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ORIGINAL ARTICLE

Sinomenine Attenuates Cartilage Degeneration by Regulating miR-223-3p/NLRP3 Inflammasome Signaling

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Abstract— Sinomenine (SIN) has been shown to protect against IL-1β-induced chondrocyte apoptosis *in vitro*. However, the role of SIN in the anterior cruciate ligament transection (ACLT)-induced osteoarthritis (OA) mouse model and its underlying molecular mechanisms remain unclear. In the present study, the protective effect of SIN on ACLT-induced articular cartilage degeneration and IL-1β-induced chondrocyte apoptosis miR-223-3p/NLRP3 signaling regulation was investigated. Safranin O staining was performed to evaluate the pathological changes of articular cartilage. Chondrocyte apoptosis was measured with Annexin V-fluorescein isothiocyanate/polyimide (annexin V-FITC/PI) staining using flow cytometry. Gene and protein expression were detected by RT-qPCR and Western blotting, respectively. SIN administration markedly improved articular cartilage degradation in mice undergoing ACLT surgery. In addition, SIN treatment downregulated the levels of inflammatory cytokines and the protein expression of NLRP3 inflammasome components and upregulated the expression of miR-223-3p in OA mice and IL-1β-stimulated chondrocytes. *In vitro*, we found that NLRP3 was a direct target of miR-223-3p, and overexpression of miR-223-3p blocked IL-1β-induced apoptosis and the inflammatory response in chondrocytes. These findings indicate that miR-223-3p/NLRP3 signaling could be used as a potential target of SIN for the treatment of OA.

KEY WORDS: sinomenine; osteoarthritis; miR-223-3p; NLRP3; chondrocyte.

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Abbreviations: SIN, sinomenine; ACLT,anterior cruciate ligament transection; OA, osteoarthritis; TNF-α, tumor necrosis factor-α; IL-1β, interleukin-1β; TLR4, toll-like receptor 4; NF-κB, nuclear factor kappa B; TRAF6, tumor necrosis factor receptor-associated factor 6; NLRP3, NOD-like receptor family, pyrin domain containing 3; ASC, caspase-recruitment domain; 3′-UTR, 3′-untranslated regions

INTRODUCTION

Osteoarthritis (OA) is one of the most common diseases worldwide, particularly among the elderly, and it is characterized by degradation of articular cartilage, thickening of subchondral bone and formation of osteophytes, leading to severe joint pain and loss of function [1]. In general, aging, vitamin, and mineral deficiencies and the incidence of drug abuse lead to a markedly increased risk of OA development [2]. Although OA has high morbidity, there is currently no effective therapeutic strategy for postponing the progression of OA, which may be attributed to the lack of exact OA etiology. Currently, conventional therapeutic medications, including analgesics and

nonsteroidal anti-inflammatory drugs, are widely applied in the clinic for the treatment of OA, while emerging side effects, such as serious gastrointestinal, cardiovascular, and renal adverse events, limit their clinical applications [3, 4]. Nevertheless, anti-inflammatory drugs are still an interesting avenue for OA treatment. Accumulated evidence shows that inflammatory cytokines, including tumor necrosis factor-α (TNF-α), interleukin-1β (IL-1β) and IL-6, and inflammation signaling pathways, including toll-like receptor 4 (TLR4), nuclear factor-kappa B (NF-κB), and tumor necrosis factor receptor-associated factor 6 (TRAF6), are activated during the progression of OA [5–7]. Therefore, it is urgent to investigate potential therapeutic medications and their anti-inflammatory molecular mechanisms, which may be effective approaches to ameliorate undesirable OA situations in clinical practice.

Recently, traditional herbs and their bioactive components, including baicalin [8], cryptotanshinone [9], and curcumin [10], have received considerable attention because of their anti-inflammatory biological effects and low toxicity in the treatment of OA. Sinomenine (7,8-didehydro-4-hydroxy-3,7-dimethoxy-17-methylmorphinane-6-one; SIN), as the main bioactive ingredient of *Sinomenium acutum*, has a variety of biological functions, including anti-inflammatory [11], anti-oxidative [12], and anti-neoplastic activities [13]. *In vivo* and *in vitro* studies indicate that SIN has a beneficial effect on cartilage degradation and protects against IL-1β-induced chondrocyte apoptosis [14]. However, the underlying molecular mechanisms of SIN have not been clarified in the progression of OA and chondrocyte apoptosis.

Recently, a few reports have revealed that the NOD-like receptor family, pyrin domain-containing 3 (NLRP3) inflammasome, which contains a caspase-recruitment domain (ASC), procaspase-1, and NLRP3, has been implicated in the pathogenesis of OA [10, 15, 16]. Canonical NLRP3 inflammasome signaling is activated by the assembly of the TLR4/NF-κB and NLRP3/ASC/procaspase-1 complex, which processes pro-IL-1β and pro-IL-18 into their mature active forms of IL-1β and IL-18 that are secreted and induce the inflammatory response [17, 18]. Further studies show that the NLRP3-modulated inflammatory response can be regulated by microRNAs (miRs) at the posttranscriptional level by binding to the 3′-untranslated region (3′-UTR) of NLRP3, resulting in the downregulation of NLRP3 expression at the level of transcription or translation [19–21]. Among the miRs, miR-223, as a mainly posttranscriptional regulator, functions as an important rheostat controlling NLRP3 inflammasome activity [19, 20]. Intriguingly, upregulation of miR-223 can prevent IL-1β-induced degradation of chondrocyte extracellular matrix in chondrocytes [22]. Notably, the role played by miR-223 in the progression of OA by targeting NLRP3 remains unclear. The aim of the present study was to investigate whether SIN has a protective effect on articular cartilage by regulating the miR-223-3p/NLRP3 signaling pathway. The roles of SIN were investigated *in vivo* using a mouse model of anterior cruciate ligament transection (ACLT)-induced experimental OA and *in vitro* using an IL-1β-induced cell model in chondrocytes to mimic the progression of OA.

MATERIALS AND METHODS

Animal Experiments

Male 8-week-old ICR mice ($n = 36$; 20 ± 2 g) were acquired for the Experimental Animal Center of Dalian Medical University (Dalian, China) and were allowed to acclimate to the environment for 1 week. Subsequently, they were given free access to food and tap water and were caged individually under a controlled temperature (23 ± 2 °C) and humidity ($55 \pm 5\%$) with an artificial 12 h light/dark cycle. All experimental procedures were conducted in accordance with the guidelines of the Dalian Medical University (Dalian, China) on animal care. The anterior cruciate ligament (ACL) was transected to induce OA of the left knee. Animals were sacrificed at 12 weeks post-surgical operation, and samples of the knee joints were collected for further molecular and histological analyses. The mice were randomly divided into three groups: (i) sham group, mice that underwent the control operation, which was completed by opening the joint capsule and then suturing the incision in the left knee of each mouse ($n = 12$); (ii) OA group, mice that underwent the ACLT operation ($n = 12$); and (iii) SIN group, mice that underwent ACLT operation in combination with SIN (100 mg/kg; Hunan Zhengqing Pharmaceutical Co. Ltd., Huaihua, Hunan, China) intraperitoneal injection ($n = 12$).

Safranin O Staining

Following fixation in 4% formaldehyde for 7 days, the tibia specimens were decalcified in 0.5 M EDTA (pH = 8.0) and then embedded in paraffin by standard histological procedures. Sections of 5 μm thickness were cut and stained with a Safranin O staining kit (Nanjing SenBeiJia Biological Technology Co., Ltd., China) and visualized under a microscope (Leica DM 2500; Leica Microsystems GmbH, Wetzlar, Germany). The Osteoarthritis Research

Society International (OARSI) score was performed to assess cartilage damage as previously described [23].

Enzyme-Linked Immunosorbent Assay

The levels of inflammatory cytokines, TNF-α, IL-1β, IL-6, and IL-18, in the serum and supernatant were measured by ELISA kit (Elabscience Biotechnology Co., Ltd., Wuhan, China) on a SpectraMax M5 ELISA plate reader (Molecular Devices, LLC, Sunnyvale, CA, USA) according to the manufacturer's instructions.

Western Blotting

Protein was extracted in NP-40 buffer (Beyotime Institute of Biotechnology, Haimen, China). Western blotting was performed as described previously [24]. The primary antibodies purchased were as follows: NF-κB/p65 (cat. no. 3034; dilution 1:500; Cell Signaling Technology, Inc., USA), NF-κB/p-p65 (cat. no. 3037; dilution 1:500; Cell Signaling Technology, Inc., USA), NLRP3 (cat. no. ab214185; dilution 1:1000; Abcam, Cambridge, UK), ASC (cat. no. GTX105780; dilution 1:1000; GeneTex, Southern California, USA), caspase-1 (cat. no. 2225; dilution 1:2000; Cell Signaling Technology, USA), IL-1β (cat. no. sc-12742; dilution 1:1000; Santa Cruz Biotechnology, Santa Cruz, CA, USA), and IL-18 (cat. no. ESAP10527; dilution 1:1000; Elabscience Biotechnology Co., Ltd., Wuhan, China). After primary antibody incubation, the membranes were incubated with the appropriate horseradish peroxidase-conjugated secondary antibody (dilution 1:10,000; Santa Cruz Biotechnology) at room temperature for 2 h and visualized by chemiluminescence (Thermo Fisher Scientific, Inc.). Signals were analyzed with Quantity One® software version 4.5 (Bio-Rad Laboratories, Inc., Hercules, CA, USA). β-Actin (cat. no. sc-130065; dilution 1:2000; Santa Cruz Biotechnology) was used as the control antibody.

Reverse Transcription-Quantitative Polymerase Chain Reaction

Total RNA was extracted using TRIzol® (Invitrogen; Thermo Fisher Scientific, Inc., Waltham, MA, USA) according to the manufacturer's protocol. TaqMan® RT (Applied Biosystems; Thermo Fisher Scientific, Inc.) and TaqMan® MicroRNA assay kits (Applied Biosystems; Thermo Fisher Scientific, Inc.) were used to perform reverse transcription-quantitative polymerase chain reaction (RT-qPCR) of miR-223-3p, according to the manufacturer's protocol. U6 small nuclear RNA was used as an endogenous control.

Total RNA (2 μg) was used to synthesize cDNA with Moloney murine leukemia virus reverse transcriptase (Invitrogen; Thermo Fisher Scientific, Inc.). RT-qPCR was performed by Applied Biosystems 7300 Real-Time PCR System (Thermo Fisher Scientific, Inc.) with the TaqMan Universal PCR Master Mix (Thermo Fisher Scientific, Inc.). The relative mRNA expression levels were calculated using the $2^{-\Delta\Delta Cq}$ method [25] and normalized to glyceraldehyde 3-phosphate dehydrogenase (GAPDH). The primers were as follows: miR-223-3p forward 5′-GCGT GTATTTGACAAGCTGAGTT-3′ and reverse 5′-GTGTCAGTTTGTCAAATACCCCA-3′; U6 forward 5′-GCTTCGGCAGCACATATACTAAAAT-3′ and reverse 5′-CGCTTCACGAATTTGCGTGTCAT-3′; NLRP3 forward 5′-AAAGCCAAGAATCCACAGTG TAAC-3′ and reverse 5′-TTGCCTCGCAGGTA AAGGT-3′; and GAPDH forward 5′-ACAG GGGAGGTGATAGCATT-3′ and reverse 5′-GACC AAAAGCCTTCATACATCTC-3′.

Cell Culture

Murine primary chondrocytes were isolated from postnatal mice (days 5 to 6), as previously described [26]. Cells were cultured in Dulbecco's modified Eagle's medium (DMEM; Invitrogen, Carlsbad, CA, USA) with 5% fetal bovine serum (Thermo Scientific HyClone, Beijing, China), 5% CO_2, and a 95% air atmosphere in a humidified incubator (Thermo, USA).

Luciferase Reporter Gene Assay

The sequences of miR-NC, miR-223-3p mimics, scrambled, and miR-223-3p inhibitors were synthesized by RiboBio (Guangzhou, China) and transfected into chondrocytes using Lipofectamine™ RNAiMAX (Life Technologies, USA) according to the manufacturer's instructions. The wild-type (WT) or mutant-type (MUT) 3′-UTR of NLRP3 was inserted into the multiple cloning sites of the luciferase-expressing pMIR-REPORT vector (Ambion; Thermo Fisher Scientific, Inc.). The luciferase activity was measured using the Dual-Luciferase Reporter® Assay System (cat.no: E1960; Promega, USA) on a Luminoskan™ Ascent Microplate Luminometer (Thermo Fisher Scientific, Waltham, MA, USA).

3-(4,5-Dimethyl-2-yl)-2,5-Diphenyltetrazolium Bromide (MTT) Assay for Cell Viability

Cell viability was monitored using the MTT assay (Beyotime Institute of Biotechnology, Haimen, China), as described previously [27]. The absorbance at 490 nm was obtained using a SpectraMax M5 ELISA plate reader (Molecular Devices, LLC, Sunnyvale, CA, USA).

Flow Cytometry for Apoptosis

An Annexin V-FITC/PI apoptosis detection kit was purchased from Invitrogen (Carlsbad, CA, USA). Cell apoptosis assays were performed by flow cytometry (FACScan, BD Biosciences, San Jose, CA, USA) and analyzed by CELL Quest 3.0 software (BD Biosciences).

Statistical Analysis

Data are presented as the mean ± standard deviation for each group. All statistical analyses were performed using PRISM version 7.0 (GraphPad Software, Inc., La Jolla, CA, USA). Intergroup differences were analyzed by one-way analysis of variance, followed by *post hoc* Tukey's test for multiple comparisons. $P < 0.05$ was considered to indicate a statistically significant difference.

RESULTS

SIN Alleviates Articular Cartilage Degeneration in a Mouse Model of OA

To investigate whether SIN has a beneficial effect on articular cartilage, the ACL was transected to induce abnormal OA of the knee in a mouse model, and safranin O staining was performed to evaluate the pathological changes of articular cartilage in the proximal tibia. The results demonstrated that smooth and intact cartilage surfaces were observed in the sham-operated mice. The articular cartilage in ACLT mice exhibited accelerated proteoglycan loss compared with that of the mice in the sham group. However, SIN treatment markedly improved articular cartilage degradation in the mice undergoing surgery (Fig. 1a, b).

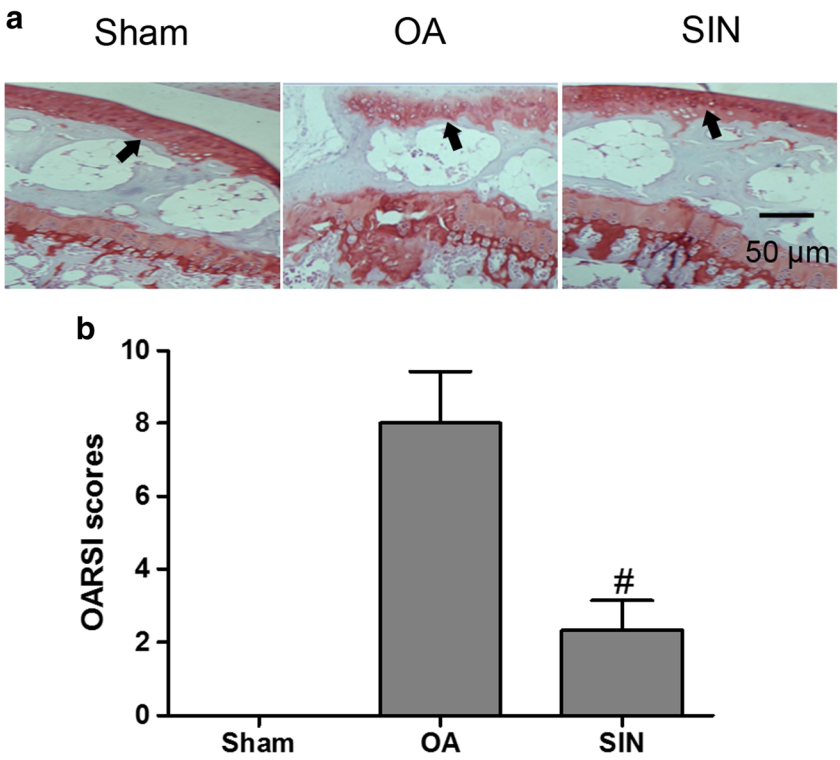

Fig. 1. SIN alleviates articular cartilage degeneration in a mice model of OA. Articular cartilage degeneration in ACL-induced OA is measured by safranin O staining (magnification × 100; **a**) in a mice model with or without SIN treatment. Osteoarthritis Research Society International (OARSI) scores demonstrated a drastic increase in cartilage damage in OA mice (**b**). $^{\#}P < 0.05$ compared with OA group; $n = 6$ in each group.

SIN Blocks the OA-Induced Inflammatory Response

To determine whether inflammation is a potential pathogenic factor of OA-related articular cartilage injury, we performed ELISA assays to measure inflammatory cytokine (TNF-α, IL-1β, IL-6, and IL-18) levels in serum from OA mice. Notably, the upregulation of TNF-α (Fig. 2a), IL-1β (Fig. 2b), IL-6 (Fig. 2c), and IL-18 (Fig. 2d) in serum was detected in ACLT-operated mice compared to that of the mice in the sham group. However, the upregulation of TNF-α, IL-1β, IL-6, and IL-18 was significantly reversed by SIN administration in ACLT-operated mice.

SIN Inhibits NLRP3 Inflammasome Signaling in OA Mice

Evidence suggests that NLRP3 can directly contribute to the pathogenesis of OA, leading to cartilage degradation and synovial inflammation [15]. Consistent with these reports, we identified that the protein expression levels of NLRP3, ASC, and caspase-1 were significantly upregulated in the proximal tibia of ACLT-operated mice compared with the sham group. However, the increase in NLRP3, ASC, and caspase-1 was reversed by SIN administration in the proximal tibia of ACLT-operated mice (Fig. 3a). NF-κB plays a crucial role in the NLRP3 inflammasome-modulated inflammatory response, including the enhancement of NLRP3 transcription and acceleration of pro-IL-1β and pro-IL-18 production [28]. Ultimately, overactivation of NLRP3 inflammasome signaling activates caspase-1, which causes maturation of the pro-inflammatory cytokines IL-1β and IL-18 [28]. In the present study, we found NF-κB/p-p65 (Fig. 3b), IL-1β, and IL-18 (Fig. 3c) protein expression to be more susceptible to ACLT operation, while the upregulation of p-p65, IL-1β, and IL-18 could be partly blocked by SIN.

NLRP3 Is a Direct Target of miR-223-3p

We next investigated the molecular mechanisms by which SIN suppressed NLRP3 inflammasome signaling *via* a posttranscriptional regulatory mechanism. Previous studies have validated that miR-223 can reduce inflammation by targeting NLRP3 [19, 20]. However, the roles miR-223 plays in targeting NLRP3 in the pathogenesis of OA remain to be elucidated. First, we found that the expression of miR-223-3p was significantly reduced in the tibiae of ACLT-operated mice compared to the mice in the sham-operated group, while SIN treatment evoked the expression of miR-223-3p in OA mice (Fig. 4a). To investigate whether NLRP3 was a direct target of miR-223-3p, we performed a bioinformatics analysis to predict the potential binding sites between miR-223-3p and NLRP3. The results revealed that NLRP3 RNA contained one conserved target site for miR-223-3p, and we found that NLRP3 was a candidate target gene for miR-223-

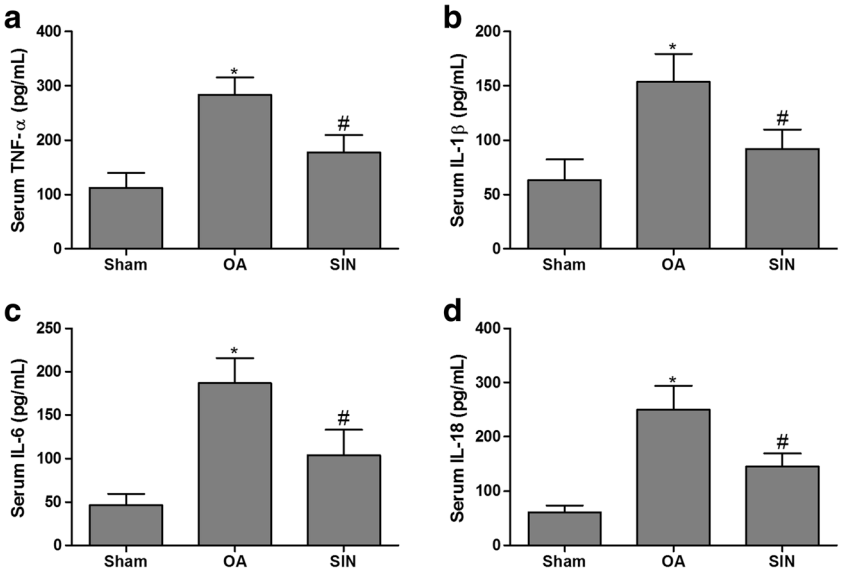

Fig. 2. SIN blocks OA-induced inflammatory response. ELISA assays are performed to measure serum levels of TNF-α (**a**), IL-1β (**b**), IL-6 (**c**), and IL-18 (**d**). $^{*}P < 0.05$ compared with sham group; $^{\#}P < 0.05$ compared with OA group; $n = 3$ in each group.

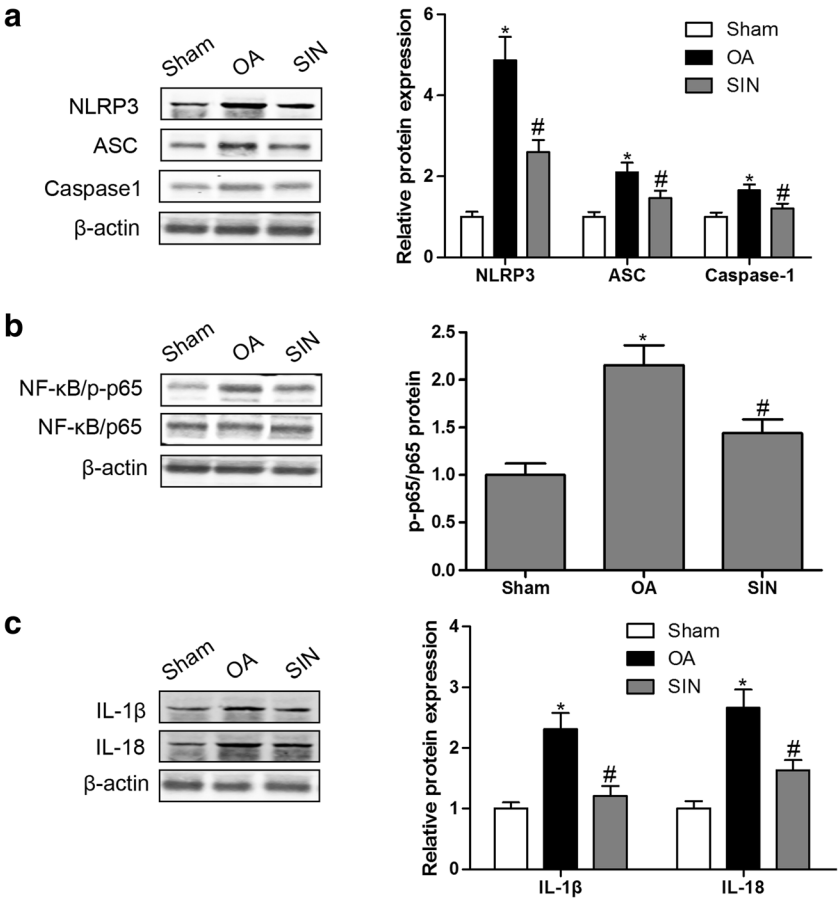

Fig. 3. SIN inhibits NLRP3 inflammasome signaling in OA mice. The protein expression of NLRP3, ASC, and caspase-1 (**a**), p-p65 and p65 (**b**), and IL-1β and IL-18 (**c**) is measured by western blotting. $^{*}P < 0.05$ compared with sham group; $^{\#}P < 0.05$ compared with OA group; $n = 6$ in each group.

3p (Fig. 4b). To further explain this assumption, we constructed luciferase reporter vectors containing either the WT or MU 3'UTR of NLRP3, which was cotransfected with miR-223-3p mimics or miR-Con. We performed a dual-luciferase reporter assay to examine the relative luciferase activities after 48 h transfection. Our findings showed that miR-223-3p mimics significantly reduced the luciferase activity compared with the control group in chondrocytes containing the WT 3′UTR of NLRP3 (Fig. 4c). However, miR-223-3p mimics had no obvious effect on the luciferase activity in chondrocytes containing the MU 3′UTR of NLRP3 (Fig. 4c). RT-qPCR and Western blotting assays demonstrated that miR-223-3p mimics significantly downregulated and miR-223-3p inhibitors significantly upregulated the mRNA and protein expression of NLRP3 in the chondrocytes (Fig. 4d, e). These findings suggest that NLRP3 is a direct target of miR-223-3p.

SIN Reverses IL-1β-Induced Growth Inhibition and Apoptosis in Chondrocytes by Regulating miR-223-3p/NLRP3 Signaling

The cytotoxicity tests showed that a concentration of SIN less than 100 μM had no toxic effects on chondrocyte growth after 48 h of treatment. However, the concentration of SIN at 200 and 500 μM showed a significant reduction of proliferation in chondrocytes. Interestingly, IL-1β-induced inhibition of chondrocyte growth was reversed by SIN treatment at a concentration of 100 μM (Fig. 5a). We also found that IL-1β-induced apoptosis in chondrocytes could be retarded by SIN treatment (Fig. 5b, c). Mechanistically, we found that the expression of miR-223-3p was markedly reduced in chondrocytes with IL-1β stimulation (Fig. 6a). In addition, NLRP3 inflammasome signaling was activated, reflecting that the protein expression of NLRP3 (Fig. 6b, c), ASC (Fig. 6b, c),

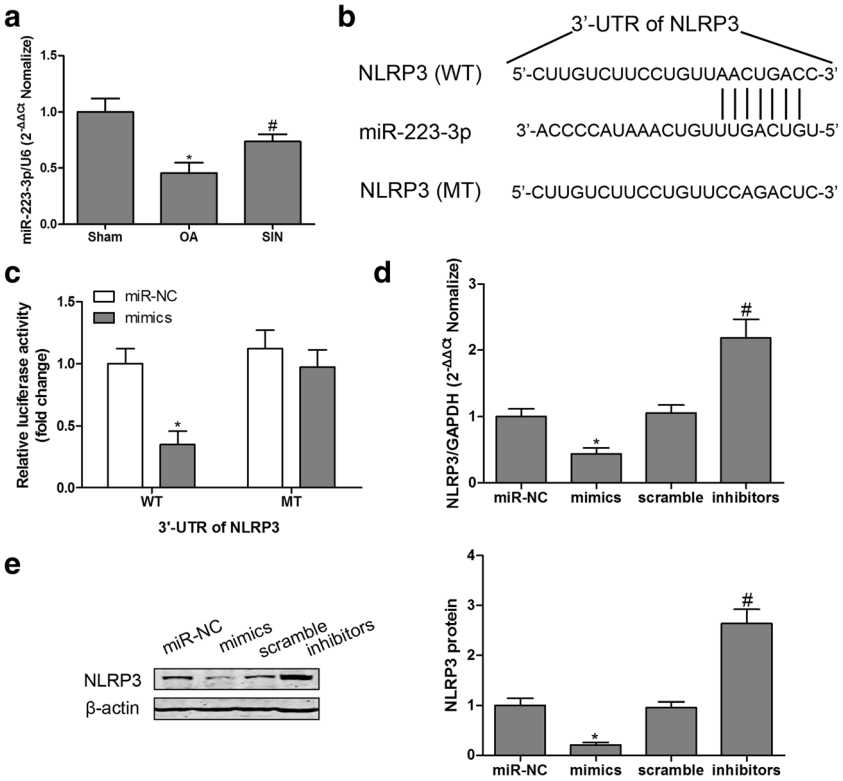

Fig. 4. NLRP3 is a direct target of miR-223-3p. The expression of miR-223-3p is measured by RT-qPCR (**a**). Schematic represents the putative binding sites of miR-223-3p in the 3′-UTR of NLRP3 (**b**). Luciferase activity assay is measured in mice chondrocytes (**c**). After transfected with miR-223-3p mimics or inhibitors, the mRNA (**d**) and protein (**e**) expression of NLRP3 are measured by RT-qPCR and western blotting, respectively. $^{*}P < 0.05$ compared with sham or miR-NC group; $^{\#}P < 0.05$ compared with OA or scramble group.

and caspase-1 (Fig. 6b, e) was increased in chondrocytes with IL-1β stimulation. However, SIN administration reversed the upregulation of NLRP3, ASC, and caspase-1 and the downregulation of miR-223-3p in IL-1β-treated chondrocytes. Furthermore, our results revealed that overexpression of miR-223-3p inhibited IL-1β-induced apoptosis (Fig. 6f, g) and the upregulation of IL-1β and IL-18 levels (Fig. 6h).

DISCUSSION

The results of the present study indicated that miR-223-3p was downregulated and NLRP3 inflammasome signaling was activated in the tibiae of ACLT-operated mice and IL-1β-stimulated chondrocytes. In addition, overexpression of miR-223-3p was demonstrated to inhibit IL-1β-induced apoptosis and inflammation in chondrocytes. Notably, SIN showed a beneficial effect on ACLT-induced articular cartilage degeneration in mice and inhibited IL-1β-induced growth inhibition and apoptosis in chondrocytes; the underlying mechanism was mediated, at least partially, through the activation of miR-223-3p, which subsequently suppressed NLRP3 inflammasome signaling.

Cartilage erosion is a prominent feature of OA, and in this process, inflammation plays a crucial role in promoting metabolic disorder and apoptosis in chondrocytes [8, 29]. Previous studies indicate that chondrocytes are highly responsive to inflammatory cytokines, including IL-1β and TNF-α, which can accelerate the catabolism of chondrocytes by upregulating matrix metalloproteinase activity and degrading type II collagen [8, 30]. On the other hand, chondrocyte apoptosis is usually induced in inflammatory conditions, reflecting the increased DNA fragmentation and caspase-3 activity [14, 31]. Consistent with these findings, our results also found a significant increase in chondrocyte apoptosis when exposed to IL-1β. However, both overexpression of miR-223-3p and SIN inhibited IL-1β-induced chondrocyte apoptosis.

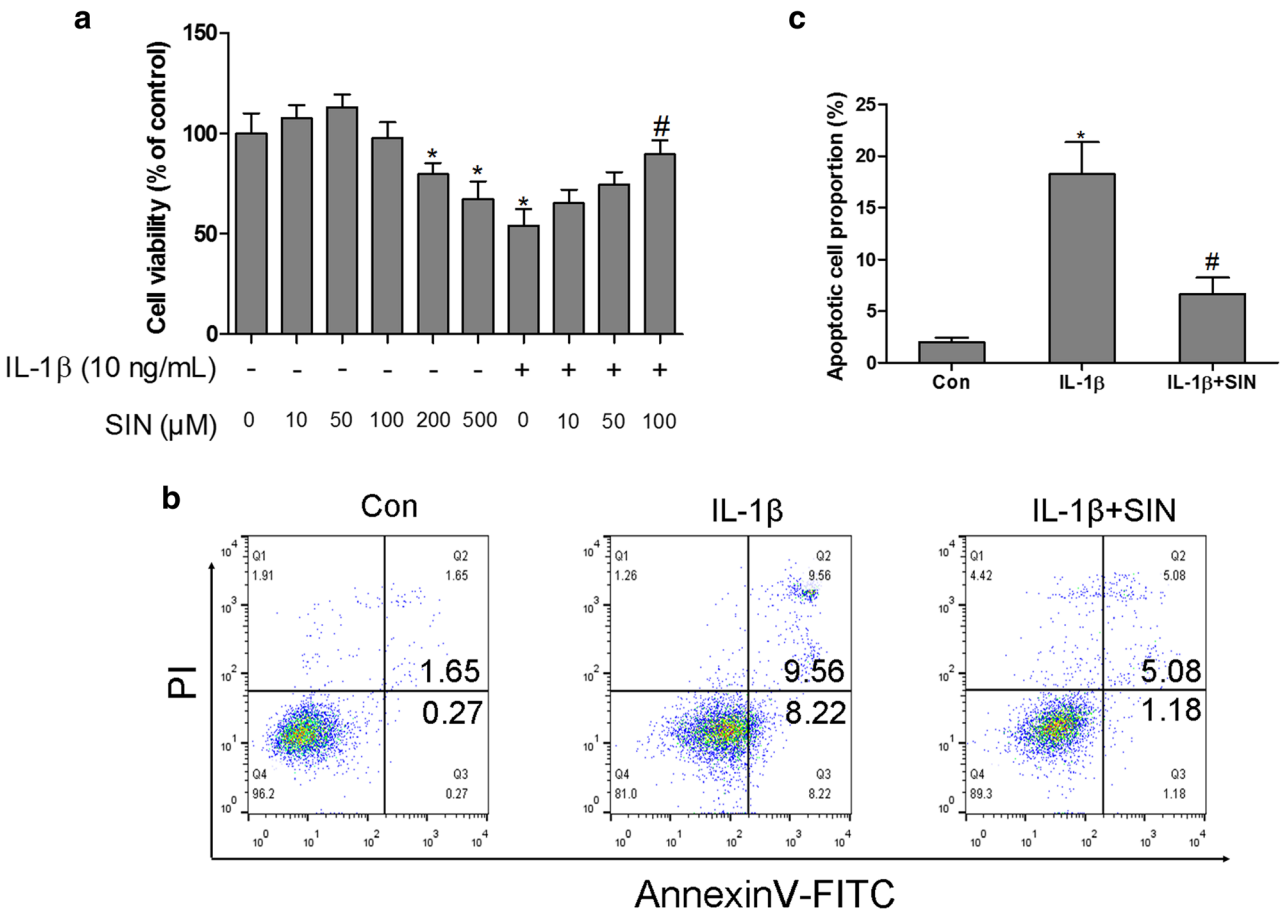

Fig. 5. SIN inhibits IL-1β-induced apoptosis in chondrocytes. Chondrocyte exposure to SIN, IL-1β, or IL-1β combined with SIN, and cell viability is detected using MTT assay (**a**); chondrocyte apoptosis is monitored by flow cytometry (**b**, **c**). $^{*}P < 0.05$ compared with control group; $^{\#}P < 0.05$ compared with IL-1β treatment group; $n = 3$ in each group.

In the present study, we found that overactivation of NLRP3 inflammasome signaling was closely associated with ACLT-induced articular cartilage degeneration and IL-1β-induced chondrocyte apoptosis. By activating caspase 1, the NLRP3 inflammasome causes the maturation of the pro-inflammatory cytokines IL-1β and IL-18, which are secreted into the extracellular matrix and induce the inflammatory response, leading to a rapid form of inflammatory cell death called pyroptosis [15, 32]. An NLRP3-activating mutation leads to growth plate dysplasia with loss of chondrocytes and growth arrest in the epiphyses of neonatal-onset multisystem inflammatory disease mice [33, 34]. Nasi et al. indicated that NLRP3, IL-1α, or IL-1β knockout exacerbates or has no effect on meniscectomy-induced OA in a murine model, suggesting that NLRP3, IL-1α, or IL-1β are not involved in the pathogenesis of OA [35]. Bougault et al. showed that stress-induced cartilage degradation might be independent of NLRP3 inflammasome activity [36]. Clavijo-Cornejo et al. showed that the protein expression of NLRP3 was 5.4-fold higher in synovial membranes from patients with knee OA than in the control group [37]. In our study, NLRP3 protein expression was shown to be respectively increased 4.9-fold and 3.3-fold in the tibiae of ACLT-induced posttraumatic OA mice and IL-1β-treated chondrocytes compared with the corresponding control group. The differentially expressed NLRP3 in the above studies may be associated with different animal models and pathological tissue, such as tibia, knee, and synovial fluid. In addition, blockage of NLRP3 inflammasome signaling by SIN attenuated articular cartilage degeneration in the ACLT rodent model of OA.

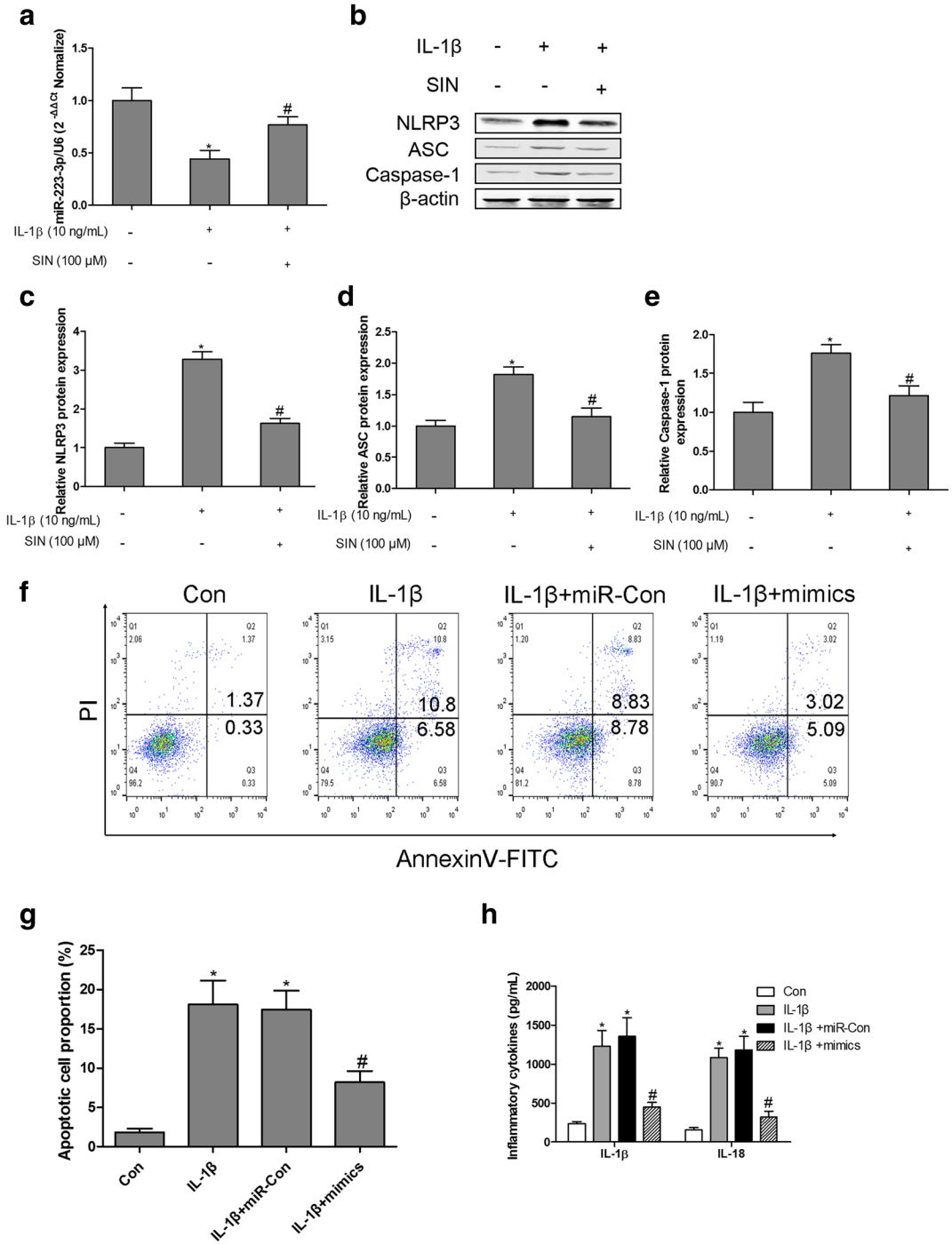

Fig. 6. SIN reverses IL-1β-induced growth inhibition and apoptosis in chondrocytes by regulating miR-223-3p/NLRP3 signaling. The expression of miR-223-3p is measured by RT-qPCR (**a**). The protein expression of NLRP3 (**b**, **c**), ASC (**b**, **d**), and caspase-1 (**b**, **e**) is measured by western blotting. After transfected with miR-223-3p mimics, the cell apoptosis of IL-1β-stimulated chondrocytes is monitored by flow cytometry (**f**, **g**); IL-1β and IL-18 levels (**h**) in the supernatant are detected by ELISA assay. $^{*}P < 0.05$ compared with control group; $^{\#}P < 0.05$ compared with IL-1β treatment group; $n = 3$ in each group.

Mechanistically, our findings revealed that miR-223-3p, as a posttranscriptional regulator targeting NLRP3, inhibited cartilage degeneration *in vivo* and IL-1β-induced chondrocyte apoptosis *in vitro*. In a recent study, miR-223-3p was shown to suppress ischemia/reperfusion-induced cardiac necroptosis by directly inhibiting the expression of NLRP3 [38]. Moreover, overexpressed miR-223-3p could attenuate inflammation-associated cytokines, NLRP3, IL-1β, and IL-18, to inhibit cell proliferation and migration in glioblastomas [39]. Overexpressed miR-223-3p protects against mitochondrial damage-associated molecular pattern-induced acute lung injury by inhibiting NLRP3 inflammasome signaling [40]. These results suggest that miR-223-3p plays a beneficial role in inflammation-induced organ damage and cell death. Here, we report, for the first time, that overexpression of miR-223-3p *in vitro* inhibits IL-1β-induced chondrocyte apoptosis *via* inactivation of the NLRP3-mediated inflammatory response.

In conclusion, NLRP3, as one of the key cytokines responsible for articular cartilage degeneration and chondrocyte apoptosis, could be inhibited by SIN and overexpression of miR-223-3p. In addition, SIN administration increased miR-223-3p expression *in vivo* and *in vitro* to block the progression of OA. These results suggest that miR-223-3p/NLRP3 could be used as a potential target of SIN for the treatment of OA.

AUTHORS' CONTRIBUTIONS

Study design: HAI-CAO DON; Literature research, Data acquisition, and Data analysis: HAI-CAO DON, PEI-NAN LI, CHANG-JIAN CHEN, XIN XU, HONG ZHANG, GANG LIU, LIAN-JIE ZHENG, PENG LI; Manuscript preparation and Manuscript editing: HAI-CAO DONG, PEI-NAN LI and CHANG-JIAN CHEN; Manuscript review: T HAI-CAO DON; Cell experiments: CHANG-JIAN CHEN, XIN XU and HONG ZHANG; Animal experiments: GANG LIU, LIAN-JIE ZHENG and PENG LI. All authors read and approved the final manuscript.

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COMPLIANCE WITH ETHICAL STANDARDS

Ethics Approval and Consent to Participate. This study was permitted by the Ethics Committee of the Dalian Medical University (Dalian, China) on June 1st in 2017 (Approval number 20170019a).

Conflict of Interest. The authors declare that they have no competing interests.

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