Altered expression of microRNA-98 in IL-1β-induced cartilage degradation and its role in chondrocyte apoptosis

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Abstract. Osteoarthritis (OA) is a multifactorial disease characterized by degeneration of the articular cartilage due to genetic and epigenetic components. The pathogenesis of OA is complex and the mechanism of chondrocyte homeostatic regulation remains to be fully elucidated. Previous studies have demonstrated that microRNAs (miRNAs/miR) contribute to cartilage dysfunction. However, the functional role of miR-98 in interleukin-1β (IL-1β)-induced chondrocyte apoptosis in OA cartilage remains to be investigated. The present study aimed to identify and characterize the expression profile of miR-98 and apoptosis-associated proteins in healthy and OA chondrocytes, and western blot analysis and TUNEL staining were used to evaluate the role of miR-98 in the regulation of chondrocyte apoptosis. The present study demonstrated that miR-98 expression was increased in OA chondrocytes in response to IL-1β stimulation, and the expression levels of apoptosis-associated proteins, including Fas cell surface death receptor, caspase-3, caspase-8 and B-cell lymphoma-2 (Bcl-2)-associated X protein, were also increased in IL-1β-stimulated chondrocytes. In addition, it was revealed that upregulation of miR-98 was accompanied by reduced expression of Bcl-2 following exposure to IL-1β. IL-1β-induced downregulation of Bcl-2 was associated with miR-98-mediated translational repression. Transfection of OA chondrocytes with a miR-98 inhibitor had an inhibitory effect on IL-1β-induced cell apoptosis, increased cell proliferation and upregulated Bcl-2 expression. It is possible that miR-98 inhibited IL-1β-induced chondrocyte apoptosis by modulating Bcl-2 expression levels. The findings of the present study indicated that the effects of miR-98 on chondrocyte apoptosis were induced by regulation of Bcl-2 expression. In addition, the present study confirmed that miR-98 targeted the 3'-untranslated region of Bcl-2. In conclusion, miRNA-coordinated regulation of apoptosis-associated protein expression has been identified in OA chondrocytes following IL-1β induction.

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Introduction

Osteoarthritis (OA) is a prevalent degenerative joint disease, which is characterized by progressive destruction of the articular cartilage and subchondral bone. Numerous etiological factors are implicated in the pathogenesis of OA; however, cartilage destruction appears to be due to chondrocyte apoptosis (1). It has previously been suggested that microRNAs (miRNAs/miR) are involved in the pathogenesis of OA (2). miRNAs are single-stranded, non-coding small RNAs that negatively regulate the expression of target genes in a post-transcriptional manner; >700 miRNAs have been identified to serve a role in the regulation of cellular processes, including proliferation, apoptosis and differentiation (3,4). Previous studies that used miRNA microarrays to compare the expression of miRNAs between normal and OA human articular cartilage revealed that miRNAs were comprehensively involved in OA generation (5,6). Diaz-Prado *et al* (6) previously generated cartilage-specific Dicer-null mice, which exhibited reduced chondrocyte proliferation and severe growth defects, thus indicating the importance of miRNAs in regulating cartilage function. However, the expression and functional role of miRNAs in OA remains to be fully elucidated. Therefore, the miRNA expression pattern and functions that are essential for chondrocyte development and OA damage require further investigation. The inflammatory cytokine interleukin (IL)-1β, which is a major catabolic inducer, is one of the most prominent catabolic cytokines, which serves a crucial role in OA pathogenesis (7,8). IL-1β promotes the production of proteases, such as matrix metallo proteinases (MMPs), and inhibits the synthesis of proteoglycans and collagens by chondrocytes (9,10). Previous studies have suggested that various miRNAs are involved in IL-1β-induced OA cartilage destruction, including miR-9, miR-98 (11), miR-140 (12), miR-146a (13) and miR-558 (14).

Previous studies have investigated the role of miR-98 in the regulation of tumor growth, invasion, angiogenesis and anti-inflammation in various disease models. Siragam *et al* (15) defined a regulatory role for miR-98 in tumor angiogenesis and invasion via the suppression of activin A receptor type 1B and MMP11 expression. Du *et al* (16) suggested that miR-93, miR-98 and miR-197 exert a negative regulatory effect on the expression of tumor suppressor gene FUS1 in lung cancer. Li *et al* (17) demonstrated that the expression levels of miR-98 were reduced in melanoma tissues at a higher tumor stage and in melanoma with metastasis, and suggested that miR-98 inhibited melanoma metastasis via a novel miR-98-IL-6-negative feedback loop. However, the expression pattern and functional role of miR-98 in chondrocyte apoptosis of OA cartilage remains to be fully elucidated. The present study aimed to determine the molecular mechanism underlying the pathogenesis of OA. Therefore, the expression levels of miR-98 in normal chondrocytes and IL-1β-treated OA chondrocytes were detected. In addition, the role of miR-98 in an IL-1β-induced OA disease model was identified, suggesting its potential involvement in the regulation of apoptosis. The role of miR-98 in the regulation of chondrocyte proliferation and apoptosis was investigated using an *in vitro* OA model. The findings of the present study confirmed that miR-98 targeted the 3'-untranslated region (3'-UTR) of B-cell lymphoma 2 (Bcl-2). These findings suggested that miR-98 serves a crucial role in the coordinated regulation of the expression of apoptosis-associated proteins in chondrocytes in response to IL-1β-induction.

Materials and methods

Mouse primary chondrocyte culture. The present study was approved by the Ethics Committee of the First People's Hospital of Yunnan Province (Kunming, China). A total of 30 2-week old male C57BL/6 mice (~10 g) were purchased from the Shanghai Animal Center, Chinese Academy of Sciences and housed in a specific pathogen free facility with a constant humidity and temperature at 12:12 h light:dark cycle with free access to food and water for 2 days in the animal research facility in accordance with an approved protocol. Mice were anesthetized with diethyl ether (60297; Sinopharm Chemical Reagent Co., Ltd., Shanghai, China) and sacrificed, then cartilage sections of mice were aseptically removed and washed in PBS (pH 7.4; Gibco; Thermo Fisher Scientific, Inc., Waltham, MA, USA) containing 1% penicillin-streptomycin (Gibco; Thermo Fisher Scientific, Inc.). Subsequently, cartilage slices were minced with sterile ophthalmic scissors and transferred into digestion buffer containing 0.25% trypsin (Sigma-Aldrich, Merck KGaA, Darmstadt, Germany) for 30 min at 37˚C. The supernatant containing trypsin was discarded following centrifugation at 4˚C for 10 min at 1,000 x g and the trypsinized cartilage was digested again using 2 mg/l type IV collagenase (Sigma-Aldrich; Merck KGaA) for 4-6 h at 37˚C. Following the digestion, the cells were washed with Dulbecco's modified Eagle's medium (DMEM; Gibco; Thermo Fisher Scientific, Inc.) containing 10% fetal bovine serum (FBS; Gibco; Thermo Fisher Scientific, Inc.) and centrifuged at 800 x g for 10 min at 4˚C prior to being cultured. The quantity of chondrocytes obtained was determined using a hemocytometer, and 0.4% Trypan blue dye (Sigma-Aldrich, Merck KGaA) was used to assess the viability of the cells. Chondrocytes were cultured in a 25 cm^2 culture flask (Corning, Inc., Corning, NY, USA) in DMEM supplemented with 1% glutamine and 10% FBS at 37˚C in 5% CO_2. Half of the medium was replaced once every 3 days with an equal quantity of fresh medium. Cells between passages 3 and 10 were used in the present study.

Normal chondrocytes were treated with 10 ng/ml IL-1β for 12, 24 and 48 h for OA chondrocyte preparation. The cell treatment groups including the control group, IL-1β treatment group, IL-1β treatment with miR-98 inhibitor transfection group and corresponding control, miR-98 mimic transfection group and miR-98 mimic control group.

Transfections with miRNA mimics and inhibitors. In order to manipulate the cellular function of miR-98 in mouse chondrocytes, the present study used specific antisense oligonucleotides to miRNAs in order to inhibit miRNA function, and specific miRNA precursors to increase miRNA expression. Briefly, following culture of mouse chondrocytes to 90% confluence, they were transfected with 3-100 nM miR-98 mimic, miR-98 inhibitor or negative controls (Sangon Biotech Co., Ltd., Shanghai, China) using Lipofectamine 2000 (Invitrogen; Thermo Fisher Scientific, Inc.) according to the manufacturer's protocol. The expression levels of miR-98 were detected 24 h post-transfection in order to confirm the optimal concentration of miR-98 mimic, miR-98 inhibitor or negative controls had been used. The final concentration used in the present study was 50 nM.

RNA extraction and reverse transcription-quantitative polymerase chain reaction (RT-qPCR). Total RNA was extracted using TRIzol reagent (Invitrogen; Thermo Fisher Scientific, Inc.) according to the manufacturer's protocol, and the concentration of total RNA was determined using NanoDrop 2000 (Thermo Fisher Scientific, Inc.). cDNA synthesis for the detection of target genes and miRNA was performed with 1 μg total RNA using PrimeScript™ 1st Strand cDNA Synthesis kit (Takara Bio, Inc., Otsu, Japan) according to the manufacturer's protocol. The mRNA expression levels of Fas cell surface death receptor (Fas), caspase-3, caspase-8, Bcl-2-associated X protein (Bax), Bcl-2 and GAPDH, and the expression levels of miR-98 and U6 were analyzed using SYBR Green PCR Master Mix kit (Takara Bio, Inc.) according to the manufacturer's protocol. Primer sequences are presented in Table I. All oligonucleotide primers were synthesized by Sangon Biotech Co., Ltd. (Shanghai, China). qPCR reactions (denaturation: 95˚C, 30 sec 1 cycle; PCR reaction: 95˚C, 5 sec, 60˚C, 40 sec 45 cycles) were performed on an ABI7900 Real-Time system (Applied Biosystems; Thermo Fisher Scientific, Waltham, MA, USA). The Cq values were analyzed using the comparative Cq (ΔΔCq) method (18) and the quantity of target mRNA was obtained by normalizing to the endogenous references (GAPDH or U6), relative to the control. All experiments were repeated at least six times.

Western blotting. Whole cell lysates were obtained using RIPA Protein Extraction reagent (Beyotime Institute of Biotechnology, Inc., Haimen, China) plus EDTA-free

Protease Inhibitor Cocktail tablets (Roche Diagnostics, Basel, Switzerland). Protein concentrations were detected using the BCA Protein Assay kit. Equal amounts of protein (25 μg) were separated by 12.5% SDS-PAGE. Total proteins were electrotransferred onto polyvinylidene fluoride membranes (Merck KGaA) and blocked with 3% bovine serum albumin (Beyotime Institute of Biotechnology, Inc., Haimen, China) in Tris-HCl buffered saline following electrophoresis. Bcl-2 (1,5071; 28 kD; 1:2,000) and β-actin antibodies (12262; 42 kD; 1:2,000) were purchased from Cell Signaling Technology, Inc. (Danvers, MA, USA). Following the primary antibody incubation at 4˚C overnight, membranes were incubated with horseradish peroxidase-conjugated species-specific secondary antibody (sc-2031, 1:5,000; Santa Cruz Biotechnology, Inc.) at 37˚C for 1 h and the proteins were visualized using an enhanced chemiluminescence kit (Pierce; Thermo Fisher Scientific, Inc.). The results of the western blotting were analyzed using Gel-Pro Analyzer version 4.0 (Media Cybernetics, Inc., Rockville, MD, USA). All experiments were repeated at least three times. Densitometric levels of target protein were semi-quantified and normalized to β-actin, relative to untransfected cells.

Terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) staining. TUNEL staining was performed 24 h post-transfection according to the manufacturer's protocol (Merck KGaA). The chondrocytes were fixed in cold 4% paraformaldehyde in PBS for 20 min at room temperature and washed three times in cold PBS. Subsequently equilibration buffer was added at room temperature for 10 min. The fixed and permeabilized samples were incubated with DNaseI (300 U/ml in 50 mM Tris-HCl, pH 7.5) for 10 min at room temperature in order to induce DNA strand breaks prior to labeling procedures. Reaction mix (10-50 μl/well to immerse all samples) was added to the cells and incubated at 37˚C for 2 h in a humidified atmosphere in the dark. Subsequently, the chondrocytes were rinsed with PBS three times for 5 min. Hoechst 33342 solution was applied for 1 min in order to visualize all nuclei under a fluorescent microscope (Leica Microsystems GmbH, Wetzlar, Germany).

Cell proliferation assay. The cell proliferation rate was evaluated using an MTT proliferation assay. Briefly, following exposure to IL-1β (PHC0813; Thermo Fisher Scientific, Inc.) or transfection with recombinant plasmids, cells were cultured in 96-well plates and were washed twice with PBS at 24 and 48 h. Subsequently, 0.5 mg/ml MTT was added to each well and the cells were incubated for 4 h at 37˚C in 5% CO_2. The medium was replaced with 200 μl dimethyl sulfoxide, the plates were agitated for 15 min at room temperature and the absorbance was determined at 490 nm using a microplate spectrophotometer.

Luciferase reporter constructs and luciferase assay. cDNA oligonucleotides (Sangon Biotech Shanghai, Co., Ltd., Shanghai, China) containing the putative miR-98 target site according to miRBase sequence database (http://www.mirbase.org) within the 3'-UTR of the target gene were synthesized with Hind III and Sac I restriction enzyme digestion (Takara Biotechnology Co., Ltd., Dalian, China) sites and cloned into the multiple cloning site of the pMIR-REPORT Luciferase vector (Ambion; Thermo Fisher Scientific, Inc.). An additional pMIR-REPORT Luciferase construct containing mutant 3'-UTR was generated as a control. Subsequently, HEK293 cells (Cell bank of Chinese Academy of Sciences, Shanghai, China) were cultured in 24-well plates with DMEM and 10% FBS for 24 h and then were transfected with each reporter construct, alongside miR-98 mimic or miR-98 inhibitor, using Lipofectamine 2000 reagent (Invitrogen; Thermo Fisher Scientific, Inc.) according to the manufacturer's protocol. Firefly luciferase activity was quantified at 48 h post-transfection using the dual-luciferase reporter assay system (Thermo Fisher Scientific, Inc.).

Statistical analysis. Statistical analysis was conducted with SPSS 18.0 (SPSS, Inc., Chicago, IL, USA). All of the experiments were performed with samples in triplicate or greater. Data are presented as the mean ± standard deviation of six independent experiments. One-way analysis of variance with Tukey HSD post hoc t tests were used to determine levels of statistical significance. Analysis of variance was used to determine statistical significance of the differences between the treatment groups. $P<0.05$ was considered to indicate a statistically significant difference.

Results

Relative expression of miR-98 and apoptotic factors in chondrocytes following IL-1β treatment. In order to investigate whether the expression of miR-98 was induced by IL-1β, mouse primary chondrocytes were incubated with IL-1β (10 ng/ml) for 12, 24 and 48 h. The expression levels of miR-98 were upregulated 2.75-fold at 24 h and 3.61-fold at 48 h. The expression of miR-98 was significantly increased following incubation with 10 ng/ml IL-1β for 24 and 48 h compared with in the 0 h treatment group ($P<0.05$; Fig. 1A). The effects of IL-1β on apoptotic factors were also determined by examining their expression levels, including Fas, caspase-3, caspase-8, Bax and Bcl-2. Exposure of primary chondrocytes to IL-1β (10 ng/ml) for 48 h significantly reduced the expression levels of Bcl-2 at the mRNA and protein level ($P<0.05$; Fig. 1A and B). The mRNA expression levels of Fas, caspase-3, caspase-8 and Bax were significantly greater following IL-1β stimulation for 48 h ($P<0.05$; Fig. 1A).

miR-98 affects apoptosis and proliferation of chondrocytes. In order to determine the effects of silencing miR-98 on mouse chondrocyte apoptosis and proliferation, miR-98 inhibitor was transfected into primary chondrocytes, and the chondrocytes were subsequently treated with 10 ng/ml IL-1β for 24and 48 h. A TUNEL assay was performed to detect the number of apoptotic cells. As presented in Fig. 2A, transfection with the miR-98 inhibitor reduced the number of TUNEL-positive cells compared with transfection with miR-98 inhibitor control or treatment withIL-1β only. Therefore, it is possible that downregulation of miR-98 expression in OA may be effective in the prevention of IL-1β-induced chondrocyte apoptosis. An MTT assay was used to examine the proliferation rate of chondrocytes under

Table I. Primer sequences for quantitative polymerase chain reaction.

Gene	Sense (5'-3')	Antisense (5'-3')
miR-98	ACACTCCAGCTGGGTGAGGTAGTAAGT	CTCAACTGGTGTCGTGGAGTC
U6	CTCGCTTCGGCAGCACATTGC	AACGCTTCACGAATTTGCGT
Fas	AGACTGCGTGCCCTGCCAAGA	GGCCTGCCTGTTCAGTAACT
Caspase-3	CTCGCTCTGGTACGGATGTG	TCCCATAAATGACCCCTTCATCA
Caspase-8	CGTGCCTAATGGCGTTAACCA	AGCCTTGGCCAGCCGACCTT
Bax	GACCCGGTGCCTCAGGATGC	GTCTGTGTCCACGGCGGCAA
Bcl-2	GCTACCGTCGTGACTTCGC	CCCCACCGAACTCAAAGAAGG
GAPDH	GACCCCTTCATTGACCTCAACTACA	GTCCACCACCCTGTTGCTGTAGCCA

miR-98, microRNA-98; Fas, Fas cell surface death receptor; Bcl-2, B-cell lymphoma 2; Bax, Bcl-2-associated X protein.

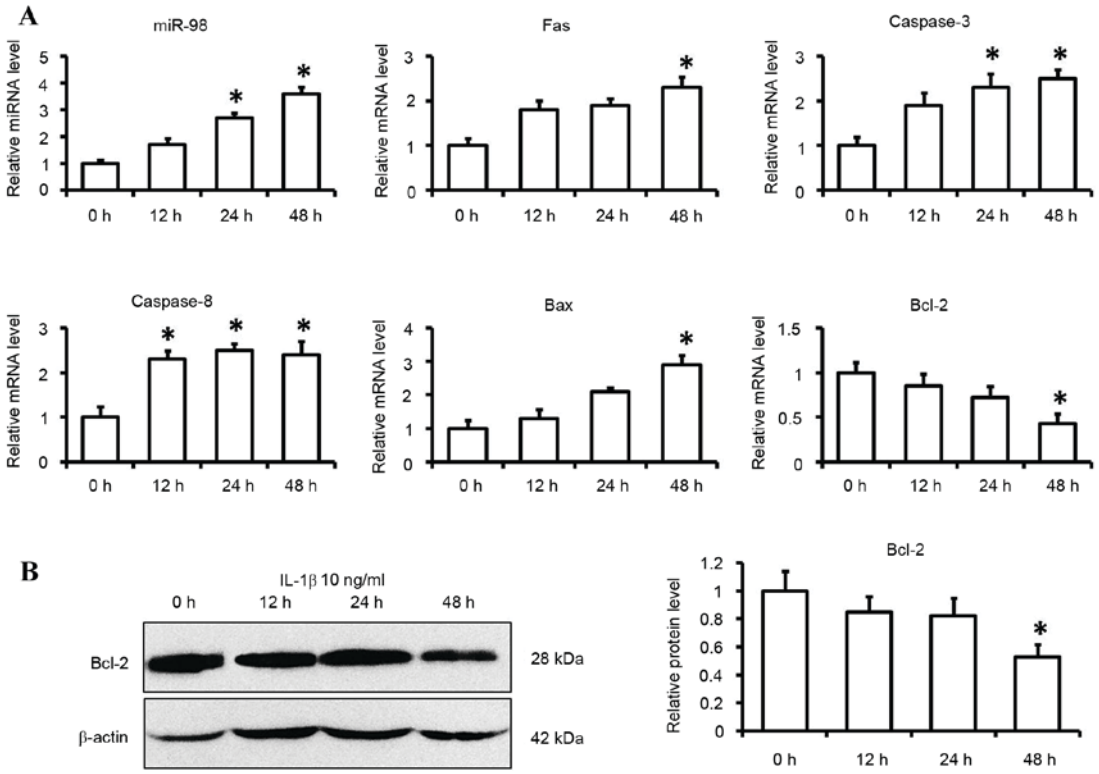

Figure 1. Expression levels of miR-98 and apoptotic factors in IL-1β-induced chondrocytes. Cells were cultured with IL-1β (10 ng/ml) or control medium for 12, 24 and 48 h. (A) miR-98, Fas, caspase-3, caspase-8, Bax and Bcl-2 mRNA expression levels were determined by reverse transcription-quantitative polymerase chain reaction using U6 and GAPDH as internal controls. (B) Bcl-2 protein levels were determined by western blotting. Each data point was normalized to the control. Data are presented as the mean ± standard error from six independent experiments. *P<0.05 vs. control group (0 h). miR-98, microRNA-98; IL-1β, interleukin 1β; Fas, Fas cell surface death receptor; Bcl-2, B-cell lymphoma 2; Bax, Bcl-2-associated X protein.

various conditions and it was determined that the rate of cell proliferation was significantly reduced following treatment withIL-1β at 24 and 48 h and this decrease was more pronounced after 48 h compared with at 24 h (P<0.05; Fig. 2B). However, transfection with the miR-98 inhibitor significantly alleviated the IL-1β-induced decrease in cell proliferation at 48 h (P<0.05; Fig. 2B).

miR-98 negatively regulatesBcl-2 expression in mouse chondrocytes. The mRNA expression levels of miR-98 and apoptotic factors were compared following exposure to IL-1β in cultured chondrocytes using RT-qPCR, and an increase inmiR-98 expression was identified with a corresponding decrease in Bcl-2 expression following exposure to IL-1β. In order to examine the effects of silencing miR-98 on Bcl-2 regulation, RT-qPCR and western blotting were performed following transfection of mouse chondrocytes with miR-98 inhibitor or mimic for 24 h. RT-qPCR (Fig. 3A) and western blotting (Fig. 3B) revealed that the miR-98 mimic significantly reduced the mRNA and protein expression levels of Bcl-2 (P<0.05). Conversely, the miR-98 inhibitor significantly alleviated the IL-1β-induced downregulation of Bcl-2 mRNA and protein expression (P<0.05; Fig. 3). These findings indicated that Bcl-2 may be a target gene of miR-98.

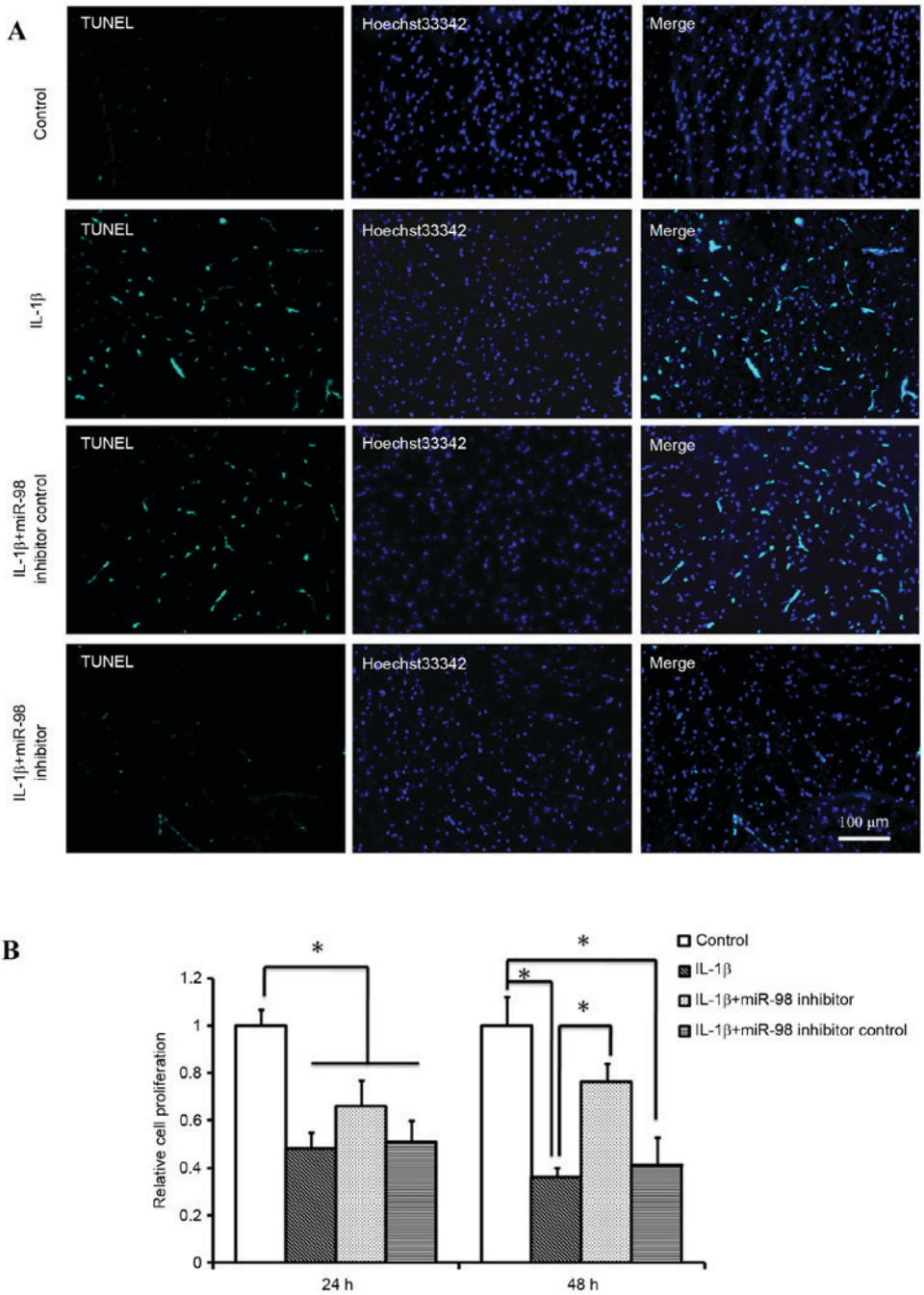

Figure 2. Effects of miR-98 on chondrocyte apoptosis and proliferation following IL-1β treatment. (A) Detection of apoptotic cells using TUNEL staining and (B) cell proliferation by MTT assay. Transfection with miR-98 inhibitor reduced the number of TUNEL-positive chondrocytes and promoted chondrocyte proliferation compared with transfection with miR-98 inhibitor control or treatment with IL-1β only (n=6/group). *P<0.05. IL-1β, interleukin 1β; TUNEL, terminal deoxynucleotidyl transferase dUTP nick end labeling; miR-98, microRNA-98.

miR-98 targets the 3'-UTR ofBcl-2 and suppresses translation. The present study hypothesized that miR-98 may potentially target Bcl-2, and the results revealed that transfection with a miR-98 inhibitor significantly increased the expression levels of Bcl-2 following treatment with IL-1β. To validate the hypothesis that miR-98 regulates Bcl-2 expression following IL-1β stimulation, miR-98 mimic/mimic control were transfected into HEK293 cells. To confirm the efficiency of transfection, the expression levels of miR-98 were quantified using RT-qPCR. The mRNA and protein expression levels of Bcl-2 were also evaluated 24 h after miR-98 mimic/mimic control transfection. The relative expression levels of miR-98 were significantly increased following transfection of HEK293 cells with miR-98 mimic (P<0.05; Fig. 4A).RT-qPCR and western blot analysis revealed that the relative expression of Bcl-2 was significantly reduced in the miR-98 mimic transfection group compared with the mimic control group (P<0.05; Fig. 4A). A dual luciferase reporter assay was performed to investigate whether miR-98 directly binds to the 3'-UTR of Bcl-2. It was determined that co-transfection with miR-98 mimic and pMIR-Bcl-2-wild type reporter plasmids significantly reduced the luciferase activity in HEK293 cells compared with in the

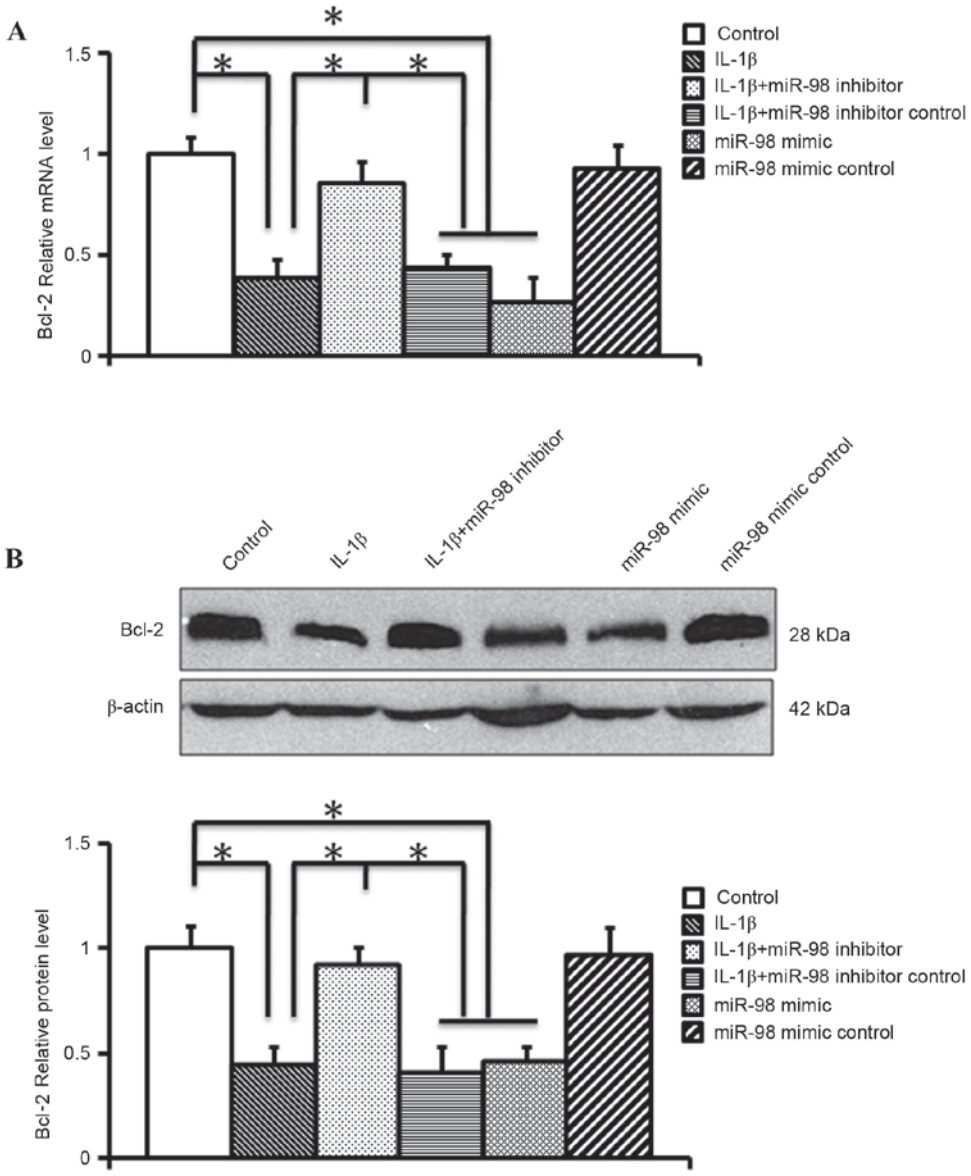

Figure 3. Analysis of the role of miR-98 in the expression of Bcl-2 in IL-1β-treated chondrocytes. (A) Reverse transcription-quantitative polymerase chain reaction analysis of the mRNA expression of Bcl-2. (B) Western blot analysis of the protein levels of Bcl-2. miR-98 inhibitor significantly alleviated IL-1β-induced downregulation of Bcl-2 at the mRNA and protein level (n=6/group). *P<0.05. Bcl-2, B-cell lymphoma 2; IL-1β, interleukin-1β; miR-98, microRNA-98.

control group (P<0.05; Fig. 4B). The results for the control luciferase assay was identical with the mimic control (data not shown).

Discussion

OA is the most prevalent arthropathy and frequently leads to chondrocyte apoptosis. Age, obesity, history of joint trauma, repeated injury, overuse and joint dysplasia are possibly involved in the etiology of OA, which may lead to serious joint pain, functional impairment or irreversible functional disability, along with reduced quality of life (19). The pathogenesis of OA is primarily associated with age and has been confirmed to increase with the median age of the population (20). Previous studies have indicated that numerous human diseases are associated with alterations in the expression of miRNAs, and several reports on miRNA profiling of human cartilage lesions have already been published (5,21). Various algorithms may be used to predict potential mRNA targets; however, only a few miRNAs have been validated and assigned to specific mRNAs. To the best of our knowledge, the present study is the first to suggest that miR-98 serves an important role in chondrocyte apoptosis by regulating Bcl-2 expression in an IL-1β conditional cultured system *in vitro*. Previous studies identified various miRNAs that may be involved in chondrogenesis and OA (14,22). The role of miR-98 in tumor apoptosis remains to be elucidated. Previous studies have reported that miR-98 expression is associated with tumor cell growth (15,23). Clinical studies have suggested that miR-98 expression is associated with head and neck cancer development (24) and may be downregulated in nasopharyngeal carcinoma (25). In addition, microarrays and qPCR have confirmed that miR-98 is upregulated in primary breast cancer specimens (26). However, contradictory findings have been reported in two other recent

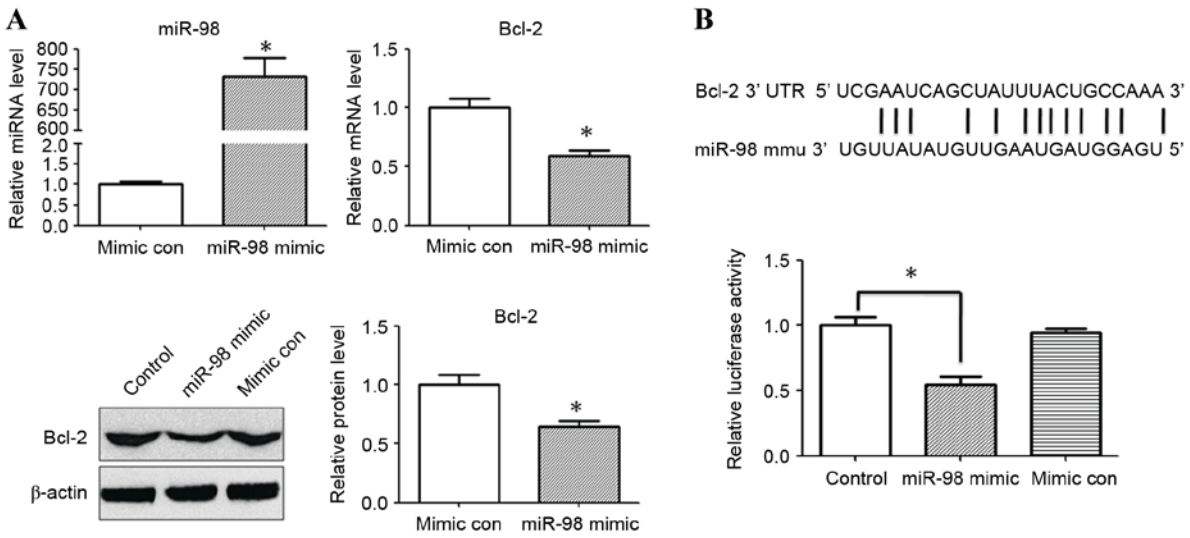

Figure 4. Bcl-2 was identified as a target gene of miR-98. Cells were transfected with 50 nM miR-98 mimic or control mimic for 24 h. (A) Reverse transcription-quantitative polymerase chain and western blotting were performed to evaluate the expression levels of miR-98 and Bcl-2. (B) Sequence alignment (miRBase sequence database; http://www.mirbase.org) of miR-98 with 3'-UTR of Bcl-2. Luciferase reporter activity was significantly decreased following transfection with the miR-98 mimic compared with in the control group (lower panel) (n=6/group). *P<0.05 vs. control group. miR-98, microRNA-98; Bcl-2, B-cell lymphoma 2; 3'-UTR, 3'-untranslated region; con, control.

studies. Chen *et al* (27) demonstrated that miR-25 was upregulated in OA chondrocytes, whereas Miyaki *et al* (28) stated that this miRNA was downregulated in OA cartilage. Using an *in vitro* model of mouse OA chondrocytes, the present study detected the expression levels of miR-98 using RT-qPCR; the results demonstrated that the expression levels of miR-98 were increased in OA chondrocytes following *in vitro* treatment with IL-1β, as previously reported (11). The expression levels of apoptotic factors following IL-1β stimulation were also detected in order to determine the effects of miR-98 on the apoptosis of mouse chondrocytes.

Removal of undesirable cells is a biological process that functions to maintain homeostasis, and is termed apoptosis or programmed cell death (29). Apoptosis is controlled by two primary molecular signaling pathways: The extrinsic and intrinsic pathways (30,31). The extrinsic apoptotic pathway refers to Fas ligand (FasL) binding to Fas receptor (32,33). This forms the death-inducing signaling complex, which contains the Fas-associated death domain, caspase-8 and caspase-10. Subsequently, cleavage and activation of executive caspase-3 is induced by cleaved caspase-8; activated capase-3 consequently cleaves DNA molecules, leading to apoptosis (34,35). It has previously been demonstrated that the Fas/FasL pathway exerts a principle role in the induction of apoptosis (36), and in many tumor and inflammation related diseases an alteration of the Fas/FasL pathway has been observed. The Bcl-2 protein family, which includes anti-apoptotic proteins, such as Bcl-2, Bcl-extra large and myeloid cell leukemia 1, and pro-apoptotic members, including Bcl-2 antagonist/killer 1 (Bak), Bax and Bcl-2-like protein 11, are important mediators of the intrinsic pathway. Overexpression of any of the Bcl-2-like pro-survival members, or the loss of the multi-Bcl-2-homology domain proteins Bak and Bax, may inhibit apoptosis through the intrinsic pathway (37). The present study used RT-qPCR to identify the mRNA expression levels of apoptotic factors. Fas, caspase-3, caspase-8 and Bax expression levels were all increased following IL-1β stimulation; however, a decrease in Bcl-2 and a corresponding increase in miR-98 expression was detected following IL-1β exposure. Western blot analysis confirmed that Bcl-2 protein expression levels were significantly reduced. In order to determine whether reduced Bcl-2 expression was regulated by the upregulation of miR-98 RT-qPCR and western blotting were performed, and revealed that IL-1β treatment reduced Bcl-2 expression via post-transcriptional gene regulation involving the activation of miR-98. A dual luciferase reporter assay confirmed that miR-98 may target the 3'-UTR of Bcl-2, which leads to translational suppression.

Chondrocytes are an important component of arthrosis, which serve a role in maintaining normal cartilage homeostasis and structural integrity, and account for only 5% of the total cartilage volume; production of the extracellular matrix and its enzymatic degradation are maintained by chondrocytes (38). The tilting of this balance in favor of catabolic events may lead to the loss of articular cartilage, as observed in OA. Therefore, maintaining the integrity of the articular cartilage is the primary function of chondrocytes. In addition, chondrocyte damage may lead to matrix degeneration, and may be associated with the onset and progression of OA (39,40). Previous studies have indicated that chondrocyte apoptosis may be associated with OA pathogenesis, and studies performed *in situ* have identified a higher number of apoptotic chondrocytes in OA compared with in normal samples (41,42). Therefore, the effective prevention of chondrocyte apoptosis may be a potential therapeutic strategy for the treatment of OA. In the present study, miR-98 inhibited the upregulation of Bcl-2 expression, which may be one of the primary causes of articular chondrocyte apoptosis. However, the function of miR-98 in chondrocyte apoptosis via the inhibition of its target genes remains to be fully elucidated, as computational analyses frequently state that one miRNA may have hundreds of target genes (43). Therefore, chondrocyte apoptosis may

be involved in numerous molecular networks associated with apoptosis and growth.

In conclusion, the present study demonstrated that the expression of miR-98 was induced by IL-1β treatment in mouse primary chondrocytes and that a miR-98 inhibitor may prevent the downregulation of Bcl-2 induced by IL-1β in chondrocytes. These results suggested that silencing miRNA-98 may prevent chondrocyte apoptosis.

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