Platelet-rich plasma protects rat chondrocytes from interleukin-1β-induced apoptosis

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Abstract. Interleukin (IL)-1β-induced chondrocyte apoptosis is associated with the pathogenesis of arthritis. Platelet-rich plasma (PRP), which is derived from the patient's own blood and contains numerous growth factors, has the potential for arthritis treatment. Therefore, the present study aimed to determine the effects of PRP on chondrocyte apoptosis, under IL-1β-induced pathological conditions. Chondrocytes isolated from the knee joint of Sprague Dawley rats were used in the present study. Cell viability was determined using the Cell Counting kit-8 assay, cell apoptosis was evaluated by flow cytometry, and the expression of apoptosis-, anabolism- and catabolism-associated genes were detected by quantitative polymerase chain reaction; protein expression was detected by western blot analysis. The results demonstrated that 10% PRP in the culture medium increased chondrocyte proliferation, whereas IL-1β induced cell apoptosis. Treatment with PRP significantly attenuated cell apoptosis in IL-1β-treated chondrocytes, and altered apoptosis-associated expression at the gene and protein level. Furthermore, treatment with PRP significantly reduced matrix metalloproteinase production and promoted anabolism of cartilage extracellular matrix under IL-1β treatment. The present study demonstrated the protective effects of PRP on chondrocyte apoptosis and extracellular matrix anabolism, and provided scientific evidence to support the potential use of PRP as a promising therapeutic strategy for the treatment of arthritis.

Introduction

Arthritis, which includes osteoarthritis (OA) and rheumatoid arthritis, is a chronic inflammatory disease characterized by pain and inflammation, progressive joint destruction, and disability (1). The proinflammatory cytokine interleukin (IL)-1β has a key role in arthritis (2); IL-1β is able to stimulate the production of matrix metalloproteinases (MMPs), which are enzymes that degrade all components of the extracellular matrix (3). It has also been suggested that IL-1β may affect chondrocyte apoptosis (4), which is correlated with the severity of cartilage damage and is associated with the pathogenesis of arthritis (5).

Therefore, inhibition of IL-1β-dependent apoptosis and the function of MMPs may be considered potential therapeutic strategies. Platelet-rich plasma (PRP) consists of blood plasma enriched with platelets, and is a rich source of autologous growth factors, including platelet-derived growth factor (PDGF) and transforming growth factor (TGF)-β1 (6). PRP is able to promote cell proliferation, angiogenesis and collagen synthesis, thus contributing to healing of skeletal muscle, tendon, bone and ligaments (7,8). PRP (9-11) is widely used in clinical practice, particularly during aesthetic plastic surgery and the treatment of soft-tissue ulcers (12-14), and may improve the survival of surgically implanted adipose tissue in patients (15,16). Notably, it has been reported that treatment of chondrocytes with PRP may enhance cell division and collagen synthesis (17). In light of the role of PRP in arthritis therapy, determination of its effects on chondrocyte apoptosis may provide evidence for future clinical applications.

The present study aimed to determine the effects of PRP on IL-1β-dependent chondrocyte apoptosis and extracellular matrix anabolism. The results demonstrated that PRP was able to inhibit chondrocyte apoptosis induced by IL-1β and promote matrix anabolism. These results suggested that PRP may protect chondrocytes from apoptosis, thus indicating a potential therapeutic strategy for the treatment of arthritis.

Materials and methods

Ethics approval. The present study was approved by the ethics committee of Beijing Friendship Hospital of the Capital Medical University of China.

Cell culture. Chondrocytes were isolated from cartilage tissue in the knee joints of Sprague-Dawley rat neonates. Cartilage samples from three rat neonates were harvested and pooled for use in the present study. The animals used were 4-week-old male Sprague-Dawley rats (70-100 g; obtained from Charles River Laboratories, Inc., Wilmington, DE, USA), and they were housed in a wire mesh cage in an animal room under controlled conditions (temperature, 23±3˚C; relative humidity,

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50±20%, 12-h light/dark cycle). Male Sprague-Dawley rats, on reaching a weight of 180-250 g, were sacrificed by cervical dislocation. Briefly, cartilage was isolated and digested in 1 mg/ml collagenase (cat. no. C9263; Sigma-Aldrich, St. Louis, MO, USA) in Dulbecco's modified Eagle's medium (DMEM; cat. no. 11965092; Gibco; Thermo Fisher Scientific, Inc., Waltham, MA, USA) supplemented with 10% Gibco™ fetal bovine serum (FBS) for 16 h at 37˚C. Undigested tissue were removed using a 70 μm cell strainer. Chondrocytes were washed with phosphate-buffered saline (PBS) and were resuspended in basic medium (DMEM supplemented with 10% FBS, 100 U/ml penicillin and 100 mg/ml streptomycin). Chondrocytes were subsequently cultured in basic medium in culture flasks at 37˚C in an incubator containing 5% CO_2 (BPX-82 incubator; Shanghai Boxun Medical Biological Instrument Corp., Shanghai, China).

Cultured chondrocytes were characterized by immunohistochemical staining of collagen type II (COL2). Briefly, chondrocytes ($1x10^6$) were plated on glass cover slips in six-well plates. After 24 h, the cells were washed with PBS and were fixed in 4% paraformaldehyde for 30 min at room temperature. Permeabilization and blocking were performed by incubating the cells with 1% Triton-X 100 and 1% bovine serum albumin for 15 min. Cover slips were then incubated overnight at 4˚C with rabbit polyclonal anti-COL2 primary antibodies (cat. no. Bs-11929R; dilution, 1:500; Bioss, Inc., Woburn, MA, USA). Sequentially, primary antibodies were visualized using an EnVision Detection system (cat. no. GK400315; Dako Belgium Nv, Leuven, Belgium) and a DMLP-MP30 microscope (Leica, Heidelberg, Germany).

PRP preparation. A total of 10 ml blood was collected in a syringe containing 1 ml 3.8% sodium citrate from the artery of one male rat (age, 10 weeks). In addition, blood samples were collected from three rats and processed separately. Blood samples were gently agitated to thoroughly mix the sodium citrate with the blood. Subsequently, the samples were centrifuged at 1,500 rpm for 10 min at 4˚C. The plasma and buffy coat layer were then transferred to another centrifuge tube, and were centrifuged at 3,000 rpm for 10 min at 4˚C. PRP was obtained after discarding 3/4 of the upper layer of plasma. The concentration of platelets in PRP was $1.0\text{-}1.5x10^{12}$/l. To release growth factors from alpha granules, 8 ml PRP was mixed with 10% $CaCl_2$ (containing 1,000 U/ml thrombin) with a ratio of 9:1 (volume/volume). The mixture was centrifuged at 4,000 rpm for 10 min at 4˚C. Approximately 7.5 ml of supernatant was obtained from one preparation. The supernatant was then mixed with DMEM (high-glucose) supplemented with 10% FBS to prepare medium with various concentrations of PRP.

Cell counting kit-8 (CCK8) assay. Cell proliferation was monitored using the colorimetric water-soluble tetrazolium salt (CCK8) assay. Briefly, 100 μl cell mixtures ($2x10^5$ cells) were seeded onto 96-well plates. After adding various concentrations of PRP (1, 2, 5, 10 and 25%, volume/volume), all samples were incubated for 24, 48 or 72 h (37˚C, 5% CO_2). Following incubation with 10 μl CCK solution for 2 h, the number of viable cells was assessed by measuring the absorbance at 450 nm. Cells cultured in basic medium (DMEM supplemented with 10% FBS, 100 U/ml penicillin and 100 mg/ml streptomycin) were used as a control group.

PRP was prepared and diluted with PBS to obtain five different concentrations (1, 2, 5, 10 and 25%, volume/volume), which was added to the chondrocytes and incubated for 24, 48 and 72 h. Chondrocytes were incubated with three concentrations of IL-1β (5, 10 and 50 ng/ml) for 24, 48 and 72 h.

Quantitative polymerase chain reaction (qPCR). Total RNA was isolated from the cells using TRIzol® reagent (1 ml per 10^7 cells; Invitrogen; Thermo Fisher Scientific, Inc.). Reverse transcription was conducted using the Takara PrimeScript II 1st Strand cDNA Synthesis kit (Takara Biotechnology Co., Ltd., Dalian, China), according to the manufacturer's protocol.

All primers used in the present study were designed using Primer 3.0 software (Premier Biosoft, Palo Alto, CA, USA). The primer sequences used were as follows: B-cell lymphoma 2 (Bcl-2), forward 5'CTGGTGGACAACATCGCTCT3', reverse 5'GCATGCTGGGGCCATATAGT3'; Bcl-2-associated X protein (Bax), forward 5'AGGACGCATCCACCAAGAAG3', reverse 5'CAGTTGAAGTTGCCGTCTGC3'; caspase-3, forward 5'GGAGCTTGGAACGCGAAGAA3', reverse 5'ACACAAGCCCATTTCAGGGT3'; poly (ADP-ribose) polymerase PARP, forward 5'ACCACGCACAATGCCTATGA3', reverse 5'AGTCTCCGGTTGTGAAGCTG3'; MMP1, forward 5'CACCAATCAGTTCAACGCAGA3', reverse 5' TGACTTGGTAATGGGTTGCC3'; MMP3, forward 5'TTTGGCCGTCTCTTCCATCC3', reverse 5'GCATCGATCTTCTGGACGGT3'; MMP9, forward 5'GATCCCCAGAGCGTTACTCG3', reverse 5'GTTGTGGAAACTCACACGCC3'; MMP13, forward 5'TGCATACGAGCATCCATCCC3', reverse 5'CTCAAAGTGAACCGCAGCAC3'; tissue inhibitor of metalloproteinases (TIMP), forward 5'TCCCCAGAAATCATCGAGAC3', reverse 5'GATGTGCAAATTTCCGTTCC3'; SRY-box 9 (SOX9), forward 5'CCAGAGAACGCACATCAAGA3', reverse 5'TCTGGTGGTCGGTGTAGTCA3'; COL2, forward 5'GGCCAGGATGCCCGAAAATTA3', reverse 5'ACCCCTCTCTCCCTTGTCAC3'; hypoxia-inducible factor-1α (HIF-1α), forward 5'TGCTCATCAGTTGCCACTTC3', reverse 5'CCATCCAGGGCTTTCAGATA3'; and glyceraldehyde 3-phosphate dehydrogenase (GAPDH), forward 5'TGCCACTCAGAAGACTGTGG3', reverse 5'TTCAGCTCTGGGATGACCTT3'.

The reaction mixture was prepared according to the instructions provided with Takara SYBR *Premix Ex Taq* II (Takara Biotechnology Co., Ltd.). The amplification curve and melting curve were produced using the Real-Time PCR system (CFX96; Bio-Rad Laboratories, Inc., Hercules, CA, USA). PCR primers were obtained from GenePharma Co., Ltd. (Shanghai, China). Amplification conditions were as follows: 10 min at 95˚C for one cycle, followed by 45 cycles at 95˚C for 10 sec, 60˚C for 20 sec and 70˚C for 30 sec, and a final extension step at 70˚C for 5 min. Blank controls were included to determine the amplification efficiency within each experiment. Quantification was conducted using the $2^{-\Delta\Delta Cq}$ method (18). GAPDH was used for normalization.

Western blotting. Cells were harvested and washed with PBS (pH 7.4) three times. The pellets were incubated with 100 μl lysis buffer (P0013C; Beyotime Institute of Biotechnology, Shanghai, China) for 30 min, and were centrifuged at

Table I. List of antibodies used in western blotting.

Antibody	Manufacturer	Catalog no.
Anti-MMP1	Bioss	Bs-0424R
Anti-MMP3	Bioss	Bs-0413R
Anti-MMP13	Bioss	Bs-0575R
Anti-MMP9	Bioss	Bs-4593R
Anti-TIMP	Bioss	Bs-4600R
Anti-Bax	Bioss	Bs-0127R
Anti-caspase-3	Bioss	Bs-0081R
Anti-PARP	Bioss	Bs-2138R
Anti-SOX9	Bioss	Bs-4177R
Anti-COL2	Bioss	Bs-11929R
Anti-HIF-1α	Bioss	Bs-0737R
Anti-Bcl-2	Bioss	Bs-0032R
Anti-GAPDH	Abcam	ab9485

Bioss, Inc., Woburn, MA, USA. MMP, matrix metalloproteinase; TIMP, tissue inhibitor of metalloproteinases; Bcl-2, B-cell lymphoma 2; Bax, Bcl-2-associated X protein; PARP, poly (ADP-ribose) polymerase; SOX9, SRY-box 9; COL2, collagen type 2; HIF-1α, hypoxia inducible factor-1α; GAPDH, glyceraldehyde 3-phosphate dehydrogenase.

12,000 rpm for 15 min at 4˚C. The supernatants were used for western blotting. Sample loading was adjusted according to protein concentration, which was determined using a bicinchoninic acid kit (Pierce Biotechnology; Thermo Fisher Scientific, Inc.). The protein samples (3 μg/μl) were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (10% gels). Following electrophoresis, the proteins were transferred to polyvinylidene fluoride membranes at 70 V for 20-70 min at 4˚C. The membranes were blocked with Tris-buffered saline (TBS) wash solution containing 5% nonfat milk and 0.1% Tween 20 for 1 h. The membranes were then incubated with the primary antibodies (1:500), with agitation overnight at 4˚C, followed by incubation with the horseradish peroxidase-conjugated secondary antibody (1:2,500) for 1 h at room temperature. Primary antibodies used in the present study are listed in Table I. The secondary antibody was goat-anti-rabbit immunoglobulin G (IgG)-horseradish peroxidase (HRP)-conjugated secondary antibody (cat. no. sc-2004; Santa Cruz Biotechnology, Inc., CA, USA). The membranes were washed three times (15 min each wash) between antibody incubations with TBS containing 0.1% Tween 20, and the blots were developed using ECL Plus Western Blotting Substrate (Pierce Biotechnology; Thermo Fisher Scientific, Inc.). Blots were semi-quantified using ImageJ 2x software (Rawak Software, Inc., Stuttgart, Germany).

Flow cytometry. The cell suspension and culture medium were mixed in a six-well plate, and were incubated with various concentrations (5, 10 and 50 ng/ml) of IL-1β (cat. no. SRP3083; Sigma-Aldrich) for 1 h (37˚C, 5% CO_2). Subsequently, the cells were digested with trypsin (without EDTA) for 5 min, and centrifuged at 300 x *g* for 5 min (4˚C). PBS was then used to wash the mixture twice, and the cells were further centrifuged at 300 x *g* for 5 min (4˚C). The cells were then resuspended with 100 μl binding buffer, and were incubated with 5 μl Annexin V-fluorescein isothiocyanate and 5 μl propidium iodide staining solution for 10 min (using an Annexin V-FITC and PI kit; cat. no. 556547, BD Biosciences, Mountain View, CA, USA). After mixing with 400 μl binding buffer, the samples were examined using a flow cytometer (BD FACSVerse; BD Biosciences, San Jose, CA, USA).

Table II. Viability of chondrocytes treated with various concentrations of PRP, as measured by Cell Counting kit-8 assay. Numbers indicate percentage of control groups.

Group	24 h	48 h	72 h
Control	100±4.63	100±3.69	100±7.03
1% PRP	91.63±2.31	116.77±1.88	123.09±3.78[a]
2% PRP	91.97±4.28	129.44±4.4[a]	149.64±6.37[a]
5% PRP	131.81±19.28[a]	155.1±3.78[a]	176.62±17.06[a]
10% PRP	159.31±12.49[a]	195.57±13.68[a,b]	204.17±19.23[a,b]
25% PRP	132.05±10.91[a]	171.4±7.65[a]	235.39±8.5[a]

[a]P<0.05, vs. the control group; [b]P<0.05, vs. the 25% PRP group. PRP, platelet-rich plasma.

Statistical analysis. For all experiments, three mice donors of chondrocytes were used. For each donor, three technical replicates were tested. Data are presented as the mean ± standard deviation. For evaluation of differences between two groups, Student's t-test was applied. Analysis of variance (ANOVA) tests were used for multiple comparisons. P<0.05 was considered to indicate a statistically significant difference. Data analysis was conducted using SAS 9.3 software (SAS Institute, Inc., Cary, NC, USA).

Results

PRP increases chondrocyte viability. Chondrocytes were isolated from rat joint cartilage, and were verified by immunostaining with the cartilage-specific matrix component, COL2 (Fig. 1). PRP was prepared and diluted with PBS or basic medium to obtain five different concentrations (1, 2, 5, 10 and 25%, volume/volume), which was added to the culture medium for 24, 48 and 72 h. Cell number increased in the PRP treatment groups in a dose-dependent manner (Table II). However, 10% PRP in the culture medium resulted in increased cell growth compared with in the 25% PRP group at 24 and 48 h (P<0.05). Therefore, 10% PRP treatment was used for subsequent experiments.

Model establishment of IL-1β-induced chondrocyte apoptosis. Chondrocytes were treated with three concentrations of IL-1β (5, 10 and 50 ng/ml) for 24, 48 and 72 h. Subsequently, flow cytometric analysis was used to determine the percentage of apoptotic cells. Apoptotic chondrocytes were defined as Annexin V-positive cells (Fig. 2A), and the apoptotic rate of the chondrocytes in various conditions are presented in

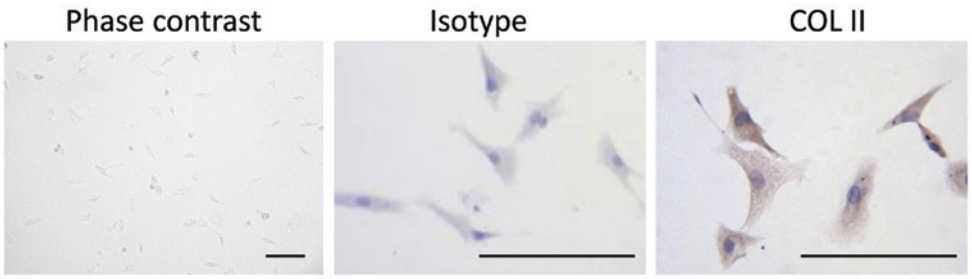

Figure 1. Characterization of rat chondrocytes. Phase contrast image shows the morphology of articular chondrocytes isolated from the knee joints of rat neonates. Following *in vitro* culture for two passages, cells were positive for collagen type II (COL II) as indicated by immunohistochemistry. Bar=50 μm.

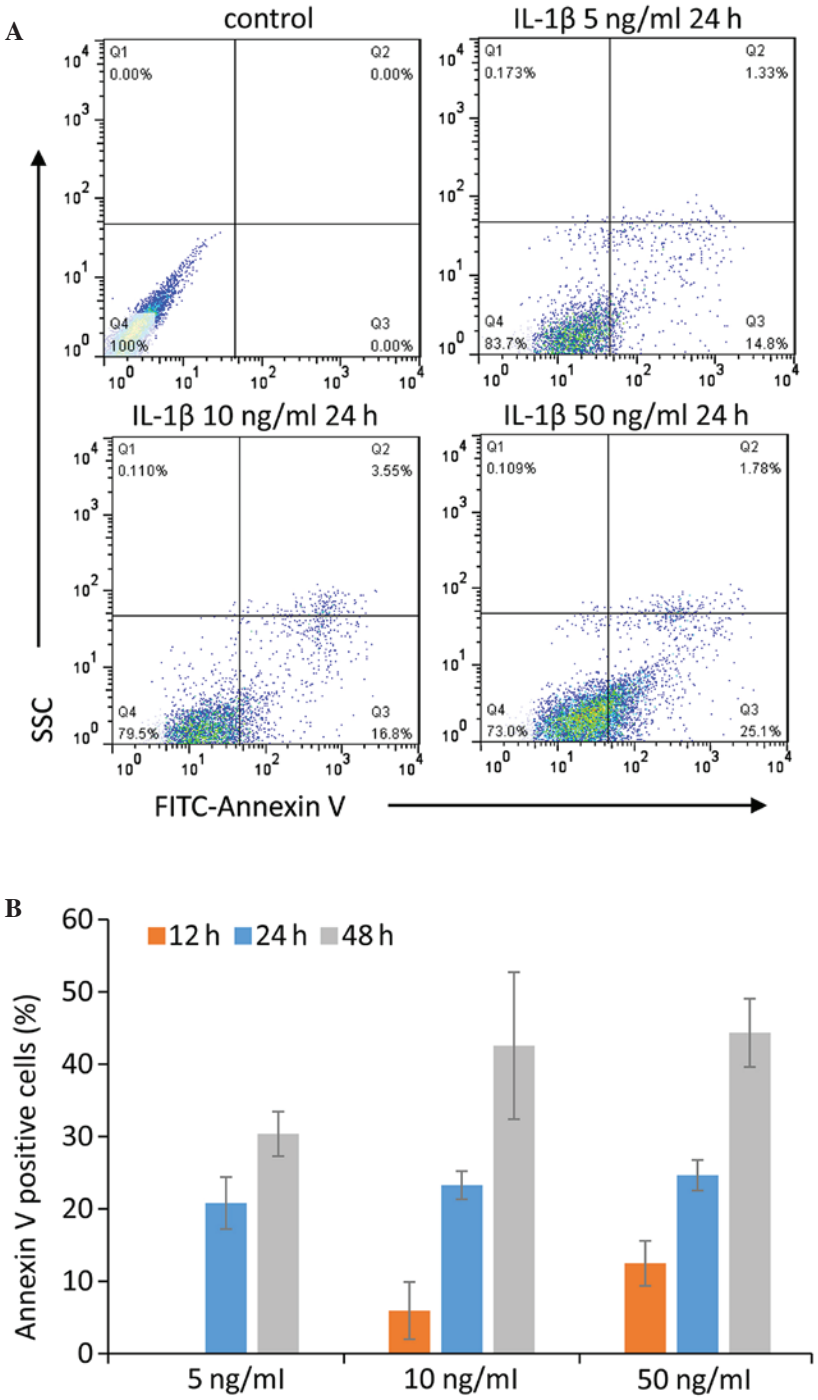

Figure 2. Interleukin (IL)-1β induces apoptosis of rat chondrocytes. (A) Annexin V staining was performed 24 h following treatment of chondrocytes with IL-1β. (B) Quantification of Annexin V-positive cells. The sum of gate Q2 and Q3 cells was used to determine the number of Annexin V-positive cells (N=3). FITC, fluorescein isothiocyanate; SSC, side scatter.

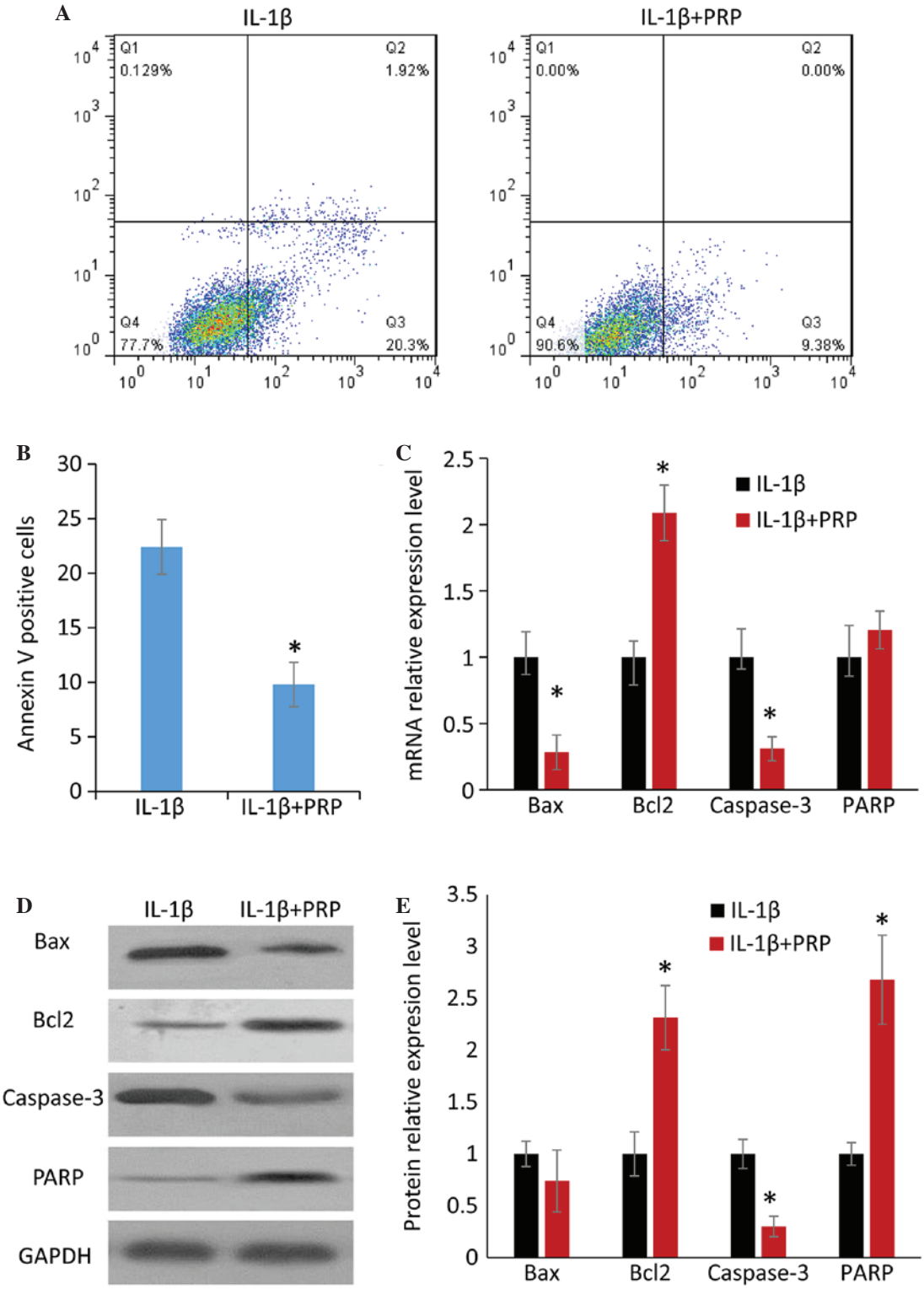

Figure 3. Platelet-rich plasma (PRP) treatment reduced apoptosis, and the expression of apoptotic genes in rat chondrocytes. (A) Annexin V staining was conducted 24 h after incubation with interleukin (IL)-1β or IL-1β + PRP. (B) Quantification of Annexin V-positive cells. The sum of gate Q2 and Q3 cells was used to determine the number of Annexin V-positive cells (N=3). (C) Quantitative polymerase chain reaction (qPCR) was performed 24 h after treatment of chondrocytes (N=3). (D) Western blotting exhibited similar gene expression results as qPCR. (E) Semi-quantification of blots confirmed the expression of apoptotic proteins (N=3). *P<0.05, vs. the IL-1β group. Bcl-2, B-cell lymphoma 2; Bax, Bcl-2-associated X protein; PARP, poly (ADP-ribose) polymerase; GAPDH, glyceraldehyde 3-phosphate dehydrogenase.

Fig. 2B. Treatment with 10 ng/ml IL-1β for 24 h was selected for further experiments, since it induced an increase in Annexin V-positive cells, saved time, and used a relatively low concentration of IL-1β.

PRP inhibits IL-1β-induced chondrocyte apoptosis. To evaluate the effects of PRP on IL-1β-induced apoptosis, flow cytometry was performed to determine the number of Annexin V-positive chondrocytes. Treatment with PRP

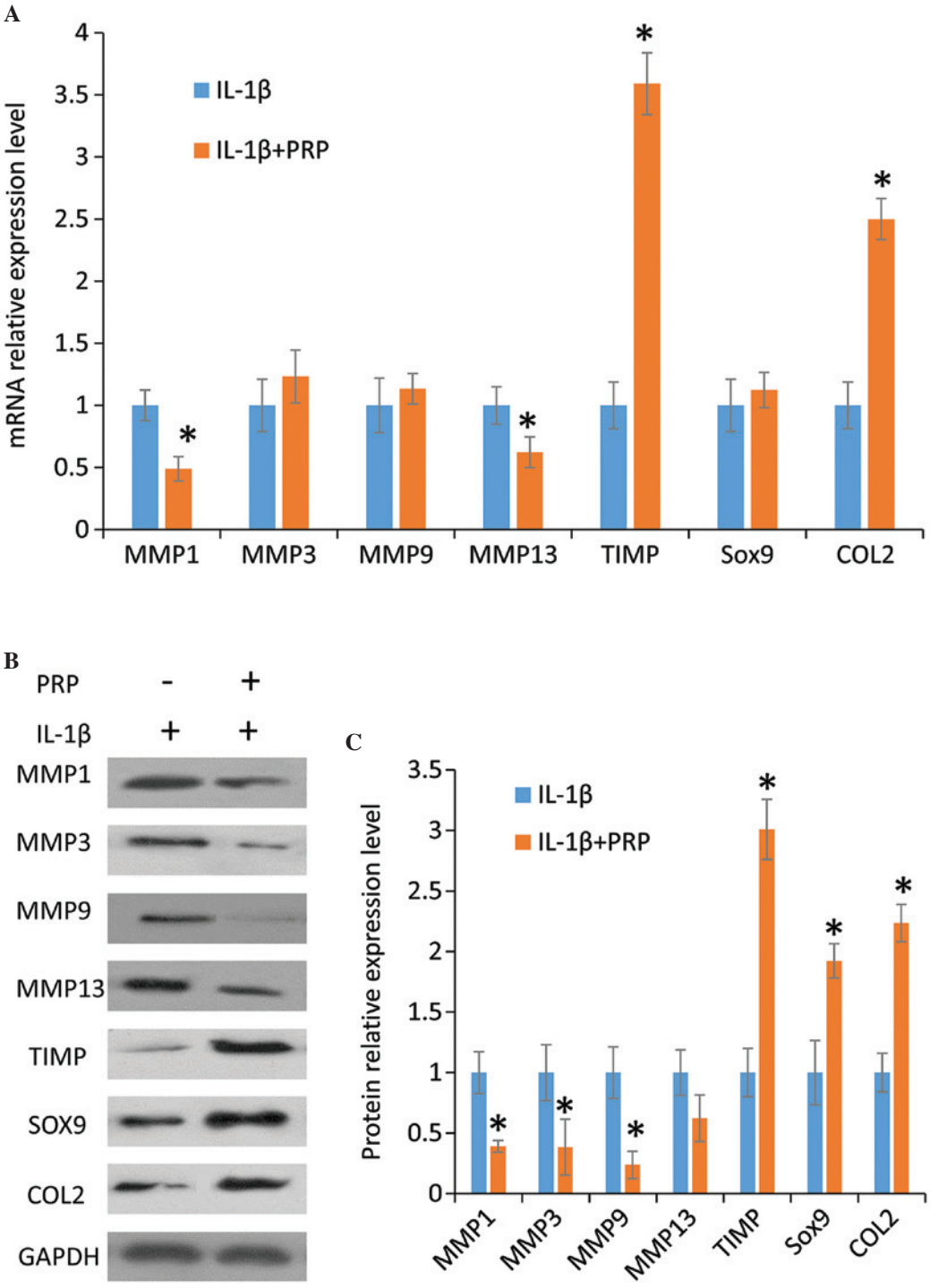

Figure 4. Platelet-rich plasma (PRP) inhibits the expression of catabolic genes induced by interleukin (IL)-1β. (A) Quantitative polymerase chain reaction was performed 24 h following treatment of chondrocytes (N=3). (B) Western blotting detected downregulation of matrix metalloproteinases (MMPs), and upregulation of tissue inhibitor of metalloproteinases (TIMP), SRY-box 9 (SOX9) and collagen type II (COL2) in the IL-1β + PRP group compared with the IL-1β group. (C) Semi-quantification of blots confirmed the expression of anabolic proteins was increased by PRP (N=3). *P<0.05, vs. the IL-1β group. GAPDH, glyceraldehyde 3-phosphate dehydrogenase.

reduced the Annexin V-positive cell population (Fig. 3A). The difference between the IL-1β group and the IL-1β + PRP group was statistically significant (P<0.05; Fig. 3B). qPCR and western blotting were conducted to detect the expression of apoptosis-related factors at the mRNA and protein level, respectively. In the IL-1β + PRP group, the mRNA and protein expression levels of Bax and caspase-3 were markedly down-regulated, whereas the expression levels of Bcl-2 and PARP were markedly upregulated (Fig. 3C-E). These results indicate that PRP may inhibit IL-1β-induced chondrocyte apoptosis.

PRP protects the chondrocyte extracellular matrix. To evaluate the effects of PRP on synthesis of the chondrocyte extracellular matrix in the presence of IL-1β, qPCR and western blotting were conducted to determine the mRNA and protein expression levels of the relevant factors, respectively. Results

from the qPCR indicated that compared with the IL-1β group, the addition of PRP significantly downregulated the expression levels of the MMPs, MMP1 and MMP13, and modestly increased the expression levels of MMP3 and MMP9; and upregulated the expression of the cartilage matrix-producing proteins, SOX9 and COL2, and TIMP (Fig. 4A). Western blotting results exhibited a similar pattern (Fig. 4B and C). These results indicate that PRP reduced the expression of catabolic genes and increased the expression of anabolic genes.

Discussion

Cartilage degradation and loss are the major characteristics of arthritis. For the treatment of cartilage damage and to prevent cartilage loss, PRP is considered a promising treatment strategy, due to its high content of growth factors, which may act as a regenerative stimulus for cartilage tissues and cells (19). Treatment of chondrocytes from human osteoarthritic cartilage with PRP resulted in an inhibitory effect on matrix loss (20). However, the effects of PRP on cell apoptosis remain unknown. The results of the present study demonstrated that PRP promoted chondrocyte survival by inhibiting IL-1β-induced chondrocyte apoptosis. This result provides scientific evidence for the beneficial effects of PRP at the cellular level. In addition, the present study revealed that the rate of matrix degradation was inhibited by PRP.

IL-1β is considered a major proinflammatory cytokine involved in arthritis. The levels of IL-1β are increased in the synovial fluid of patients with OA. In addition, it is thought to be associated with the degradation of cartilage extracellular matrix (21). IL-1β can be secreted by chondrocytes, mononuclear cells, osteoblasts and synovial cells. Previous studies have reported that IL-1β may stimulate apoptosis of chondrocytes and tendon cells (22,23) by upregulating the expression of Bax and caspase-3 (24), and suppressing the expression of Bcl-2 and PARP (25). Several studies (21,26,27) have demonstrated that IL-1β promotes catabolic activity of chondrocytes through two major mechanisms: IL-1β suppresses the synthesis of major extracellular matrix proteins, such as aggrecan and COL2; and IL-1β stimulates the release of several proteolytic enzymes, including MMPs such as MMP1, MMP3 and MMP13. Inhibition of IL-1β may be beneficial in preventing the progression of OA pathogenesis; therefore, IL-1β antagonists and their underlying mechanisms are currently under active investigation (28,29). However, simply inhibiting the IL-1β receptor does not appear to be an effective solution. For example, an IL-1 receptor antagonist, Anakinra™, was launched into clinical trials to treat OA; however, improvements in symptoms were not detected compared with the placebo group (30,31). Data from the present study indicated that PRP treatment of rat chondrocytes downregulated the expression of proapoptotic factors and upregulated the expression of anti-apoptotic factors. Along with the well-known effects of PRP on inhibiting the activity of catabolic enzymes, these anti-apoptotic and anti-catabolic effects may partially explain the clinical efficacy of PRP on treating cartilage injury.

It has previously been reported that high concentrations of PDGF, endothelial growth factor and TGF, together with the anti-inflammatory and proinflammatory cytokines IL-4, IL-8, IL-13, IL-17, tumor necrosis factor-α and interferon-α, are present in PRP (32). The present study demonstrated that PRP was able to counteract the apoptotic and catabolic effects of IL-1β in chondrocytes. The anti-inflammatory factors present in PRP may induce several effects on IL-1β-activated signaling pathways. However, the interaction between PRP-derived anti-inflammatory factors and IL-1β requires a more thorough investigation.

The expression levels of two catabolic enzymes, MMP3 and MMP9, were decreased at the protein level in the IL-1β + PRP-treated chondrocytes, as compared with in the IL-1β-treated chondrocytes. However, the expression of these two enzymes at the mRNA level was not significantly different between the IL-1β and IL-1β + PRP groups. This inconsistency between expression at the mRNA and protein level may reflect the complexity of interactions between IL-1β and growth factors present in PRP. Some factors in PRP may be able to regulate the expression of catabolic enzymes by inhibiting their translation from mRNA. In addition, 10% PRP in the culture medium resulted in increased cell growth compared with in the 25% PRP group at 24 and 48 h. These results indicated that the beneficial effects of PRP on cell growth do not linearly increase with higher PRP concentrations, at least in a short time period. Considering all growth factors have best working concentrations, better understanding of the concentrations of growth factors in PRP is crucial to explain these observations.

In conclusion, the present study demonstrated that PRP is able to protect chondrocytes from IL-1β-dependent apoptosis, and can promote anabolism of chondrocyte extracellular matrix. The effects of PRP on IL-1β-treated chondrocytes support the beneficial effects of PRP application in the treatment of arthritis.

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