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Original Article

Osteopontin can decrease the cartilage cellular inflammatory reaction induced by LPS

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Abstract: Objective: This study aims to explore the effect of osteopontin on the inflammatory reaction of cartilage cells stimulated by LPS and its possible signal pathway. Methods: The inflammatory reaction of cartilage cells was induced by LPS, and then the cells were treated with OPN and PD98059 respectively. The expression of TNF-α, IL-1β and ERK1/2 were detected by ELISA or Western-blotting methods. Results: Osteopontin could decrease the cartilage cellular inflammatory reaction induced by LPS with dose dependent, while PD98059 could reverse the inhibition of osteopontin. Conclusion: Osteopontin could decrease the cartilage cellular inflammatory reaction induced by LPS, which may be associated with the ERK1/2 signal pathway.

Keywords: Osteopontin, inflammatory reaction, ERK1/2 signal pathway

Introduction

Cartilage covers the surfaces of synovial joints where it prevents direct bone to-bone contact, A variety of causes such as hereditary, developmental, metabolic and mechanical deficits may initiate processes leading to loss of cartilage, when bone surfaces become less well protected by cartilage, bone may be exposed and damaged. Osteoarthritis (OA), also known as degenerative arthritis or degenerative joint disease is the most common form of arthritis that affects the joints and surrounding tissues. The development of OA involves distinct morphological changes, depending on the extent of degeneration. Its symptoms include joint pain, tenderness, stiffness, locking and sometimes an effusion [1-3].

OA is a global problem and it reduces quality of life because of no cure, which results in significant economic impact. The treatment of OA generally involves a combination of exercise, lifestyle modification and analgesics. It is challenging since cartilage impedes the local and systemic delivery of therapeutic compounds. With the accelerated development of aging society, 9.6% of men and 18% of women over 60 years old suffer from symptomatic OA. It is expected to be the fourth leading cause of disability by 2020 [4-7].

Osteopontin (OPN) is a glycosylated phosphoprotein, which exist in various body tissues. Studies have found that there was a positive correlation between OPN content and joint damage degree in the synovial fluid and cartilage of patients with primary total knee prosthesis [8], which indicated that OPN might be potential biomarker for OA patients. In this study, we explored whether OPN can inhibit the inflammatory reaction of cartilage cell, which aims to find a potential treatment target of OA, to find a new direction for the treatment of patients with OA.

Materials and methods

Cartilage cells cultured in vitro

Briefly, intervertebral disc tissue was taken from New Zealand rabbits and nucleus pulposus were separated from the tissue. The nucleus pulposus were digested in 0.25% type II col-

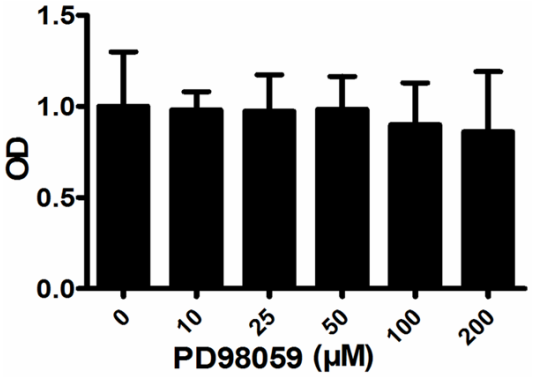

Figure 1. Effects of PD98059 with different concentrations on the proliferation of cartilage cells.

lagenase solution (Sigma, USA) at 37°C for 20 min. They were filtered with 70 μm of stainless steel wire mesh and the suspension was centrifuged for 8 min at 1 000 r/min. Cells were counted and 4×10^5 cells were cultured in 25 cm^2 flask at 37°C with 5% CO_2.

Detection of the effect of ERK1/2 signal pathway inhibitor PD98059 on the growth of Cartilage cells using MTT method

Briefly, the primary cartilage cells in good growth condition were seeded in 96-well plates at a density of 3×103 cells/well followed by incubation at 37°C with 5% CO_2 in a humidified atmosphere. Each growth group contained 3 wells of 6 groups. The medium was discarded and different concentrations (0 μM, 10 μM, 25 μM, 50 μM, 100 μM and 200 μM) of PD98059 (Sigma, USA) diluted with F12-DMEM (Gibco, USA) were added into the wells after incubation for 24 h. Then 20 μl MTT (5.0 mg/ml) were added to these wells after incubation for 24 h. After incubation for 4 h, the supernatant was discarded and 200 μl DMSO was added to these wells. The optical density (OD) was measured by NanoDrop ND-1000 spectrometer (Nanodrop Technologies, Wilmington, USA).

ELISA detection

The expression of TNF-α and IL-1β was detected according to the manual of TNF-α and IL-1β ELISA kits (BD, USA). Briefly, A total of 50 μl cell supernatant and 50 μl sample dilution were added into 96 well plate coated with specific antibody and incubation at 37°C for 2 h. The plates were washed 5 times, 100 μl substrate of enzyme reaction was added and incubation at 37°C for 2 h. Then plates were washed 5 times and 100 μl chromogenic reaction liquid was added and incubation at 3°C for 30min, the termination liquid was added to terminate the reaction. The absorbance was detected using microplate reader at 450 nm wavelength.

Western-blotting

Total proteins were extracted and analyzed with SDS-PAGE electrophoresis. Then it was electrotransferred to the PVDF membrane (Millipore Co., Billerica, MA, USA). The membrane containing the proteins was used for immunoblotting with required antibodies. They were blocked with 5% non-fat milk in TBST (10 mM Tris-HCl (pH 8.0), 150 mM NaCl, and 0.1% Tween-20) for 2 h, then incubated with the primary antibodies: rabbit anti- ERK1/2 antibodies (1:200), rabbit anti- p-ERK1/2 antibodies (1:200) and anti-GAPDH (1:5000) (CST, Beverly, MA, USA) at 4°C overnight. Then they were incubated with secondary antibodies conjugated with horseradish peroxidase at room temperature for 1 h. Antibody binding was detected using Odessey Infrared Imaging system.

Statistical analysis

Data are presented as Mean ± SD. The SPSS software package was used for the statistical tests. Comparisons of continuous variables among groups were performed by a one-way ANOVA and t-test was used to specify differences between two groups. $P<0.05$ was considered statistically significant.

Results

Effect of PD98059 on the proliferation of cartilage cells

There was no significant difference between the groups indicated that PD98059 had no effect on the proliferation of cartilage cells (**Figure 1**, $P>0.05$).

Effects of OPN on the inflammatory reaction of cartilage cells induced by LPS

The results of ELISA showed that the expression of TNF-α and IL-1β in culture supernatant of cartilage cells increased significantly when

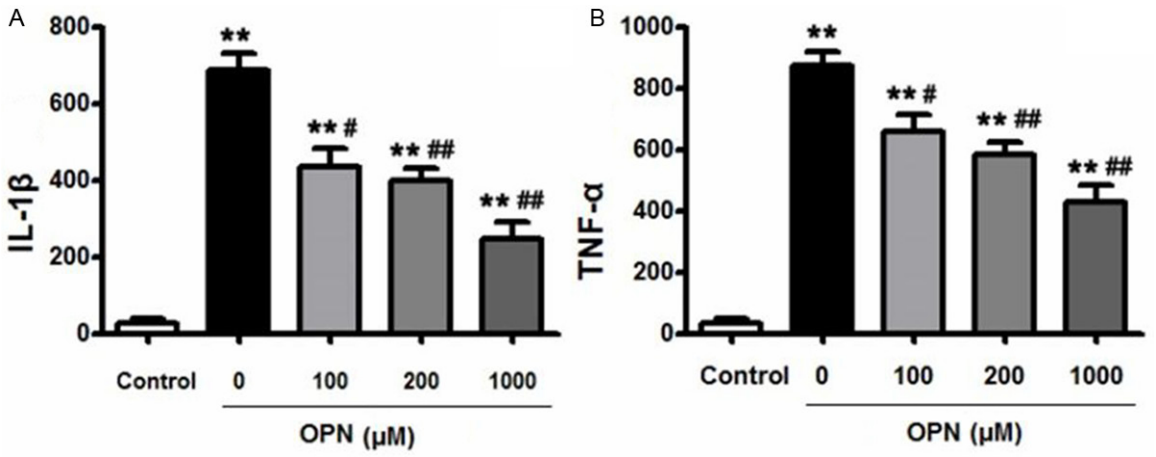

Figure 2. Effects of OPN with different concentrations on the inflammatory reaction of cartilage cells induced by LPS. A. Effects of OPN with different concentrations on the expression of IL-1β in inflammatory reaction induced by LPS; B. Effects of OPN with different concentrations on the expression of TNF-α in inflammatory reaction induced by LPS. $^{**}P<0.01$ vs. control; $^{\#}P<0.05$, $^{\#\#}P<0.01$ vs. OPN (0 μM).

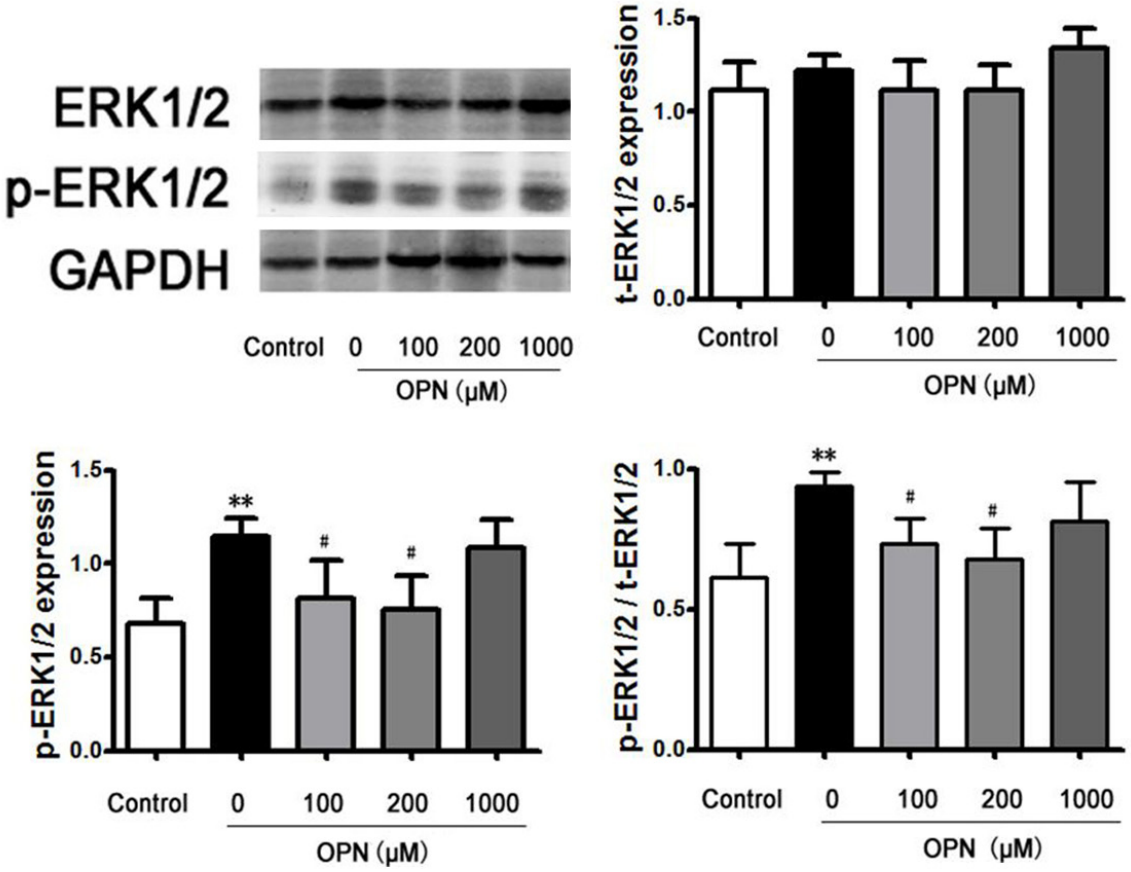

Figure 3. Effects of OPN with different concentrations on the expression of ERK1/2 in inflammatory reaction induced by LPS. $^{**}P<0.01$ vs. control; $^{\#}P<0.05$ vs. OPN (0 μM).

the cells were treated with 1 ng/ml LPS. They decreased significantly with a dose dependent when the cells were treated with different concentrations OPN (**Figure 2**).

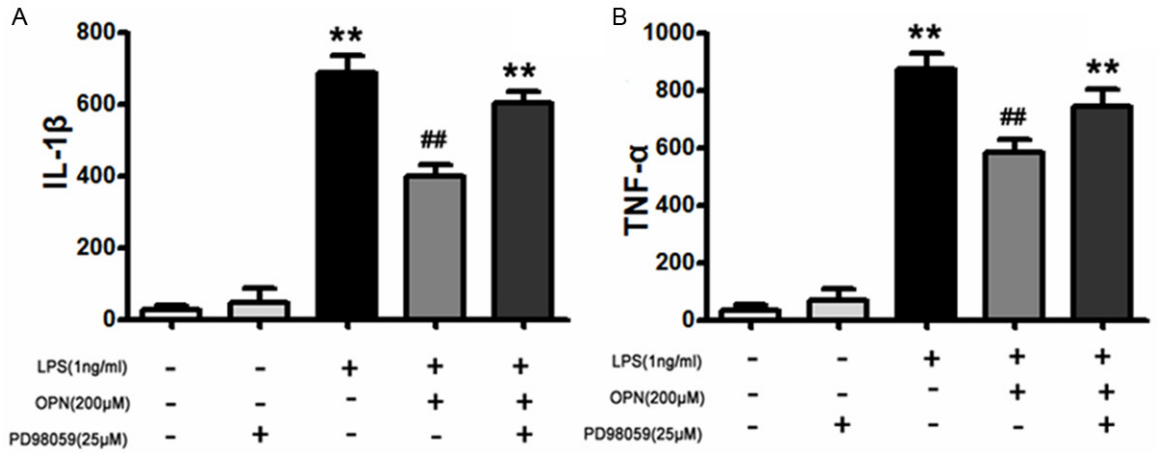

Figure 4. Effects of OPN and PD98059 with different concentrations on the expression of TNF-α and IL-1β in inflammatory reaction induced by LPS. A. Effects of OPN and PD98059 with different concentrations on the expression of IL-1β in inflammatory reaction induced by LPS; B. Effects of OPN and PD98059 with different concentrations on the expression of TNF-α in inflammatory reaction induced by LPS. PD98059 could reverse the inhibition of OPN, $^{**}P<0.01$ vs. control; $^{\#\#}P<0.01$ vs. LPS.

Effects of OPN on the expression of ERK1/2 in inflammatory reaction induced by LPS

The results of Western-blotting showed that there was no effect on the expression of t-ERK1/2 while the expression of p-ERK1/2 increased when the cartilage cells were treated with LPS. When the cells were treated with 100 μM and 200 μM of OPN, the expression of t-ERK1/2 had no significant difference while the expression of p-ERK1/2 decreased significantly (**Figure 3**).

Effects of OPN and PD98059 on the expression of TNF-α and IL-1β in inflammatory reaction induced by LPS

The results of ELISA showed that there was no inflammation reaction in cartilage cells when they were treated with PD98059, the expression of TNF-α and IL-1β in culture supernatant of cartilage cells increased significantly when the cells were treated with 1 ng/ml LPS. The expression of TNF-α and IL-1β decreased significantly when the cells were treated with 200 μM OPN. PD98059 can reverse the inhibition of OPN on cartilage cellular inflammation after inhibiting ERK1/2 signaling pathway (**Figure 4**).

Discussion

OA is a common disease articular cartilage degeneration, more and more patients suffer from this disease and it has become a problem of medical and social widespread concern. It is important for economic and social development to study on the pathogenesis of osteoarthritis, look for the potential therapeutic targets, delay the onset of joint degeneration and improve the level of prevention and treatment.

The pathogenesis of OA is very complex; it is now generally thought that cytokines and inflammatory mediators play a key role in the occurrence and development of OA. The excessive secretion of cytokines can promote the apoptosis of cartilage cells and synthesis of articular cartilage degenerate gradually [9-11]. Other factors such as aging, hormone disorder and mechanical damage also can promote the development of OA [12-14].

In this study, we observed the effects of OPN on cartilage cellular inflammation induced by LPS. We found that there was no significant effect of PD98059 with different concentrations on the proliferation of cartilage cells and PD98059 did not induce inflammatory reaction of cartilage cells. The expression of TNF-α, IL-1β and p-ERK1/2 increased significantly in cartilage cells induced by LPS. However, they decreased significantly with a dose dependent when the cells were treated with different concentrations OPN. It indicated that OPN may reduce the inflammatory reaction induced by LPS by regulating the expression of p-ERK1/2. Inhibition of

OPN on cartilage cellular inflammation could be reversed when using PD98059 to inhibit ERK1/2 pathway. OPN may regulate inflammatory reaction induced by LPS through regulating ERK1/2 signaling pathway.

OPN is involved in many physiological and pathological processes such as wound healing, bone metabolism, tumorigenesis and inflammation and immune response [15-17]. Attur et al found that OPN was highly expressed in the joint cartilage in patients with OA [18]. Other studies found that there was a positive correlation between the OPN concentration in the synovial fluid and the degree of pain and articular cartilage injury of the tibial side in patients with the cartilage degenerative lesions [19]. The related animal experiments also proved that OPN played an important role in age or damage related OA. OPN can regulate the degeneration of articular cartilage through regulating the expression of MMP-13 and degradation of glycosaminoglycans [20]. These indicated that OPN might be potential biomarkers for OA patients. In clinical studies in patients with hypertension, Wolak et al found that N terminal of OPN was related to the formation of carotid atherosclerosis inflammatory plaque [21]. The activation of thrombin with OPN participation was related to knee osteoarthritis and other inflammatory diseases [22]. ERK1/2 signal pathway played an important role in the regulation of cartilage specific gene expression [23]. Dai et al found that OPN induced endothelial cellular angiogenesis through the activation of ERK1/2 signal pathway [24]. Prasadam et al found that interaction between osteoblasts and articular cartilage cells in the cartilage of osteoarthritis patients affected the expression of specific thrombospondin of cartilage and bone and MMPs, which involving the MAPK-ERK1/2 signaling pathway [25].

In a word, OPN could reduce the inflammatory reaction induced by LPS by ERK1/2 signaling pathway. Further study on whether OPN can inhibit inflammatory reaction and its mechanism can provide new ideas for looking for new direction for the treatment of OA patients.

Disclosure of conflict of interest

None.

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