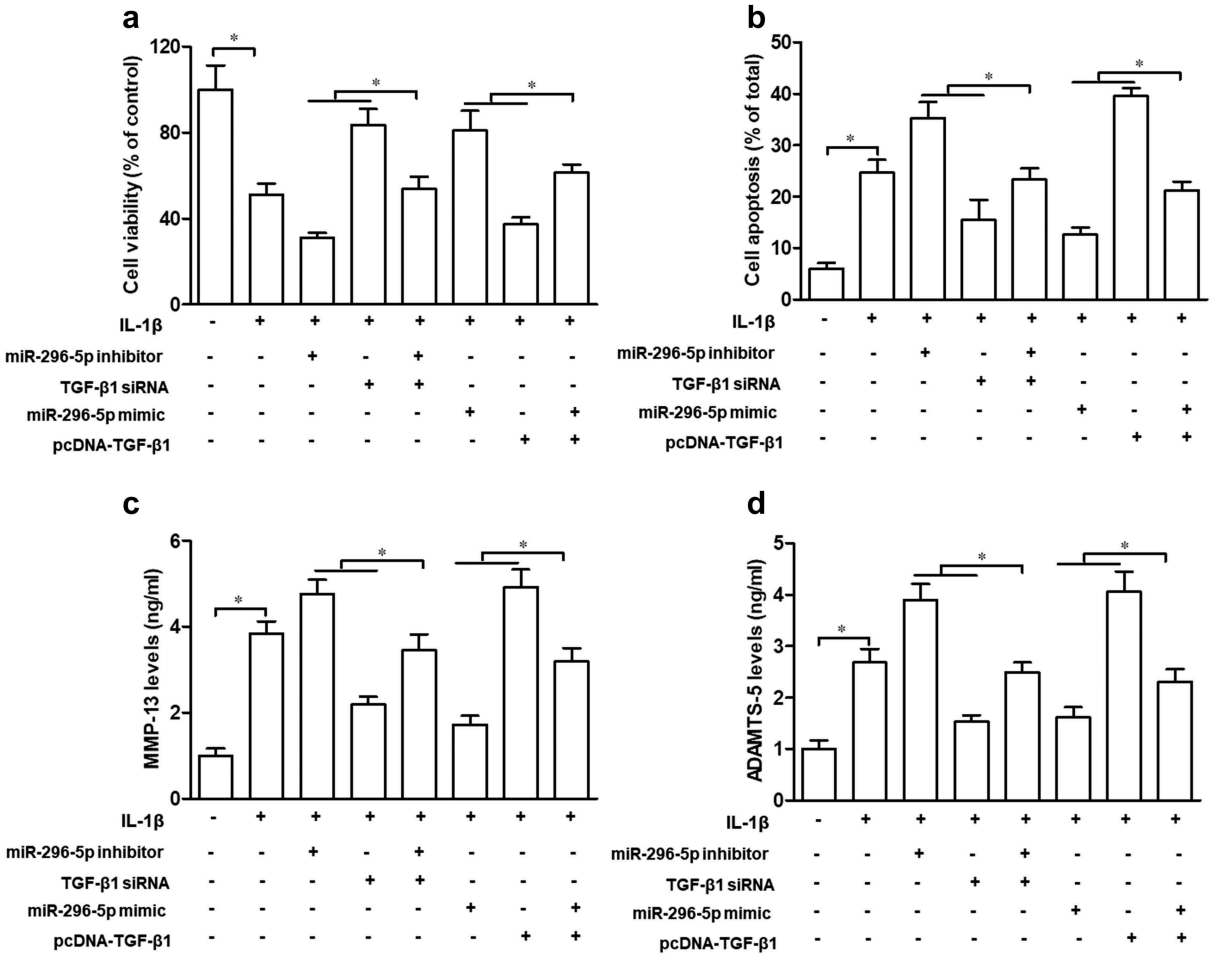

Figure 5. TGF-β1 partly attenuates the suppressive effect of miR-296-5p on IL-1β-induced chondrocyte apoptosis and cartilage degradation.

NHAC-kn cells were transfected with miR-296-5p inhibitor, TGF-β1 siRNA, miR-296-5p mimics or/and pcDNA-TGF-β1, before IL-1β treatment. (a) Cell viability was examined by CCK-8 assay. (b) Apoptosis was analyzed by Annexin V FITC/PI assay. (c–d)The levels of MMP-13 and ADAMTS-5 in the culture medium were analyzed by ELISA. *$p < 0.05$ vs control.

who found that miR-296-5p expression was decreased in OA tissues [16]. Previous studies have reported that miR-296-5p is aberrantly expressed in a variety of human diseases and exhibits tissue-specific differences [28–30]. Notably, by acting on different target genes, miR-296-5p plays a different role in many pathophysiological processes, such as cellular proliferation and apoptosis [31,32]. On the one hand, miR-296 suppresses cell proliferation and induces apoptosis in hepatocellular carcinoma and prostate cancer by targeting NRG1/ERBB2/ERBB3/RAS/MAPK/Fra-2 signaling and peptidyl-prolyl isomerase (Pin1), respectively [15,33]. On the other hand, miR-296-5p promotes cell proliferation in vascular smooth muscle cells (VSMCs) through inhibition of p53, $p21^{WAF1}$ and p27; miR-296-5p attenuates apoptosis of gastric cancer by interacting with *Caudal*-related homeobox 1 (CDX1) [34,35]. In the present study, we found that miR-296-5p ameliorated IL-1β-induced chondrocyte apoptosis and cartilage degradation via targeting of TGF-β1.

A growing number of studies have shown that TGF-β1 plays a key role in the formation of articular cartilage during growth and development, and it can stimulate chondrocytes to synthesize and secrete proteoglycans and type II collagen [36]. Some evidence indicate that TGF-β1 downregulation can trigger the occurrence of OA, and it can play a protective role in articular cartilage [37]. However, other investigations have found that overexpression of TGF-β1 is closely related to the

degradation of proteoglycans, thereby leading to OA occurrence [38]. Zhang et al. reported that the TGF-β1 receptor inhibitor sB-505124 can significantly reduce levels of cartilage degeneration-related markers, including MMP-9 and MMP-13, in anterior cruciate ligament (ACL)-induced OA mice [39]. Zhen et al. revealed that elevating TGF-β1 levels in osteoplastic can aggravate the development of OA, whereas down-regulating TGF-β1 expression in subchondral ameliorates cartilage degradation [40]. Chen et al. also indicated that inhibition of TGF-β1 signaling in the articular chondrocyte decreases cartilage degradation in OA mice [41]. In addition, TGF-β1 has been proven to induce apoptosis in several cancers cell by blocking cyclin-dependent kinase CDK2 and Cyclin D1 activity, and the suppression of TGF-β1 could significantly reverse the suppression of chondrocyte growth and the induction of apoptosis [36,42,43]. Herein, we found that miR-296-5p inhibited IL-1β-induced chondrocyte apoptosis and cartilage degradation by negatively regulating TGF-β1.

Emerging studies have reported that CTGF is an important downstream modulator and also acts as a downstream effector molecule of TGF-β1 in various tissues. Meanwhile, TGF-β1 plays some biological functions dependent on CTGF, including promoting cell proliferation and ECM synthesis [21,44,45]. Notably, CTGF plays an important role in TGFβ activation in response to cartilage injury [45]. It has been reported that CTGF contributes to myocardial fibrosis and induces matrix protein production by activating the p38MAPK signaling pathway [22,23]. What is more, CTGF has been proved that increases the intensity and persistence of synovitis, as well as the expression of catabolic factors in OA by the potentiation of p38 activation [46]. In this study, we found that miR-296-5p inhibited CTGF/p38MAPK signaling via suppression of TGF-β1. Therefore, miR-296-5p can protect chondrocytes from IL-1β-induced injury by downregulating TGF-β1/CTGF/p38MAPK expression.

In conclusion, the present study showed that miR-296-5p was downregulated in IL-1β-induced chondrocytes, and miR-296-5p inhibited IL-1β-induced chondrocyte apoptosis and matrix-degrading enzyme expression. An in-depth analysis suggested that TGF-β1 is a direct target of miR-296-5p, and miR-296-5p mediated OA development, possibly by blocking the TGF-β1/CTGF/p38MAPK pathway. Thus, our study suggested that miR-296-5p may serve as a therapeutic target for patients with OA.

Consent to publication

All authors have obtained consent to publish the data from the study.

Availability of data and materials

All data generated or analyzed during this study are included in this published article.

Authors' contributions

XJ S designed the experiments; ZL C, WG L, XY Q, XJ S, Q Y and G C performed the experiments; ZL C and WG L analyzed the data and wrote the manuscript.

Disclosure statement

No potential conflict of interest was reported by the authors.

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