ABBS

Acta Biochim Biophys Sin, 2015, 47(6), 422–430
doi: 10.1093/abbs/gmv033
Advance Access Publication Date: 29 April 2015
Original Article

OXFORD

Original Article

Poly(ADP-ribose) polymerase 1 inhibition prevents interleukin-1β-induced inflammation in human osteoarthritic chondrocytes

Yujie Sun†, Lugang Zhou†, Dongmei Lv, Hongzhi Liu, Tian He, and Xin Wang*

Orthopedics Department, Yantai Yuhuangding Hospital, Affiliated by Qingdao University Medical College, Yantai 264000, China

†These authors contributed equally to this work.
*Correspondence address. Tel: +86-535-6270526; Fax: +86-535-6618738; E-mail: wangxinqd@sina.cn

Received 12 December 2014; Accepted 3 March 2015

Abstract

Osteoarthritis (OA) is an age-related joint disease that is characterized by the degeneration of articular chondrocytes. Nuclear enzyme poly(ADP-ribose) polymerase 1 (PARP-1) is associated with inflammation response. We investigated the role of PARP-1 in interleukin-1β (IL-1β)-stimulated human articular chondrocytes and its underlying mechanism. Cell viability and apoptosis were evaluated by using 3-(4,5)-dimethylthiahiazo(-z-y1)-3,5-di-phenytetrazoliumromide assay and flow cytometry, respectively. Tumor necrosis factor-α (TNF-α) level was measured by enzyme-linked immunosorbent assay. The mRNA and protein expression levels of PARP-1, IL-1 receptor (IL-1R), inducible nitric oxide synthase (iNOS), matrix metalloproteinases (MMPs), and tissue inhibitor of metalloproteinases-1 (TIMP-1) were determined by real-time reverse transcriptase-polymerase chain reaction and western blot analysis, respectively. The expression and phosphorylation of NF-κB p65 were measured by western blot analysis. Results showed that stimulation of chondrocytes with IL-1β caused a significant up-regulation of PARP-1 and IL-1R, resulting in NF-κB p65 nuclear translocation and phosphorylation associated with an increase of TNF-α secretion and iNOS expression. PARP-1 was inhibited by siRNA transfection. Results showed that PARP-1 inhibition suppressed IL-1β-induced reduction of cell viability and up-regulation of cell apoptosis, with a reduced IL-1R expression. PARP-1 inhibition also effectively reversed IL-1β-induced inflammatory response through inhibiting the IL-1R/NF-κB pathway. These data suggested that PARP-1 inhibition prevents IL-1β-induced inflammation response at least partly by inhibiting the IL-1R/NF-κB signaling pathway in human articular chondrocytes. Moreover, PARP-1 inhibition reduced MMPs expression and increased TIMP-1 expression, suggesting that PARP-1 inhibition could suppress cartilage destruction by modulating the balance between MMPs and TIMP-1. Inhibition of PARP-1 might be useful in the treatment of OA.

Key words: poly(ADP-ribose) polymerase 1, chondrocyte, osteoarthritis, interleukin-1β, inflammation response, cartilage destruction

Introduction

Osteoarthritis (OA) is an age-related degenerative disease of joint [1]. Cartilage degradation is the main pathological change which is characterized by extracellular matrix (ECM) damage and tissue cellularity loss. Chondrocytes are the only cells of articular cartilage responsible for the maintenance of ECM. Inflammation plays an important role in the development and progression of OA [2]. Interleukin-1β (IL-1β), a cytokine released by synovial cells and macrophages, causes

© The Author 2015. Published by ABBS Editorial Office in association with Oxford University Press on behalf of the Institute of Biochemistry and Cell Biology, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences.

Downloaded from https://academic.oup.com/abbs/article/47/6/422/1754597 by guest on 19 April 2024

inflammation of articular cartilage, stimulates the production of matrix metalloproteinases (MMPs), down-regulates the tissue inhibitor of metalloproteinases (TIMPs), and accelerates the degradation of cartilage matrix [3,4]. Imbalance between MMPs and TIMPs is well-known to be important in the progression of OA. Besides surgical therapy, treatment of OA generally aims at maintaining the mobility in the joint and retarding the degeneration of cartilage [5].

IL-1 is a potent proinflammatory cytokine and functions as the gatekeeper for inflammation, which is produced by a variety of cells and acts on virtually every organ system of the body [6]. IL-1 consists of two distinct proteins (IL-1α and IL-1β) that bind to the type 1 IL-1 receptor (IL-1R1) to activate a downstream proinflammatory pathway. IL-1R1 associates with IL-1 receptor accessory protein (IL-1RAP) to form a transmembrane signaling complex that initiates IL-1-dependent intracellular signaling [7]. Somewhat unique to the IL-1 system is the existence of two distinct types of IL-1Rs, IL-1R1 and the type 2 IL-1 receptor (IL-1R2) [8].

Poly(ADP-ribose) polymerase 1 (PARP-1) is a nuclear protein that serves as a sensor of DNA breaks [9]. PARP-1 can be activated through binding to DNA strand breaks and facilitates damage repair through poly(ADP-ribosyl)ation of target proteins, such as histones, transcription factors, and PARP-1 itself [10]. Over-activation of PARP-1 induces cell death by exhaustion of cellular ATP in conditions of severe DNA damage [11]. Moreover, PARP-1 can regulate the expressions of various key inflammatory genes, including inducible nitric oxide synthase (iNOS), intercellular adhesion molecule 1, and vascular cell adhesion molecule 1 [12]. It has been demonstrated that PARP-1 inhibition could suppress hyperstretch-induced inflammation response [13]. MMPs, a family of 28 structurally related, multidomain Zn-dependent endopeptidases, are key enzymes in the development and remodeling of tissues and organs [14]. PARP-1 inhibition increases the stability of atherosclerotic plaques, which is associated with increased TIMP expression and decreased ECM degradation [15].

In this study, we investigated whether PARP-1 inhibition is able to inhibit inflammatory responses in an *in vitro* OA model of cultured human chondrocytes, and provided evidence for its roles in cartilage destruction.

Materials and Methods

Isolation and culture of chondrocytes

All experiments were approved by the Ethical Committee of Qingdao Medical University. The primary human chondrocytes were isolated from articular cartilage of OA patients undergoing total knee replacement surgery as previously described [16]. Briefly, harvested cartilage was minced into small pieces and incubated in a trypsin-containing solution for 2 h at 37°C. The pieces were then washed with phosphate-buffered saline (PBS) and incubated in 0.2% collagenase at 37°C overnight. After digestion, the chondrocytes were collected and cultured in Dulbecco's modified Eagle's medium (DMEM) (Gibco, Gaithersburg, USA) containing 10% fetal calf serum, 100 U/ml penicillin, and 100 μg/ml streptomycin.

Cells were serum-starved overnight before treatment. Cells from passage 1 to 3 were used in all experiments.

Cell treatment and mRNA inhibition

The primary human chondrocytes were cultured in DMEM at 37°C in an atmosphere of 5% CO_2 and treated with 10 ng/ml of IL-1β (Sigma-Aldrich, St Louis, USA). The control group was untreated except for a change of the medium. Cells were harvested after incubation for 24 h. To inhibit PARP-1 expression, cells were transfected with PARP-1 siRNA (GenePharma, Shanghai, China) or negative control by using Lipofectamine 2000 (Invitrogen, Carlsbad, USA) in 24-well plates. Briefly, before transfection, cells were plated in 500 μl of growth medium without antibiotics when they are 30%–50% confluent at the time of transfection. Then, 20 pmol siRNA was diluted in 50 μl Opti-MEM (Gibco) and mixed gently. Lipofectamine 2000 (1 μl) was diluted in 50 μl Opti-MEM, mixed gently and incubated for 5 min at room temperature. After 5 min incubation, the diluted siRNA was combined with the Lipofectamine 2000, mixed gently and incubated for 20 min at room temperature. Finally, the complexes were added to each well containing cells and mixed gently by rocking the plate back and forth. Experiments were performed 24 h after transfection.

Reverse transcriptase-polymerase chain reaction

RNA was extracted from chondrocytes with Trizol reagent (Invitrogen) according to the manufacturer's instructions. The specific transcripts were quantified by quantitative polymerase chain reaction (qPCR) using a SYBR Green PCR kit (Qiagen, Hilden, Germany). The primers for genes are shown in Table 1. The mRNA levels were normalized to the GAPDH mRNA level. Amplification, detection, and data analysis were carried out by using the iCycler real-time PCR system (Bio-Rad Laboratories, Hercules, USA). The relative mRNA expression was calculated with the comparative threshold cycle method.

Cytoplasmic and nuclear protein extraction

Protein was extracted from human chondrocytes by the use of the NE-PER Nuclear and Cytoplasmic Extraction kit (Thermo Scientific, Waltham, USA) according to the manufacturer's instructions. Briefly, cells were harvested with trypsin-ethylenediaminetetraacetic acid, centrifuged at 500 *g* for 5 min, then washed with PBS and centrifuged again at 500 *g*. The supernatant was discarded and cell pellet was left as dry as possible. Then, ice cold CERI was added and the tube was vortexed for 15 s to fully suspend the cell pellet. After incubation

Table 1. Sequences of primers used in this study

Gene	Forward (5′→3′)	Reverse (5′→3′)
PARP-1	TTGAAAAAGCCCTAAAGGCTCA	CTACTCGGTCCAAGATCGCC
IL-1R1	TCCTCCATCCTGGAGAATATGA	CTCATTTGGATACTCCGTGCAT
iNOS	GTTCTCAGCCCAACAATACAAGA	GTGGACGGGTCGATGTC AC
MMP-1	CTCCCTTGGACTCACTCATTCTA	AGAACATCACCTCTCCCCTAAAC
MMP-13	TGAGGATACAGGCAAGACTCT	CAATACGGTTACTCCAGATGC
TIMP-1	CTTCTGGCATCCTGTTGTTG	AGAAGGCCGTCTGTGGGT
GAPDH	GAAGGTGAAGGTCGGAGTC	GAAGATGGTGATGGGATTTC

Downloaded from https://academic.oup.com/abbs/article/47/6/422/1754597 by guest on 19 April 2024

on ice for 10 min, CERI was added and the tube was vortexed for 5 s. After incubation on ice for 1 min, the tube was centrifuged for 5 min at 16,000 *g*, and the supernatant (cytoplasmic extract) was immediately transferred to a clean tube and stored at −80°C until use. The ice cold NER was then added into the insoluble pellet and the tube was placed on ice and vortexed for 15 s every 10 min, for a total of 40 min. After the tube was centrifuged for 10 min at 16,000 *g*, the supernatant (nuclear extract) was immediately transferred to a clean tube and stored at −80°C until use.

Western blot analysis

The protocol and semi-quantitative analysis were carried out as described previously [17]. The following antibodies were used: rabbit anti-IL-1R1 antibody (1:100; Abcam, Cambridge, UK), rabbit anti-PARP-1 antibody (1:100; Sigma-Aldrich), rabbit anti-iNOS antibody (1:1000; Cell Signaling Technology, Beverly, USA), rabbit anti-NF-κB p65 antibody (1:1000; Cell Signaling Technology), rabbit monoclonal anti-phospho-NF-κB p65 (p-p65, 1:1000; Cell Signaling Technology), rabbit anti-histone antibody (1:1000; Cell Signaling Technology), rabbit anti-MMP-1 antibody (1:100; Abcam), rabbit anti-MMP-13 antibody (1:100; Abcam), rabbit anti-TIMP-1 antibody (1:100; Abcam), and rabbit anti-β-actin antibody (internal control; 1:1000; Cell Signaling Technology). All experiments were carried out in triplicate. Signals were detected by enhanced chemiluminescence (Millipore, Billerica, USA) and analyzed by Image-Pro Plus 6.0.

Enzyme-linked immunosorbent assay

After stimulation, tumor necrosis factor-α (TNF-α) concentration in the culture supernatants was measured using a commercial enzyme-linked immunosorbent assay (ELISA) kit (Boster Biotechnology, Wuhan, China) according to the manufacture's protocol.

PARP-1 activity

PARP-1 activity in chondrocytes was determined using a colorimetric assay, as described previously [18]. Briefly, cells were stimulated with 500 mM H_2O_2, and then the medium was replaced with PARP-1 reaction buffer. Cells were fixed with 95% ethanol and endogenous peroxidase was blocked. Then, cells were washed once with PBS and blocked with 1% bovine serum albumin (BSA). After 30 min, BSA solution was aspirated and streptavidin–peroxidase (Jackson Immunoresearch Laboratories, West Grove, USA) was added. Color was developed with 3,3′,5,5′-tetramethylbenzidine substrate (R&D Systems, Minneapolis, USA). The optical density was measured with a spectrophotometer.

Cell viability

Cell viability and proliferation were determined by the 3-(4,5)-dimethylthiahiazo(-z-y1)-3,5-di-phenytetrazoliumromide (MTT) method. Briefly, after stimulation, cells were washed with PBS for three times and then MTT (5 mg/ml) was added and incubated for 4 h at 37°C. The culture medium was then replaced with an equal volume of dimethyl sulfoxide to dissolve blue formazan crystals. Absorbance was determined at 570 nm by the use of a Varioskan Flash Multimode Reader (Thermo Scientific).

Detection of apoptosis

Cell apoptosis was detected by the Annexin V-FITC/propidium iodide (PI) Apoptosis Detection kit (BD, Franklin Lakes, USA). Briefly, chondrocytes were re-suspended in 200 µl HEPES buffer, and stained with 5 µl Annexin V-FITC and 10 µl PI for 15 min at room temperature in the dark. After incubation, 200 µl HEPES buffer was added, and the cells were measured by BD FACSCalibur Flow Cytometer (BD). All experiments were performed in triplicate.

Statistical analysis

Data are expressed as the mean ± SD. SPSS 16.0 (SPSS Inc., Chicago, USA) was used for statistical analysis. Intergroup comparisons were performed by two-tailed Student's *t*-test or one-way ANOVA. $P < 0.05$ was considered to be statistically significant.

Results

IL-1β stimulation increased the expressions of PARP-1 and IL-1R as well as PARP-1 activity

Primary human chondrocytes isolated from articular cartilage of OA patients were immunostained with anti-type II collagen antibodies and observed under an optical microscope. Results showed that cells were chondrocytes (**Supplementary Fig. S1**).

IL-1β is widely used to induce OA *in vitro* [19]. In this study, human chondrocytes were stimulated with IL-1β at 10 ng/ml for 24 h. The mRNA and protein expressions of PARP-1 and IL-1R were analyzed by reverse transcriptase-PCR (RT-PCR) and western blot analysis, respectively. As shown in **Fig. 1A–E**, IL-1β could significantly increase PAPR-1 and IL-1R expressions. In addition, PARP-1 activity was also up-regulated by IL-1β stimulation compared with the control (**Fig. 1F**).

PARP-1 inhibition protected IL-1β-stimulated chondrocytes

PARP-1 expression was inhibited by siRNA. After PARP-1 was inhibited for 24, 48, and 72 h, PARP-1 mRNA expression was determined by RT-PCR. There was no statistical difference among the three time points (**Supplementary Fig. S2**). Thus, the time point of 24 h was used in the subsequent experiment. To ensure that PARP-1 was effectively inhibited in the target cells, PARP-1 expression was measured by RT-PCR and western blot analysis. Compared with the control, PARP-1 expression was significantly reduced by siRNA (**Fig. 2A–C**).

Then, viability and proliferation of cells were analyzed by the MTT method. Compared with the control, IL-1β stimulation reduced cell viability and proliferation, while PARP-1 inhibition could alleviate the adverse effect induced by IL-1β (**Fig. 2D**). Meanwhile, PARP-1 inhibition could reduce IL-1β-up-regulated cell apoptosis (**Fig. 2E**). These results suggested that PARP-1 inhibition plays a protective role in IL-1β-stimulated chondrocytes.

PARP-1 inhibition reduced the IL-1β-induced inflammation response

Inflammation response plays a critical role in the pathogenesis of OA. Among the proinflammatory cytokines involved in OA, TNF-α is considered to be a major participant. In patients or animals with OA, the level of TNF-α is elevated in the synovial fluid and serum [20]. The iNOS can synthesize nitric oxide (NO) to induce chondrocyte apoptosis [21]. So, we then investigated the TNF-α secretion and iNOS expression in chondrocytes. TNF-α level in supernatant was significantly up-regulated after IL-1β treatment, whereas PARP-1 inhibition reduced TNF-α secretion (**Fig. 3A**). Meanwhile, PARP-1 inhibition significantly reduced IL-1β-induced up-regulation of iNOS expression (**Fig. 3B–D**). These results showed that PARP-1 inhibition could suppress IL-1β-induced inflammation response.

Downloaded from https://academic.oup.com/abbs/article/47/6/422/1754597 by guest on 19 April 2024

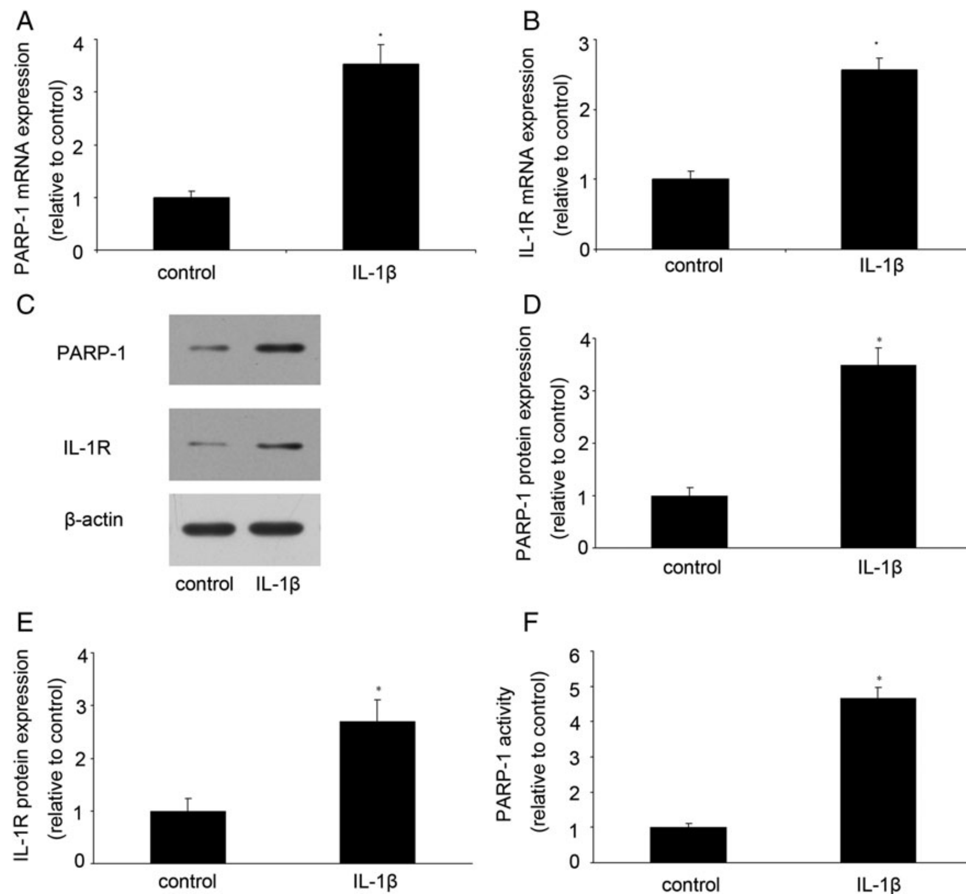

Figure 1. IL-1β increased the mRNA and protein expressions of IL-1R and PARP-1 as well as PARP-1 activity After human chondrocytes were stimulated with IL-1β, mRNA and protein expressions of IL-1R and PARP-1 were determined by RT-PCR and western blot analysis, respectively. PARP-1 activity was also analyzed. (A and B) Quantification of RT-PCR results of IL-1R and PARP-1 mRNA expressions. (C–E) Western blot analysis of IL-1R and PARP-1 protein expressions. (F) The activity of PARP-1. Data are expressed as the mean ± SD from three independent experiments. Data were analyzed by Student's *t*-test. *P< 0.05 vs. control.

PARP-1 inhibition suppressed the activation of IL-1R/NF-κB signaling pathway in IL-1β-stimulated chondrocytes

We then investigated whether IL-1R/NF-κB signaling pathway was involved in the protective effects of PARP-1 on human chondrocytes. First, IL-1R expression was analyzed after PARP-1 inhibition. As shown in **Fig. 4**, PARP-1 inhibition could reduce IL-1β-induced up-regulation of IL-1R expression. Then, a time course of NF-κB p65 nuclear translocation was analyzed. Western blot analysis showed that NF-κB p65 was mostly cytoplasmic before IL-1β stimulation, but translocated to nucleus when cells were stimulated with IL-1β (2–4 h), and then translocated to cytoplasma after 8 h of treatment (**Fig. 5**). However, PARP-1 inhibition suppressed IL-1β-induced p65 nuclear translocation (**Fig. 6A–D**). Then, p-p65 level was measured. IL-1β could significantly increase p-p65 level compared with control, and PARP-1 inhibition reduced p-p65 level (**Fig. 6E,F**). These results suggested that the anti-inflammatory response conferred by PARP-1 inhibition might be partly mediated by the IL-1R/NF-κB pathway.

PARP-1 inhibition reduced MMP expression and increased TIMP expression

An imbalance between the activity of MMPs and their inhibitors TIMP is thought to underlie cartilage destruction in OA [22]. We then investigated the expression of MMPs and TIMP-1 after PARP-1 inhibition. Compared with the control, IL-1β increased the mRNA expressions of MMP-1 and MMP-13, with a down-regulated expression of TIMP-1. PARP-1 inhibition could suppress these effects (**Fig. 7**). Then, the protein expressions of MMPs and TIMP-1 were analyzed by western blot analysis. PARP-1 inhibition reduced MMP expression and increased TIMP-1 expression (**Fig. 8**).

Discussion

OA is a degenerative joint disease characterized by the loss of chondrocyte function and ECM destruction caused by various risk factors [1]. The loss of chondrocyte function, degradation of the ECM, and inflammation response play crucial roles in the initiation and progression of OA, and thus inhibition of these factors is an important issue for the treatment of OA [23].

OA is closely associated with inflammation in the early stages of the disease. IL-1β, TNF-α, and NO are considered to be important during inflammation response. They have been reported to induce apoptosis in chondrocytes and ECM degradation [24]. In response to IL-1β, chondrocytes can secrete MMPs to induce chondrocyte apoptosis and inhibit ECM biosynthesis [25,26]. In patients or animals with OA, IL-1β, and TNF-α are elevated in the synovial fluid and serum [20]. NO, a gaseous-free radical synthesized by iNOS,

Downloaded from https://academic.oup.com/abbs/article/47/6/422/1754597 by guest on 19 April 2024

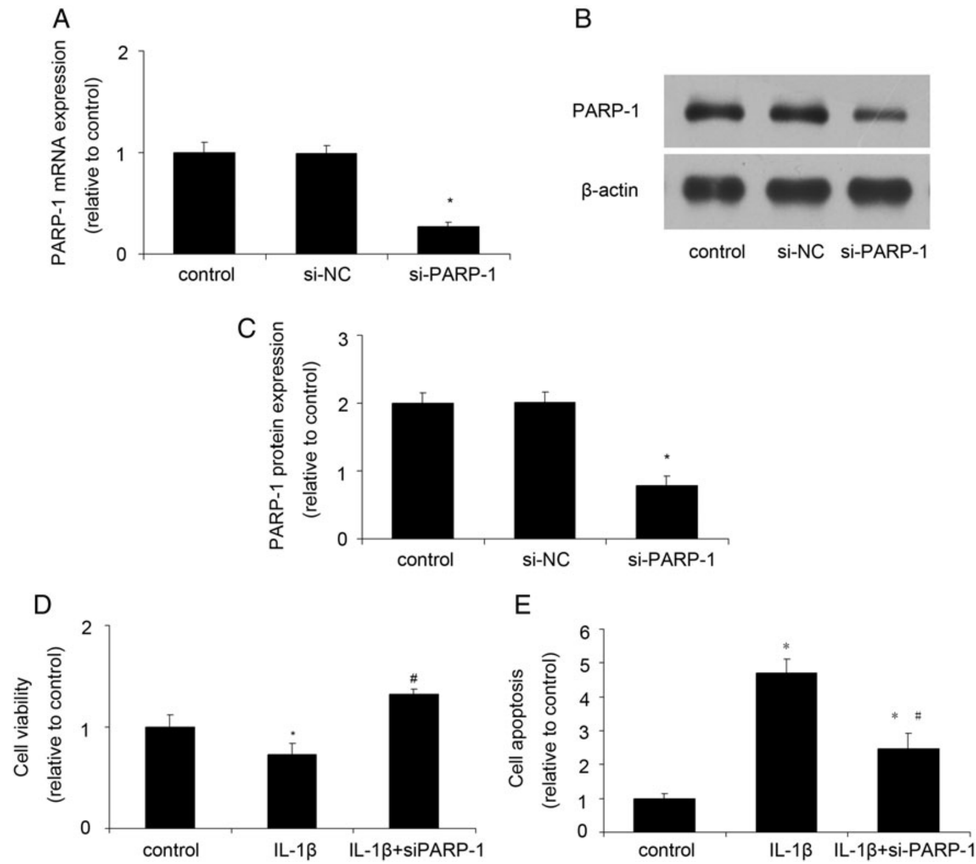

Figure 2. PARP-1 inhibition exerted protection on IL-1β-stimulated chondrocyte Chondrocytes were stimulated with IL-1β after PARP-1 inhibition by siRNA. Cell viability and apoptosis were determined by the MTT method and flow cytometry, respectively. (A–C) The mRNA and protein expressions of PARP-1 after PARP-1 inhibition. (D) Cell viability. (E) Cell apoptosis. si-NC, negative siRNA control; si-PARP-1, PARP-1 siRNA. Data are expressed as the mean ± SD. Data were analyzed by one-way ANOVA. $^{*}P < 0.05$ vs. control; $^{\#}P < 0.05$ vs. IL-1β.

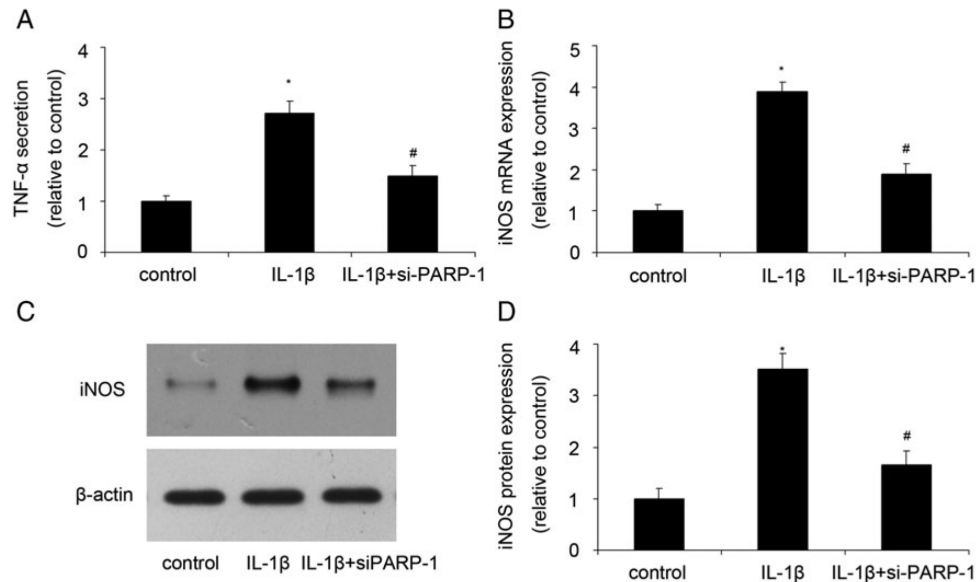

Figure 3. PARP-1 inhibition reduced IL-1β-induced inflammation response Chondrocytes were stimulated with IL-1β after PARP-1 inhibition by siRNA. TNF-α secretion was measured by ELISA. iNOS mRNA and protein expressions were analyzed by RT-PCR and western blot analysis, respectively. (A) TNF-α secretion. (B–D) iNOS mRNA and protein expressions. Data are expressed as the mean ± SD. Data were analyzed by one-way ANOVA. $^{*}P < 0.05$ vs. control. $^{\#}P < 0.05$ vs. IL-1β.

Downloaded from https://academic.oup.com/abbs/article/47/6/422/1754597 by guest on 19 April 2024

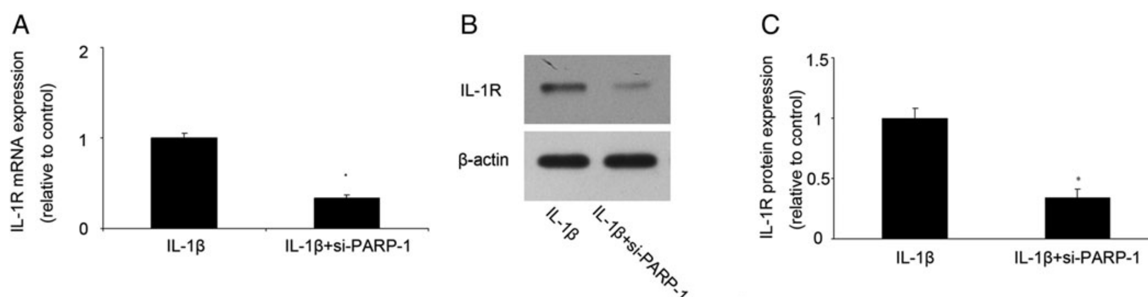

Figure 4. PARP-1 inhibition reduced IL-1R expression After PARP-1 was inhibited, cells were stimulated with IL-1β. IL-1R mRNA (A) and protein (B,C) expressions were analyzed by RT-PCR and western blot analysis, respectively. Data are expressed as the mean ± SD from three separate experiments. Data were analyzed by Student's *t*-test. $^*P < 0.05$ vs. control.

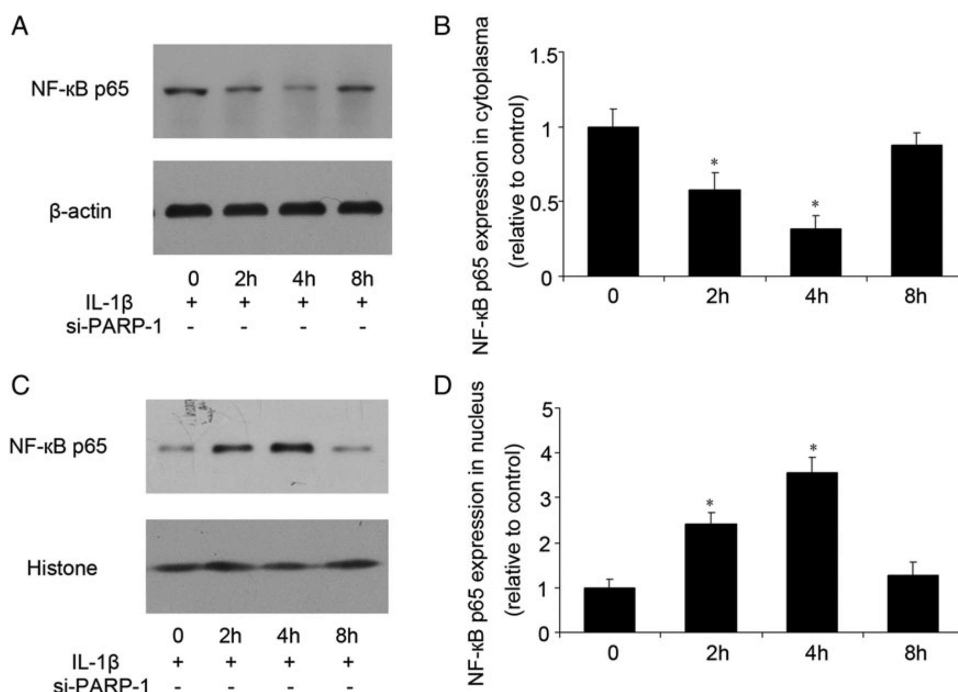

Figure 5. IL-1β-induced NF-κB p65 nuclear translocation After cells were stimulated with IL-1β for 0, 2, 4, and 8 h, NF-κB p65 expression was analyzed by western blot analysis. (A and B) Protein expression of NF-κB p65 in cytoplasma. (C and D) The protein expression of NF-κB p65 in nucleus. Data are expressed as the mean ± SD. Data were analyzed by one-way ANOVA. $^*P < 0.05$ vs. control.

can induce apoptosis and increase MMP-13 expression [21]. The results of the present study showed that compared with the control, IL-1β could significantly increase the level of TNF-α and iNOS, which was consistent with previous studies.

PARP-1, a highly conserved DNA binding protein, is important in maintaining the genomic stability, repairing the DNA damage, and regulating transcriptional processes [27]. It is closely related to various inflammatory conditions, including ischemia–reperfusion injury, hypertension, atherosclerosis, and aging [28,29]. Moreover, PARP-1 plays an important role in cell apoptosis [30]. In this study, we found that compared with the control, IL-1β stimulation could increase PARP-1 expression and activity. PARP-1 inhibition reduced IL-1β-induced inflammation response. Then, we investigated the underlying mechanisms. NF-κB, a nuclear protein, is a transcription factor for various inflammatory cytokines [12]. The NF-κB complex remains inactive in the cytoplasm through interaction with the inhibitory protein inhibitor κB (IκB). Activation of NF-κB requires phosphorylation of IκBα by IκBα kinase and subsequent degradation [31]. As shown in this study, PARP-1 inhibition reduced IL-1β-induced up-regulation of IL-1R. Moreover, NF-κB p65 was mostly cytoplasmic before IL-1β stimulation, but its localization quickly changed to the nucleus with IL-1β stimulation. After PARP-1 inhibition, p65 remained primarily cytoplasmic after IL-1β treatment. The phosphorylation of the p65 subunit is critical for binding to its target sites on DNA [32]. It was important to determine whether PARP-1 played a role in regulating the activity of NF-κB. Our results showed that compared with the IL-1β, PARP-1 inhibition significantly decreased the p-p65 level. However, the mechanism by which PARP-1 influences the phosphorylation needs further investigation. Those results suggested that IL-1β-induced

Downloaded from https://academic.oup.com/abbs/article/47/6/422/1754597 by guest on 19 April 2024

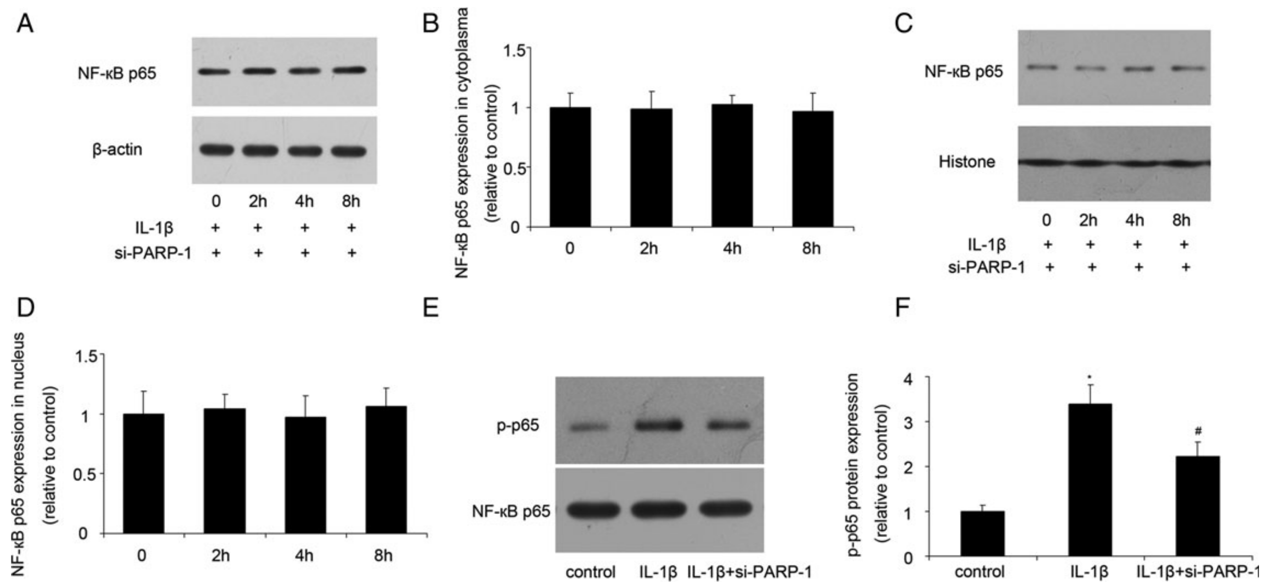

Figure 6. PARP-1 inhibition suppressed IL-1β-induced NF-κB p65 nuclear translocation After PARP-1 was inhibited, cells were stimulated with IL-1β for 0, 2, 4, and 8 h, NF-κB p65 expression and phosphorylation were analyzed by western blot analysis. (A and B) Protein expression of NF-κB p65 in cytoplasma. (C and D) Protein expression of NF-κB p65 in nucleus. Data are expressed as the mean ± SD. Data were analyzed by one-way ANOVA. $^*P<0.05$ vs. control. (E and F) Protein expression of phospho-NF-κB p65 (p-p65). Data are expressed as the mean ± SD. Data were analyzed by one-way ANOVA. $^*P<0.05$ vs. control. $^{\#}P<0.05$ vs. IL-1β.

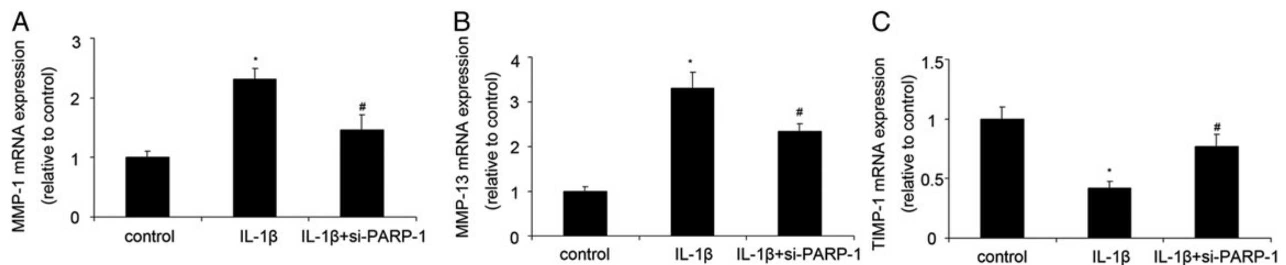

Figure 7. PARP-1 inhibition reduced MMPs and increased TIMP-1 mRNA expression Cells were stimulated with IL-1β after PARP-1 inhibition by siRNA. The mRNA expressions of MMPs and TIMP-1 were determined by RT-PCR. (A–C) RT-PCR analysis of MMPs and TIMP-1 expressions. si-PARP-1, PARP-1 siRNA. Data are expressed as the mean ± SD. Data were analyzed by one-way ANOVA. $^*P<0.05$ vs. control. $^{\#}P<0.05$ vs. IL-1β.

inflammatory response through IL-1R/PARP-1/NF-κB pathway in human chondrocytes.

The chondrocytes can synthesize appropriate ECM molecules to maintain cartilage homeostasis [33]. It is important to keep the biosynthetic activities of chondrocytes. Our results showed that IL-1β stimulation reduced the viability of chondrocytes, while PARP-1 inhibition could prevent it, suggesting the protective effect of PARP-1 inhibition. Cartilage ECM molecules consist of type II collagen and sulfated proteoglycan, which play crucial roles in regulating chondrocyte functions [34]. MMPs, especially MMP-1 and MMP-13, can degrade type II collagen and be inhibited by TIMPs. Under normal conditions, TIMPs bind to active MMPs in a 1:1 ratio to make an inactive complex. The imbalance in the ratio of TIMPs and MMPs results in continuous destruction in OA [35]. In the present study, we revealed that the IL-1β-augmented expression of MMPs was inhibited, and the protein and mRNA expressions of TIMP-1 were up-regulated by PARP-1 inhibition in the IL-1β-induced chondrocytes. Therefore, maintaining the balance of MMPs/TIMP-1 is another mechanism by which PARP-1 inhibition prevents cartilage matrix degradation.

In conclusion, we found that IL-1β stimulation could increase the protein expressions of IL-1R and PARP-1, as well as the activity of PARP-1. IL-1β reduced the viability of chondrocytes and increased cell apoptosis, while PARP-1 inhibition alleviated these. PARP-1 inhibition could reduce IL-1β-induced inflammation response, including TNF-α secretion and iNOS expression, probably through inhibiting the IL-1R/NF-κB pathway. Meanwhile, PARP-1 inhibition could keep the balance between MMPs and TIMP-1. Our results suggested that PARP-1 inhibition had effective anti-OA activity in human chondrocytes. The protection was mainly through maintaining the viability of chondrocytes, reducing the secretion of proinflammatory cytokines, suppression of MMPs expression, and simultaneous up-regulation of TIMP-1 production (**Fig. 9**). This study, to the best of our knowledge, provides the first evidence that PARP-1 inhibition can effectively treat OA. Inhibition of PARP-1 might be a new approach for the treatment of OA. However, further *in vivo* experiments are needed to identify novel and potent agents for the prevention and treatment of OA through PARP-1 inhibition.

Downloaded from https://academic.oup.com/abbs/article/47/6/422/1754597 by guest on 19 April 2024

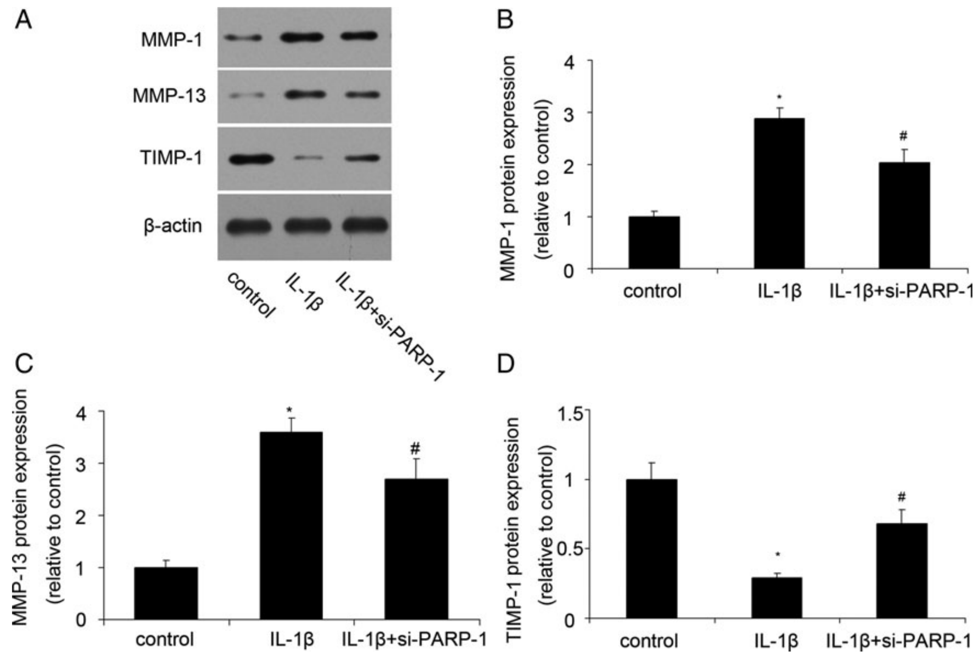

Figure 8. PARP-1 inhibition reduced MMPs and increased TIMP-1 protein expression (A–D) Western blot analysis of MMPs and TIMP-1 protein expressions. si-PARP-1, PARP-1 siRNA. Data are expressed as the mean ± SD. Data were analyzed by one-way ANOVA. $^*P < 0.05$ vs. control. $^\#P < 0.05$ vs. IL-1β.

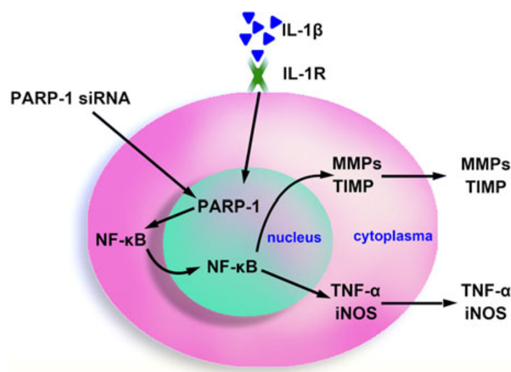

Figure 9. Schematic diagram of PARP-1 inhibition of IL-1β-induced inflammation PARP-1 inhibition by siRNA could reduce the secretion of proinflammatory cytokines, suppress MMPs expression, and increase TIMP-1 production partly through inhibition of IL-1R/NF-κB pathway.

Supplementary Data

Supplementary data is available at *ABBS* online.

Funding

This work was supported by a grant from the National Natural Science Foundation of China (No. 81400340).

References

1. Loeser RF. Aging and osteoarthritis: the role of chondrocyte senescence and aging changes in the cartilage matrix. *Osteoarthritis Cartilage* 2009, 17: 971–979.
2. Kapoor M, Martel-Pelletier J, Lajeunesse D, Pelletier JP, Fahmi H. Role of proinflammatory cytokines in the pathophysiology of osteoarthritis. *Nat Rev Rheumatol* 2011, 7: 33–42.
3. Dai SM, Shan ZZ, Nakamura H, Masuko-Hongo K, Kato T, Nishioka K, Yudoh K. Catabolic stress induces features of chondrocyte senescence through overexpression of caveolin 1: possible involvement of caveolin 1-induced down-regulation of articular chondrocytes in the pathogenesis of osteoarthritis. *Arthritis Rheum* 2006, 54: 818–831.
4. Yudoh K, Nguyen vT, Nakamura H, Hongo-Masuko K, Kato T, Nishioka K. Potential involvement of oxidative stress in cartilage senescence and development of osteoarthritis: oxidative stress induces chondrocyte telomere instability and downregulation of chondrocyte function. *Arthritis Res Ther* 2005, 7: R380–R391.
5. Alcaraz MJ, Megias J, Garcia-Arnandis I, Clerigues V, Guillen MI. New molecular targets for the treatment of osteoarthritis. *Biochem Pharmacol* 2010, 80: 13–21.
6. Dinarello CA. A clinical perspective of il-1beta as the gatekeeper of inflammation. *Eur J Immunol* 2011, 41: 1203–1217.
7. Korherr C, Hofmeister R, Wesche H, Falk W. A critical role for interleukin-1 receptor accessory protein in interleukin-1 signaling. *Eur J Immunol* 1997, 27: 262–267.
8. Ben-Sasson SZ, Caucheteux S, Crank M, Hu-Li J, Paul WE. Il-1 acts on t cells to enhance the magnitude of *in vivo* immune responses. *Cytokine* 2011, 56: 122–125.
9. Virag L, Szabo C. The therapeutic potential of poly(adp-ribose) polymerase inhibitors. *Pharmacol Rev* 2002, 54: 375–429.
10. Diefenbach J, Burkle A. Introduction to poly(adp-ribose) metabolism. *Cell Mol Life Sci* 2005, 62: 721–730.
11. Szabo C, Zingarelli B, O'Connor M, Salzman AL. DNA strand breakage, activation of poly (adp-ribose) synthetase, and cellular energy depletion are involved in the cytotoxicity of macrophages and smooth muscle cells exposed to peroxynitrite. *Proc Natl Acad Sci USA* 1996, 93: 1753–1758.
12. Sharp C, Warren A, Oshima T, Williams L, Li JH, Alexander JS. Poly adp ribose-polymerase inhibitors prevent the upregulation of icam-1 and e-selectin in response to th1 cytokine stimulation. *Inflammation* 2001, 25: 157–163.
13. Wang J, Liu L, Xia Y, Wu D. Silencing of poly(adp-ribose) polymerase-1 suppresses hyperstretch-induced expression of inflammatory cytokines *in vitro*. *Acta Biochim Biophys Sin* 2014, 46: 556–564.

Downloaded from https://academic.oup.com/abbs/article/47/6/422/1754597 by guest on 19 April 2024

14. Visse R, Nagase H. Matrix metalloproteinases and tissue inhibitors of metalloproteinases: structure, function, and biochemistry. *Circ Res* 2003, 92: 827–839.
15. Oumouna-Benachour K, Hans CP, Suzuki Y, Naura A, Datta R, Belmadani S, Fallon K, *et al*. Poly(adp-ribose) polymerase inhibition reduces atherosclerotic plaque size and promotes factors of plaque stability in apolipoprotein e-deficient mice: effects on macrophage recruitment, nuclear factor-kappab nuclear translocation, and foam cell death. *Circulation* 2007, 115: 2442–2450.
16. Culley KL, Hui W, Barter MJ, Davidson RK, Swingler TE, Destrument AP, Scott JL, *et al*. Class i histone deacetylase inhibition modulates metalloproteinase expression and blocks cytokine-induced cartilage degradation. *Arthritis Rheum* 2013, 65: 1822–1830.
17. Qin WD, Wei SJ, Wang XP, Wang J, Wang WK, Liu F, Gong L, *et al*. Poly (adp-ribose) polymerase 1 inhibition protects against low shear stress induced inflammation. *Biochim Biophys Acta* 2013, 1833: 59–68.
18. Bakondi E, Bai P, Szabo EE, Hunyadi J, Gergely P, Szabo C, Virag L. Detection of poly(adp-ribose) polymerase activation in oxidatively stressed cells and tissues using biotinylated nad substrate. *J Histochem Cytochem* 2002, 50: 91–98.
19. Iacono A, Gomez R, Sperry J, Conde J, Bianco G, Meli R, Gomez-Reino JJ, *et al*. Effect of oleocanthal and its derivatives on inflammatory response induced by lipopolysaccharide in a murine chondrocyte cell line. *Arthritis Rheum* 2010, 62: 1675–1682.
20. Hashimoto S, Nishiyama T, Hayashi S, Fujishiro T, Takebe K, Kanzaki N, Kuroda R, *et al*. Role of p53 in human chondrocyte apoptosis in response to shear strain. *Arthritis Rheum* 2009, 60: 2340–2349.
21. Del Carlo M Jr, Loeser RF. Nitric oxide-mediated chondrocyte cell death requires the generation of additional reactive oxygen species. *Arthritis Rheum* 2002, 46: 394–403.
22. Burrage PS, Mix KS, Brinckerhoff CE. Matrix metalloproteinases: role in arthritis. *Front Biosci* 2006, 11: 529–543.
23. Xu Y, Dai GJ, Liu Q, Liu ZL, Song ZQ, Li L, Chen WH, *et al*. Sanmiao formula inhibits chondrocyte apoptosis and cartilage matrix degradation in a rat model of osteoarthritis. *Exp Ther Med* 2014, 8: 1065–1074.
24. Aizawa T, Kon T, Einhorn TA, Gerstenfeld LC. Induction of apoptosis in chondrocytes by tumor necrosis factor-alpha. *J Orthop Res* 2001, 19: 785–796.
25. Attur M, Al-Mussawir HE, Patel J, Kitay A, Dave M, Palmer G, Pillinger MH, *et al*. Prostaglandin e2 exerts catabolic effects in osteoarthritis cartilage: evidence for signaling via the ep4 receptor. *J Immunol* 2008, 181: 5082–5088.
26. Abramson SB. Osteoarthritis and nitric oxide. *Osteoarthritis Cartilage* 2008, 16(Suppl 2): S15–S20.
27. Krishnakumar R, Kraus WL. The parp side of the nucleus: molecular actions, physiological outcomes, and clinical targets. *Mol Cell* 2010, 39: 8–24.
28. Martinet W, Knaapen MW, De Meyer GR, Herman AG, Kockx MM. Elevated levels of oxidative DNA damage and DNA repair enzymes in human atherosclerotic plaques. *Circulation* 2002, 106: 927–932.
29. Pacher P, Mabley JG, Soriano FG, Liaudet L, Szabo C. Activation of poly (adp-ribose) polymerase contributes to the endothelial dysfunction associated with hypertension and aging. *Int J Mol Med* 2002, 9: 659–664.
30. Zhao Y, Hongdu B, Ma D, Chen Y. Really interesting new gene finger protein 121 is a novel golgi-localized membrane protein that regulates apoptosis. *Acta Biochim Biophys Sin* 2014, 46: 668–674.
31. Ogata N, Yamamoto H, Kugiyama K, Yasue H, Miyamoto E. Involvement of protein kinase c in superoxide anion-induced activation of nuclear factor-kappa b in human endothelial cells. *Cardiovasc Res* 2000, 45: 513–521.
32. Li Q, Verma IM. Nf-kappab regulation in the immune system. *Nat Rev Immunol* 2002, 2: 725–734.
33. Roughley PJ. Articular cartilage and changes in arthritis: noncollagenous proteins and proteoglycans in the extracellular matrix of cartilage. *Arthritis Res* 2001, 3: 342–347.
34. Eyre D. Collagen of articular cartilage. *Arthritis Res* 2002, 4: 30–35.
35. Burger D, Rezzonico R, Li JM, Modoux C, Pierce RA, Welgus HG, Dayer JM. Imbalance between interstitial collagenase and tissue inhibitor of metalloproteinases 1 in synoviocytes and fibroblasts upon direct contact with stimulated t lymphocytes: involvement of membrane-associated cytokines. *Arthritis Rheum* 1998, 41: 1748–1759.

Downloaded from https://academic.oup.com/abbs/article/47/6/422/1754597 by guest on 19 April 2024