ORIGINAL RESEARCH

The Effect of Endogenous PARP-1 in Different Phases of IL-1β-Induced Chondrocyte Degeneration

Xiufan Du, MM; Shunli Zhang, MM; Chunhang Huang, MM; Guangji Wang, MM; Ning Wang, MM; Yong Hu, MM

ABSTRACT

Objective • Poly (ADP-ribose) polymerase-1 (PARP-1) is a regulatory enzyme involved in DNA damage repair, gene transcription, cell growth, death and apoptosis**.** In our study, we aimed to explore the dynamic role of PARP-1 in chondrocyte (CH) degeneration *in vitro*.

Methods • We used the primary CHs and treated them with interleukin-1 beta for up to 5 days. (IL-1β) to induce degeneration. Meanwhile, we used AG-14361 (AG) to inhibit endogenous PARP-1 expression. Cell survival and collagen II expression were used to define the cell function of CHs. In addition, other metabolic indicators were measured containing the reactive oxygen species (ROS) level, 8-Hydroxy-2'-deoxyguanosine (8-OH-dG), IL-1β, tumor necrosis factor alpha (TNF-α) and caspase 3/9 expression.

Results • With IL-1β treatment, the PARP1 expression of CHs was gradually increased from day 1 to day 5, accompanied by a reduction in cell survival and collagen II expression**,** and an increase in ROS, 8-OH-dG, IL-1β, TNF-α and caspase 3/9 levels. We suppressed PARP1 expression on the first day of IL-1β stimulation and found severe destruction of cell survival and collagen II content with a higher expression of caspase 3/9. However, when we cultured the CHs with AG from day 3 of the 5-day IL-1β stimulation, cell survival and collagen II expression were rescued, and the ROS, 8-OH-dG, IL-1β, TNF-α, and caspase 3/9 were downregulated.

Conclusions • On day 1 of degeneration, increased PARP-1 played a protective role in CHs. However, from days 3 to 5 of degeneration, the accumulated PARP-1 presented a more destructive function in CHs. (*Altern Ther Health Med.* 2023;29(5):410-416).

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INTRODUCTION

Osteoarthritis (OA) is a frequently occurring clinical disease that affects mostly large weight-bearing joints throughout the body. The knee joint is most likely to be involved, and common clinical symptoms include severe pain, swelling, joint stiffness and limited function of the knee joint.[1] The pathophysiology is characterized by increasing articular cartilage (AC) destruction, subchondral bone sclerosis, cystic changes and osteophyte formation.[2,3] Chondrocytes (CHs) are the main cellular components of AC. Their primary functions are to synthesize extracellular matrix (ECM) and maintain cartilage morphology. CH injury is a fundamental cause of AC degeneration. In the occurrence and development of OA, adverse environmental factors lead to abnormal expression levels of various cytokines, a necessary mechanism for CH catabolism, and anabolic imbalance resulting in decreased CH viability and increased apoptosis.[4,5]

Excessive accumulation of inflammation, reactive oxygen species (ROS), cell metabolites and DNA replication errors can cause DNA damage, make the genome unstable and destroy cell activity.[6] To maintain normal physiological functions, cells must have a variety of DNA damage detection and repair mechanisms so that damaged DNA can be repaired in a timely manner.[7] In patients with OA, the overexpression of ROS leads to DNA damage in CHs and thus affects joint damage and repair, which may also be a key factor at the molecular level in OA progress.[8] Poly (ADP-ribose) polymerase-1 (PARP-1) is a highly conserved, multifunctional nuclear protein that senses DNA damage. When a DNA strand breaks, PARP-1 is rapidly recruited,

activates and binds to the broken DNA, acting on target proteins such as histones and transcription factors, thereby repairing DNA, maintaining gene stability and regulating transcription.[9] Studies have confirmed that PARP-1 expression can be upregulated due to various reasons, including ischemia-reperfusion, hypertension, atherosclerosis and aging.[10] Tumor cells can self-repair by activating their DNA damage repair system, thereby generating tolerance to such anti-cancer therapies in the current tumor treatment process. Therefore, a of supplement of PARP-1 inhibitors can block the DNA repair pathway of tumor cells and thus kill tumors.[11]

In past studies of OA, PARP-1 was increased during CHs degeneration and mainly used as a marker of CH oxidative stress injury.[12, 13] In addition, Sun, et al. reported inhibition of PARP-1 alleviated the interleukin 1 beta (IL-1β)-induced reduction of cell viability and the upregulation of cell apoptosis and the inflammatory response, which suppresses AC destruction.[14] As we know, NF-κB (nuclear factor kappa-light-chain-enhancer of activated B cells) plays a vital role in the mediation of inflammatory responses, and PARP-1 has also been proven to have a positive feedback loop with NF-κB in DNA repair.[15]

Since PARP-1 is involved in the repair of DNA damage, why can suppressing its expression delay OA? Furthermore, whether or not its role in regulating inflammation is involved in the OA process is currently unknown. Therefore, our study used the IL-1β-induced CH degeneration model to clear the effect of PARP-1 during CH degeneration.

Our findings elucidated the diversiform role of PARP-1 in different CH phases and provided a treatment strategy in late OA via the PARP-1 inhibitor.

MATERIALS AND METHODS

AC Sample Collection

The original CHs were isolated from the AC tissues donated from patients with OA undergoing total knee joint replacement surgery in Hainan General Hospital in China. Before sample collection, we received approval for this project from the Ethics Committee of our hospital. A total of 10 patients (5 women, 5 men; age 48 to 63 years) signed an informed consent form and participated in this study. We conserved the tissues in a cold sterile cell growth medium immediately after cutting them from patients to isolate the CHs. All procedures were carried out according to Declaration of Helsinki guidance.

CH Isolation and Treatment

In a sterile environment, we cut the cartilage from the smooth part of the AC surface with a scalpel. Then, the samples were fragmented and digested with a mixture of 0.25% trypsin and 0.25% type I collagenase (Sigma-Aldrich, St. Louis, Missouri, USA) for 6 hours. CH pellets were re-suspended in DMEM/F12 medium (Thermo-Fisher Scientific, Waltham, Massachusetts, USA) containing 10% fetal bovine serum (FBS) and 1% penicillin-streptomycin (Gibco, Rockville, Maryland, USA). We changed the medium every 3 days and used the first pass CHs in the following experiment. To induce CH degeneration, IL-1β (10 ng/ml; R&D Systems, Minneapolis, Minnesota, USA) was used to treat the CHs for 1 to 5 days. In addition, we used AG-14361 (AG, 1 or 2 μM; Selleck, Houston, Texas, USA) to culture CHs to inhibit the PARP-1 gene expression. For the combined stimulation, we cultured the CHs with both IL-1β (10 ng/ml) and AG (2 μM) and collected the cells for the following analysis.

Cell Survival Analysis

To investigate the influence of drug treatment on the CHs, we used an MTT kit (ab211091, Abcam, Cambridge, Massachusetts, USA) to measure cell survival. CHs were seeded in 96-well plates (5000 cells/well) with 100 μl of culture medium with and without indicated drugs and incubated for 3 days. The medium was changed with serum-free medium and MTT reagent according to the manufacturer's instructions. After incubation for 3 hours in a humidified atmosphere, the absorbance was measured at OD590 nm.

Western blotting (WB)

The whole CH protein was isolated with radioimmunoprecipitation assay (RIPA) lysis buffer (Beyotime, Shanghai, China). 50 μg protein from each group was loaded into lanes of sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) gels, then transferred to a polyvinylidene fluoride (PVDF) membrane. The membranes were then blocked with 5% milk and incubated with the desired primary antibodies: Rabbit anti-NF-kB p65 antibody (ab16502, Abcam, Cambridge, Massachusetts, USA), Rabbit anti-PARP1 (ab32138, Abcam), or Rabbit anti-GAPDH as a loading control (ab9485, Abcam) overnight at 4°C. Blots were next processed with Goat Anti-Rat IgG H&L (HRP) (ab7097, Abcam) for 1 hour at room temperature. The brands were exposed using chemiluminescent electrochemiluminescence (ECL) substrate (Beyotime, Shanghai, China) and analyzed using ImageJ software (Media Cybernetics, Bethesda, Maryland, USA).

Quantitative Polymerase Chain Reaction (qPCR)

Whole RNA was isolated using TRIzol reagent (Invitrogen, Carlsbad, California , USA) and reverse-transcribed to obtain cDNA using a reverse transcript SuperScript (Roche, Basel, Switzerland) according to the manufacturer's instructions. qPCR was performed with SYBR Green supermix (TOYOBO, Osaka, Japan) to analyze the expression of PARP-1, IL-1β, TNF-α, caspase 3 and caspase 9 via normalization to the amount of GAPDH and calculated according to the 2 delta-delta Ct ($2^{-\Delta\Delta Ct}$) method. The qPCR primers are shown in Table.

Table. Primer Sequences for Reverse Transcriptase Polymerase Chain Reaction

Gene name	Forward (5'>3')	Reverse (5'>3')
PARP-1	GCCGAGATCATCAGGAAGTATG	ATTCGCCTTCACGCTCTATC
Collagen II	TGGACGATCAGGCGAAACC	GCTGCGGATGCTCTCAATCT
IL-1β	ATGATGGCTTATTACAGTGGCAA	GTCGGAGATTCGTAGCTGGA
TNF-α	CCTCTCTCTAATCAGCCCTCTG	GAGGACCTGGGAGTAGATGAG
Caspase 3	AGAACTGGACTGTGGCATTGAG	GCTTGTCGGCATACTGTTTCAG
Caspase 9	GCTCTTCCTTTGTTCATC	CTCTTCCTCCACTGTTCA
GAPDH	ACAACTTTGGTATCGTGGAAGG	GCCATCACGCCACAGTTTC

Abbreviations: IL-1β, interleukin 1 beta; TNF-α, tumor necrosis factor alpha; PARP-1, polymerase 1.

Immunofluorescence Staining (IF)

CHs were seeded on coverslips in the 24-well plates. After treatment, CHs were fixed with 4% paraformaldehyde and permeabilized with 0.1% Triton X for 15 minutes. After blocking with 5% bovine serum albumin for 30 minutes, CHs were incubated with antibodies: Mouse anti-8-Hydroxy-2'-deoxyguanosine (8-OH-dG) antibody (ab48508, Abcam) and Rabbit anti-Collagen II antibody (ab188570, Abcam) overnight at 4°C, and linked to Alexa Fluor 488 (ab150077, Abcam) or 647 (ab150115, Abcam). Then, the nuclei were counterstained with 4'-6-diamidino-2-phenylindole (DAPI; Sigma-Aldrich, St. Louis, Missouri, USA) for 1 hour in the dark at room temperature. Immunofluorescence density was measured using ImageJ software (Media Cybernetics, Bethesda, Maryland, USA).

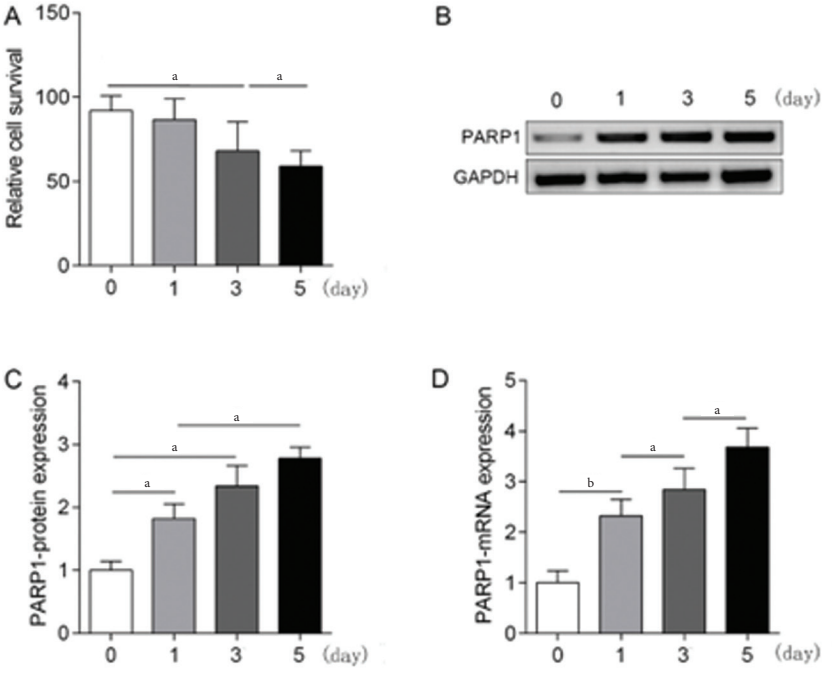
ROS Detection

ROS production in CHs was investigated by Cellular ROS detection assay kit (ab139476, Abcam) according to the manufacturer's instructions. CH suspension was stained with oxidative stress reagent orange and green for 1 hour at 37°C, then flow cytometry analyses were performed, and the absorbance was measured at 450 nm. Untreated CHs were used as controls.

Statistical Analysis

Data were analyzed by the Statistical Product and Service Solutions (SPSS) Version 22 software package (IBM Corp, Armonk, New York, USA) and expressed as mean values ± standard deviation (SD). Using one-way analysis of variance (ANOVA), the *P* value was calculated, and the statistical significance between the 2 groups with $P < .05$. All experiments were performed 3 times.

Figure 1. PARP1 gradually increased under IL-1β treatment. CHs were cultured with 10 ng/ml IL-1***β*** for up to 5 days. (**1A**) Cell survival was measured by the MTT method; (**1B**) WB analysis for the protein expression level of PARP-1, and (**1C**) its quantification measured by Image J software. (**1D**) RT-PCR analysis for the PARP-1 RNA expression by normalization to GAPDH.



[a]$P < .05$
[b]$P < .01$

Note: Results are expressed as mean ± SD.

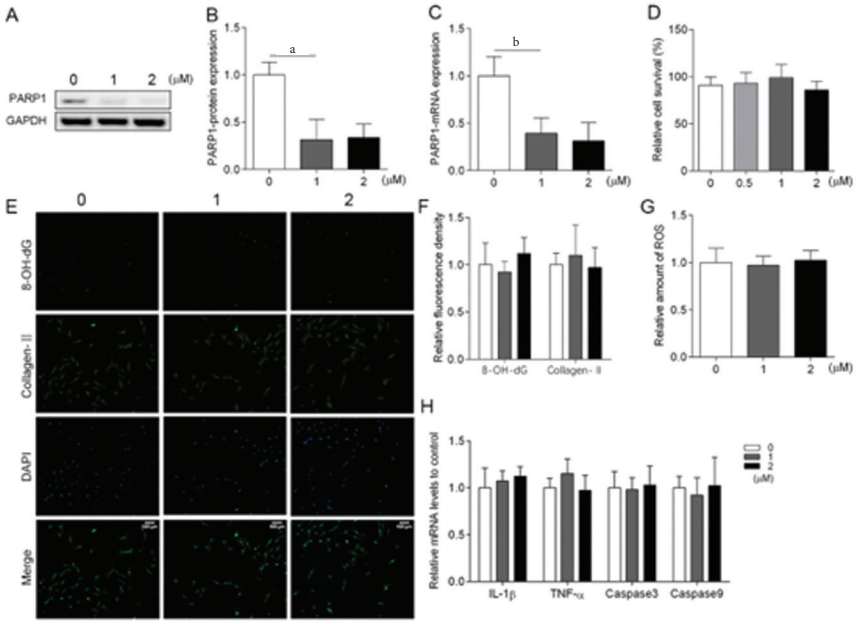
Abbreviations: CH, chondrocyte; IL-1β, interleukin-1 beta; PARP-1, polymerase 1; RT-PCR, reverse transcriptase polymerase chain reaction; SD, standard deviation; WB, Western blot.

RESULTS

PARP-1 Gradually Increased During IL-1β-Induced CH Degeneration

To clear the different expressions of PARP-1 during CHs degeneration, we cultured CHs with 10 ng/ml IL-1***β***[16] for up to 5 days and analyzed PARP-1 expression in both protein and RNA levels. As shown in Figure 1A, cell survival gradually decreased from day 0 to day 5. In addition, the

Figure 2. The effect of PARP-1 in the normal CHs. Normal CHs were treated with AG (1 μM or 2 μM) for 3 days. (**2A**) WB analysis for the protein expression of PARP-1, and (**2B**) its quantification measured by Image J software; (**2C**) RT-PCR analysis for the PARP-1 RNA expression via normalization to GAPDH; (**2D**) Cell survival was measured by the MTT method; (**2E**) IF analysis for 8-OH-dG and collagen II expression, and (**2F**) the quantification measured by Image J software; (**2G**) Cellular ROS detection; (**2H**) RT-PCR analysis for IL-1β, TNF-α, and caspase 3/9 RNA expression via normalization to GAPDH.



[a]$P < .01$;
[b]$P < .001$

Note: Results are expressed as mean ± SD.

Abbreviations: AG, AG-14361; CH, chondrocyte; IF, intrinsic factor; IL-1β, interleukin-1 beta; PARP-1, polymerase-1; ROS, reactive oxygen species; RT-PCR, reverse transcriptase polymerase chain reaction; SD, standard deviation; TNF-α, tumor necrosis factor alpha.

protein expression of PARP-1 was increased as a result of IL-1β treatment, day 5 of which was significantly higher than day 1 (Figure 1B, 1C). The RNA level of PARP-1 was also gradually increased in a time-dependent manner compared with day 0 (Figure 1D). Therefore, as previously described,[12,13] PARP-1 expression was increased, as a result of the introduction of IL-1β during CH degeneration.

The Effect of PARP-1 in Normal CHs

Since PARP-1 is sensitive to DNA damage, how PARP-1 acts in normal CHs is not explicit. To clear the effect of endogenous PARP-1 in normal CHs, we treated the CHs with a specific inhibitor of PARP-1 called AG-14361 (AG) 1 μM or 2 μM for 3 days. The cells without any treatment were set as controls. To measure the efficiency of AG, we analyzed cellular PARP-1 expression both in protein and RNA levels. The result showed AG obviously suppressed PARP-1 protein expression compared with the control, but no difference was observed between the 1 μM and 2 μM concentrations (Figure 2A, 2B). In addition, PARP-1 RNA expression was decreased when treated with AG, and 2 μM did not play a more significant role compared with the 1 μM group (Figure 2C). After treatment, cell survival was not affected, even when the concentration was increased to 2 μM (Figure 2D).

To determine the level of DNA damage, we stained the CHs with 8-OH-dG. Meanwhile, cellular collagen II protein synthesis was also co-stained to determine CH function. Compared with controls, suppression of PARP-1 by 1 or 2 μM AG did not change the DNA damage level or collagen II protein synthesis (Figure 2E, 2F). In addition, the ROS production in CHs was not affected by the deficiency of PARP-1 (Figure 2G). Furthermore, we tested IL-1β, TNF-α, caspase 3 and caspase 9 RNA expression to clarify the inflammatory response and apoptotic level of CHs, which was also not affected by the suppression of PARP-1 compared with the control group (Figure 2H). Therefore, in normal CHs, suppressing endogenous PARP-1 expression does not aggravate DNA damage and CH function. Due to no significant difference between the 1 or 2 μM supplements, we used the 2 μM concentration in the following experiments.

The Effect of PARP-1 on the Early Phase of CH Degeneration

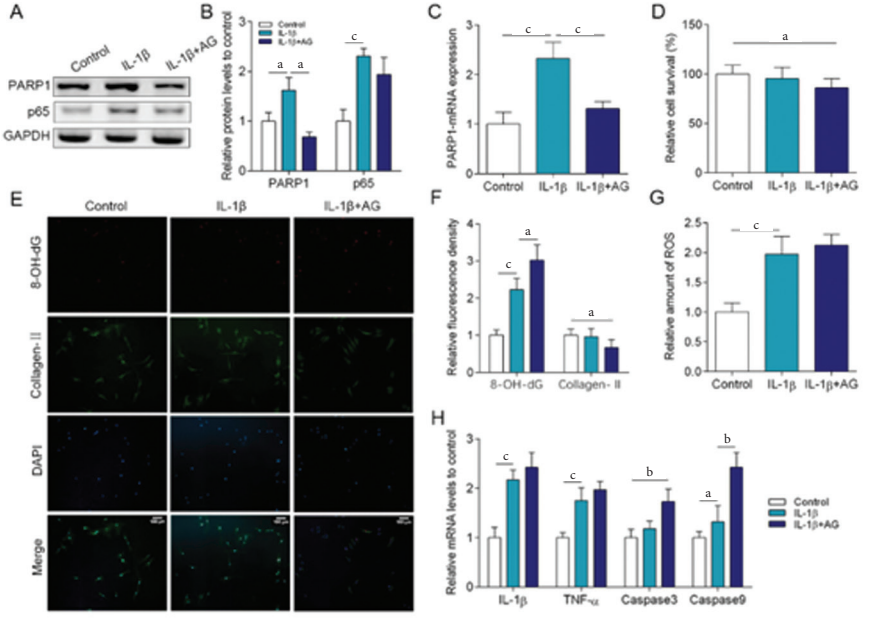
Our study results suggested that 1-day stimulation of IL-1β slightly increased PARP-1 expression compared with the control group. To determine whether the increased PARP-1 played a positive role in that phase, we suppressed the PARP-1 expression with AG while treating half the cells with IL-1β (10 ng/ml) for 1 day. Cells without treatment were set as controls. According to WB and qPCR results, the PARP-1 expression of CHs was sufficiently suppressed by the AG supplement as well. In addition, NF-κB-p65 protein levels were not affected by the reduction in PARP-1 expression (Figure 3A-3C). Unlike the survival of stable CHs without PARP-1 suppression, cell survival was reduced when the CHs were incubated with AG (2 μM) (Figure 3D). Meanwhile, the stimuli of IL-1β increased the 8-OH-dG level, which was aggravated by the lack PARP-1. Compared with controls, the CHs collagen II content was not affected after 1-day IL-1β treatment. However, it was significantly decreased when PARP-1 was suppressed (Figure 3E, 3F). In addition, the ROS level and IL-1β, and TNF-α

RNA expressions were all triggered and were higher after IL-1β treatment, and no significant difference was observed while PARP-1 was suppressed. However, caspase 3 and caspase 9 expression was upregulated, resulting from the reduction of PARP-1. In general, lacking PARP-1 in the early phase of IL-1β treatment would accelerate the degradative process of CHs.

The Effect of PARP1 on the developed phase of CHs degeneration

To clear the effect of PARP-1 on the developed phase of CH degeneration caused by IL-1β, we cultured CHs with IL-1β (10 ng/ml) for 5 days and added the AG (2 μM) to the culture medium starting on day 3. CHs cultured for 5 days without treatment were set as the control group. After 5 days of treatment, the protein level of PARP-1 and NF-κB-p65 were massively increased compared with the control group, as was PARP-1 RNA expression. However, the suppression of PARP-1 by AG obviously alleviated the upregulation of PARP-1 and NF-κB-p65 (Figure 4A-4C). In addition, the combination of the AG treatment also protected cell survival after 5 days of IL-1β treatment (Figure 4D). Furthermore, inhibition of PARP-1 also maintained collagen II protein expression compared with the IL-1β-treated CHs. However, the 8-OH-dG level was further increased after the AG supplement (Figure 4E, 4F). Cellular ROS, IL-1β, TNF-α, caspase 3 and caspase 9 RNA expression was significantly decreased when PARP-1 expression was inhibited (Figure 4G, 4H). Therefore, suppressing PARP-1 expression also leads to a higher level of DNA damage. Nevertheless, it efficiently protects CH function by maintaining cell survival, collagen II content and reducing ROS accumulation, and inflammatory and apoptotic response.

Figure 3. The effect of PARP-1 on the early phase of CH degeneration. CHs were subjected to IL-1*β* (10 ng/ml) with and without AG (2 μM) for 1 day. (**3A**) WB analysis for the protein expression of PARP-1 and p65, and (**3B**) the quantification measured by Image J software; (**3C**) RT-PCR analysis for PARP-1 RNA expression viia normalization to GAPDH; (**3D**) Cell survival was measured by the MTT method; (**3E**) IF analysis for 8-OH-dG and collagen II expression, and (**3F**) the quantification measured by Image J software; (**3G**) Cellular ROS detection; (**3H**) RT-PCR analysis for IL-1β, TNF-α and caspase 3/9 RNA expression via normalization to GAPDH.



[a]$P < .05$;
[b]$P < .01$;
[c]$P < .001$

Note: Results are expressed as mean ± SD.

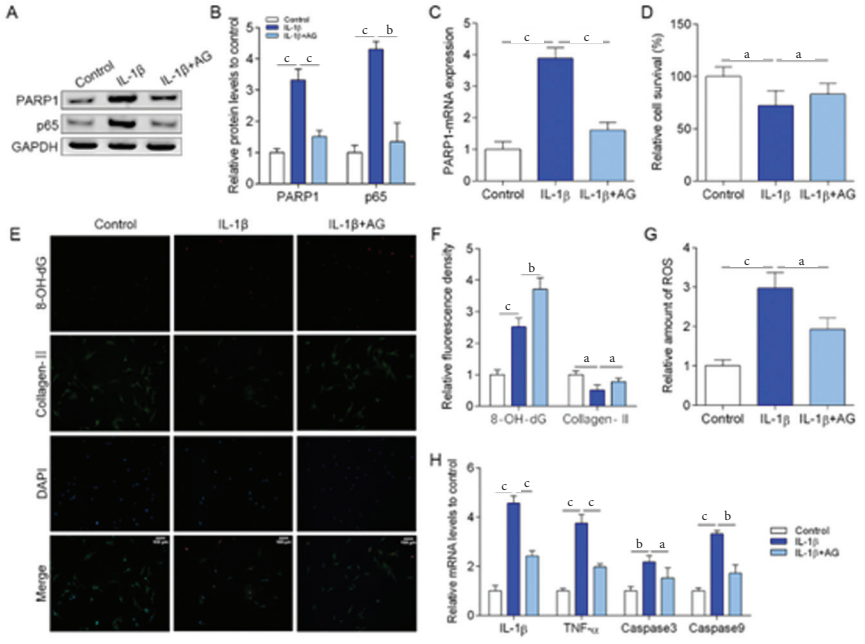
Abbreviations: AG, AG-14361; CH, chondrocyte; IF, intrinsic factor; IL-1β, interleukin-1 beta; PARP-1, polymerase-1; ROS, reactive oxygen species; RT-PCR, reverse transcriptase polymerase chain reaction; TNF-α, tumor necrosis factor alpha; WB, Western blot.

DISCUSSION

PARP-1 is a nuclear protein that senses DNA damage. When a DNA strand breaks, PARP-1 binds to the broken DNA and activates, acting on target proteins such as histones and transcription factors, thereby repairing DNA, maintaining gene stability and regulating transcription.[10] Various causes of inflammation can upregulate the expression of PARP-1. Studies have reported that the enhancement of PARP-1 activity can maintain genome stability and inhibit tumorigenesis, which is conducive to prolonging life.[17] But clinical studies have also found that inhibition of PARP-1 has a protective effect on pleurisy, arthritis, asthma, colitis, allergic encephalomyelitis and other autoimmune diseases,[18] which seems contradictory. Therefore, the mechanism of action of PARP-1 in OA remains unclear. In human CHs cultured *+0*, administration of exogenous IL-1*β* can induce similar performance in OA with both time-dependence and dose-dependence.[19, 20]

In our study, with the development of CH degeneration caused by IL-1β, we found the cell survival of CHs was challenged, and cellular PARP-1 was gradually increased. Due to the complex features and functionality of PARP-1, we wondered whether PARP-1 plays a different role in a different phase of CH degeneration *in vitro*.

Figure 4. The effect of PARP-1 on the developed phase of CH degeneration. CHs were treated with IL-1β (10 ng/ml) for 5 days, and AG (2 μM) was added to the culture medium starting on day 3. (**4A**) WB analysis for the protein expression of PARP-1 and p65, and (**4B**) its quantification measured by Image J software; (**4C**) RT-PCR analysis for the PARP-1 RNA expression via normalization to GAPDH; (**4D**) Cell survival was measured by the MTT method; (**4E**) IF analysis for 8-OH-dG and collagen II expression, and (**4F**) the quantification measured by Image J software; (**4G**) Cellular ROS detection; (**4H**) RT-PCR analysis for the IL-1β, TNF-α, and caspase 3/9 RNA expression via normalization to GAPDH.



[a]$P < .01$;
[b]$P < .01$;
[c]$P < .001$

Note: Results are expressed as mean ± SD.

Abbreviations: AG, AG-14361; CH, chondrocyte; IF, intrinsic factor; IL-1β, interleukin-1 beta; PARP-1, polymerase-1; ROS, reactive oxygen species; RT-PCR, reverse transcriptase polymerase chain reaction; TNF-α, tumor necrosis factor alpha; WB, Western blot.

8-OH-dG is a normal metabolite of the human body and is one of more than 20 oxidized DNA products. The nature of 8-OH-dG in DNA is relatively stable. The expression of 8-OH-dG in DNA can be affected by different cell phases and various enzymes. 8-OHdG is a particular form of oxidized DNA.[21] Increased 8-OHdG can cause misreading in the DNA replication process, lose the specificity of base pairing and cause the adjacent pyrimidine bases to replicate incorrectly.[22] It can be seen that the molecular mechanism of oxygen free radical damage to DNA may be the activation of nuclease, the direct reaction of hydroxyl free extreme with DNA. Therefore, 8-OHdG is an indicator of DNA oxidative damage. After 1 day of treatment with IL-1β, 8-OH-dG expression and the ROS content of CHs was upregulated. The suppression of PARP-1 by AG led to insufficient repair of damaged DNA, presenting a higher 8-OH-dG level. Meanwhile, cellular p65, ROS, IL-1β and TNF-α levels were not significantly decreased. However, the aggravated cell survival decreased collagen II content, and increased caspase 3/9 expression indicated a more severe CH condition, which suggests that lacking PARP-1 would accelerate the IL-1β-induced CH degeneration.

A total of 3 days after IL-1β treatment, PARP-1 had somehow accumulated in the cells. We continuously combined IL-1β with the PARP-1inhibitor to culture CHs for an extra 2 days. This time, suppressing PARP-1 also aggravated the DNA damage. However, cell function was protected with a rescued survival and collagen II content. The results indicated that the downregulation of PARP-1 alleviated ROS accumulation, inflammatory response and CH apoptosis.

NF-κB is a protein complex, and p65 is the most crucial subunit among these proteins.[23] It is involved in regulating inflammation, cell apoptosis and the stress response, which is also regulated by PARP-1. Zhang, et al.[24] reported pretreating human aortic endothelial cells with PARP-1 siRNA inhibited lipopolysaccharide-induced NF-κB-p65 expression and nuclear translocation. Vuong, et al.[25] found that PARP-1 enzymatic activity plays a vital role in the TNFα-induced transcriptional activation of NF-κB in mouse cortical astrocytes and microglia. In the developed phase of CH degeneration, our study potentially proved that inhibiting PARP-1 contributes to the suppression of NF-κB-p65, which could also be an explanation for the improved cell function. Apart from these effects, PARP-1 was at the onset of apoptosis by caspase 3[26] and caspase 9[27] *per* se. When PARP-1 is massively accumulated in the late phase of CH degeneration, the caspase-based apoptotic cascade is activated. Excessive PARP-1 reaction will also consume a large amount of NAD+, causing total energy adenosine triphosphate (ATP) depletion, and at the same time destroying the steady-state environment in the nucleus, reducing cell viability or leading to cell death.[28] Therefore, although the progress of DNA damage was not enhanced by suppressing PARP-1 in the developed phase of CH degeneration, the inflammatory and apoptotic processes were delayed.

Study Limitations

However, our study was limited by the fact that we hardly investigated the role of PARP1 inhibition *in vivo* in animal OA models. That requires further exploration in the future.

CONCLUSION

In conclusion, PARP-1 acts not only as the sensor and repairer of DNA damage but also as the regulator of inflammation and apoptosis. At the beginning of CH degeneration, the gradually increased PARP-1 provided CH protection. However, with the accumulation of PARP-1 in the developed phase of CH degeneration, inflammatory and apoptotic progress is significantly activated, which could be alleviated by moderate inhibition of PARP-1 expression. Our study identified a novel mechanism of PARP1 involved in the development of CHs degeneration. It provides an understanding of the physiological functions of PARP-1 in CHs and related pathological phenomena of OA, and thusPARP-1 may be a promising target for clinical therapy and medicine development.

CONFLICT OF INTEREST

None.

FUNDING

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REFERENCES

1. Loeser RF, Collins JA, Diekman BO. Ageing and the pathogenesis of osteoarthritis. *Nat Rev Rheumatol*. 2016;12(7):412-420. doi:10.1038/nrrheum.2016.65
2. Kung LHW, Mullan L, Soul J, et al. Cartilage endoplasmic reticulum stress may influence the onset but not the progression of experimental osteoarthritis. *Arthritis Res Ther*. 2019;21(1):206. doi:10.1186/s13075-019-1988-6
3. Hunter DJ, Bierma-Zeinstra S. Osteoarthritis. *Lancet*. 2019;393(10182):1745-1759. doi:10.1016/S0140-6736(19)30417-9
4. Vinatier C, Merceron C, Guicheux J. Osteoarthritis: from pathogenic mechanisms and recent clinical developments to novel prospective therapeutic options. *Drug Discov Today*. 2016;21(12):1932-1937. doi:10.1016/j.drudis.2016.08.011
5. Mariani E, Pulsatelli L, Facchini A. Signaling pathways in cartilage repair. *Int J Mol Sci*. 2014;15(5):8667-8698. doi:10.3390/ijms15058667
6. Chatterjee N, Walker GC. Mechanisms of DNA damage, repair, and mutagenesis. *Environ Mol Mutagen*. 2017;58(5):235-263. doi:10.1002/em.22087
7. Jackson SP, Bartek J. The DNA-damage response in human biology and disease. *Nature*. 2009;461(7267):1071-1078. doi:10.1038/nature08467
8. Alvarez-Garcia O, Matsuzaki T, Olmer M, Plate L, Kelly JW, Lotz MK. Regulated in development and DNA damage response 1 deficiency impairs autophagy and mitochondrial biogenesis in articular cartilage and increases the severity of experimental osteoarthritis. *Arthritis Rheumatol*. 2017;69(7):1418-1428. doi:10.1002/art.40104
9. Ray Chaudhuri A, Nussenzweig A. The multifaceted roles of PARP1 in DNA repair and chromatin remodelling. *Nat Rev Mol Cell Biol*. 2017;18(10):610-621. doi:10.1038/nrm.2017.53
10. Pascal JM. The comings and goings of PARP-1 in response to DNA damage. *DNA Repair (Amst)*. 2018;71:177-182. doi:10.1016/j.dnarep.2018.08.022
11. D'Andrea AD. Mechanisms of PARP inhibitor sensitivity and resistance. *DNA Repair (Amst)*. 2018;71:172-176. doi:10.1016/j.dnarep.2018.08.021
12. Zhang Q, Yin ZS, Zhang FW, Cao K, Sun HY. CTHRC1 mediates IL1βinduced apoptosis in chondrocytes via JNK1/2 signaling. *Int J Mol Med*. 2018;41(4):2270-2278. doi:10.3892/ijmm.2018.3403
13. Ren H, Yang H, Xie M, et al. Chondrocyte apoptosis in rat mandibular condyles induced by dental occlusion due to mitochondrial damage caused by nitric oxide. *Arch Oral Biol*. 2019;101:108-121. doi:10.1016/j.archoralbio.2019.03.006
14. Sun Y, Zhou L, Lv D, Liu H, He T, Wang X. Poly(ADP-ribose) polymerase 1 inhibition prevents interleukin-1β-induced inflammation in human osteoarthritic chondrocytes. *Acta Biochim Biophys Sin (Shanghai)*. 2015;47(6):422-430. doi:10.1093/abbs/gmv033
15. Li D, Luo Y, Chen X, et al. NF-kappaB and poly (ADP-ribose) polymerase 1 form a positive feedback loop that regulates DNA repair in acute myeloid leukemia cells. *Mol Cancer Res*. 2019;17(3):761-772. doi:10.1158/1541-7786.MCR-18-0523
16. Wongwichai T, Teeyakasem P, Pruksakorn D, Kongtawelert P, Pothacharoen P. Anthocyanins and metabolites from purple rice inhibit IL-1β-induced matrix metalloproteinases expression in human articular chondrocytes through the NF-κB and ERK/MAPK pathway. *Biomed Pharmacother*. 2019;112:108610. doi:10.1016/j.biopha.2019.108610
17. Beneke S, Bürkle A. Poly(ADP-ribosyl)ation in mammalian ageing. *Nucleic Acids Res*. 2007;35(22):7456-7465. doi:10.1093/nar/gkm735
18. Pacher P, Szabo C. Role of the peroxynitrite-poly(ADP-ribose) polymerase pathway in human disease. *Am J Pathol*. 2008;173(1):2-13. doi:10.2353/ajpath.2008.080019
19. Wang Y, Yu D, Liu Z, et al. Exosomes from embryonic mesenchymal stem cells alleviate osteoarthritis through balancing synthesis and degradation of cartilage extracellular matrix. *Stem Cell Res Ther*. 2017;8(1):189. doi:10.1186/s13287-017-0632-0
20. Castro Martins M, Peffers MJ, Lee K, Rubio-Martinez LM. Effects of stanozolol on normal and IL-1β-stimulated equine chondrocytes in vitro. *BMC Vet Res*. 2018;14(1):103. doi:10.1186/s12917-018-1426-z
21. Vorilhon S, Brugnon F, Kocer A, et al. Accuracy of human sperm DNA oxidation quantification and threshold determination using an 8-OHdG immuno-detection assay. *Hum Reprod*. 2018;33(4):553-562. doi:10.1093/humrep/dey038
22. Basu S, De D, Dev Khanna H, Kumar A. Lipid peroxidation, DNA damage and total antioxidant status in neonatal hyperbilirubinemia. *J Perinatol*. 2014;34(7):519-523. doi:10.1038/jp.2014.45
23. Jimi E, Fei H, Nakatomi C. NF-kappaB signaling regulates physiological and pathological chondrogenesis. *Int J Mol Sci*. 2019;20(24):6275. doi:10.3390/ijms20246275
24. Zhang JN, Ma Y, Wei XY, et al. Remifentanil protects against lipopolysaccharide-induced inflammation through PARP-1/NF-kappaB signaling Pathway. *Mediators Inflamm*. 2019;2019:3013716. doi:10.1155/2019/3013716
25. Vuong B, Hogan-Cann AD, Alano CC, et al. NF-κB transcriptional activation by TNFα requires phospholipase C, extracellular signal-regulated kinase 2 and poly(ADP-ribose) polymerase-1. *J Neuroinflammation*. 2015;12(1):229. doi:10.1186/s12974-015-0448-8
26. Galia A, Calogero AE, Condorelli R, et al. PARP-1 protein expression in glioblastoma multiforme. *Eur J Histochem*. 2012;56(1):e9. doi:10.4081/ejh.2012.e9
27. Mathieu J, Flexor M, Lanotte M, Besançon F. A PARP-1/JNK1 cascade participates in the synergistic apoptotic effect of TNFalpha and all-trans retinoic acid in APL cells. *Oncogene*. 2008;27(24):3361-3370. doi:10.1038/sj.onc.1210997
28. Sairanen T, Szepesi R, Karjalainen-Lindsberg ML, Saksi J, Paetau A, Lindsberg PJ. Neuronal caspase-3 and PARP-1 correlate differentially with apoptosis and necrosis in ischemic human stroke. *Acta Neuropathol*. 2009;118(4):541-552. doi:10.1007/s00401-009-0559-3