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Interleukin-1β exacerbates the catabolic effects of human nucleus pulposus cells through activation of the Nuclear Factor kappa B signaling pathway under hypoxic conditions

Z.-Y. SUN[1], Y.-T. LIU[2], H. LIANG[1], Y. LI[1], D.-G. WANG[1], J.-W. TIAN[3]

[1]Shanghai Songjiang District Central Hospital, Shanghai, China
[2]Shandong Rizhao City Chinese Medicine Hospital, Rizhao, Shandong, China
[3]Department of Orthopedics, School of Medicine, Shanghai General Hospital Affiliated to Shanghai Jiao Tong University, Shanghai, China

D.-G. Wang and *J.-W. Tian* contributed equally to this paper

Abstract. – OBJECTIVE: To determine the regulatory role of interleukin 1 beta (IL-1β) in the Nuclear Factor kappa B (NF-κB) -mediated catabolic effects of the nucleus pulposus cells in human intervertebral disc degeneration under hypoxic conditions.

PATIENTS AND METHODS: Human nucleus pulposus cells were cultured and exposed to IL-1β under hypoxic or normoxic environments, with or without NF-κB inhibition. The cell growth was determined using cell counting kit-8; gene and protein expressions were analyzed by Real-time polymerase chain reaction and Western blotting, respectively.

RESULTS: Co-treatment with IL-1β and hypoxia decreased cell viability in human nucleus pulposus cells. There was a positive effect of IL-1β on human nucleus pulposus cells under hypoxia, which was through the up-regulation of matrix metalloproteinase-3 (MMP-3), MMP-13, a disintegrin and metalloproteinase with thrombospondin motifs (ADAMTS)-4, and ADAMTS-5. IL-1β-induced expressions of MMP-3, MMP-9, ADAMTS-4, and ADAMTS-5 under hypoxia were accompanied by increased activation of NF-κB. Inhibition of NF-κBp65 by small interfering RNA or specific inhibitor BAY11-7082 blocked IL-1β–dependent gene upregulation of MMP-3, MMP-13, ADAMTS-4, and ADAMTS-5 in a hypoxic environment. The gene expression of aggrecan was decreased by IL-1β under hypoxic conditions, which was reversed by either BAY11-7082 or NF-κBp65 knockdown.

CONCLUSIONS: IL-1β and hypoxia synergetically contributed to the catabolic effects of the nucleus pulposus cells by upregulating the expression of MMP-3, MMP-13, ADAMTS-4 and ADAMTS-5 through the activation of NF-κB signaling pathway, indicating that the NF-κB signaling pathway is a key mediator of intervertebral disc degeneration.

Key Words:
Hypoxia, NF-κB, Disc degeneration, Signaling pathway, Nucleus pulposus cells, ADAMTS, Aggrecan, IL-1β, MMP-3, MMP-13, ADAMTS-4, ADAMTS-5.

Introduction

Intervertebral disc degeneration (IDD) is a major health issue worldwide, with comprehensive socio-economical consequences. IDD is generally associated with low back ache in the clinic, and a lifetime incidence of IDD can reach up to 80% in general populations[1]. Although the exact etiology and pathophysiological mechanisms of IDD are largely unknown, it has been suggested that the degenerative process happens by the slow inhibition of the physiological turnover of the extracellular matrix (ECM), which is usually balanced by anabolic factors, such as growth factors, catabolic factors, such as matrix metalloproteinases (MMPs), aggrecanases/a disintegrin and metalloproteinase with thrombospondin motifs (ADAMTSs), and other proteolytic enzymes, resulting the loss of aggrecan (Agg) and type II collagen (Col II) from the ECM in IDD[2-5]. Recently, there is an increasing interest in investigating the effectiveness of inhibition of ECM degradation for experimental therapy of IDD[6]. Intervertebral disc (IVD) is the largest avascular structure in the body[7]. The lack of blood supply in the nucleus pulposus (NP) cells and the inner annulus fibrosus (AF) cells is an important feature of IVD, which imposes a hypoxic state on the cells[8,9].

Corresponding Author: Jiwei Tian, Ph.D; e-mail: tjw609@163.com
D.G. Wang, MD; e-mail: wangdeguo@live.cn

Evidence[10-12] supports the notion that hypoxia is an important cellular stress in many diseases, such as cerebral ischemia, cancer, and chronic degenerative disorders. It has also been reported that a low-oxygen-tension environment plays an important role in maintaining the physiological functions of IVD cells, including energy metabolism, matrix synthesis and cell viability[13-15]. Low oxygen tension (5%) enhances the ECM synthesis in the porcine NP cells in a pellet culture system[16]. Although the NP cells can survive under hypoxia conditions *in vitro*, without a significant loss of cell viability[17], the mechanism underlying IDD in a hypoxic environment is not fully understood. In addition to hypoxia, pro-inflammatory cytokines may affect ECM degradation in IDD. Degenerative disc disease is characterized by elevated levels of proinflammatory cytokines, including tumor necrosis factor-beta (TNF-β), interleukin-1 beta (IL-1β), interleukin-6 (IL-6), and interleukin 6 (IL-8), and decreased levels of matrix molecules, such as aggrecan (Agg) and collagen II (Col-II) [18-21]. Many studies have shown that IL-1β promotes IDD; it is elevated in the degenerated human IVDs, up-regulates the expression of matrix degrading enzymes and down-regulates the expression of matrix genes in IVD cells[22-25]. The results from *in vitro* experiments with human cells have demonstrated that IL-1β can significantly increase the matrix-degrading enzyme activity in the NP and AF cells of normal and degenerated IVDs[26-28]. Although the role of IL-1β has been extensively studied in IDD, its effect on ECM degradation of NP cell under hypoxia conditions is still not well understood. In addition, most studies[29,30] have focused on the effect of IL-1β or hypoxia alone; the combined effect of IL-1β and hypoxia on IDD is largely unknown. Therefore, clarifying the combined effects of IL-1β and hypoxia on IDD is pivotal. Also, it is very important to identify the regulatory pathways that could be targeted to eliminate the negative effects of IL-1β and hypoxia on IDD. The present work was designed to test our hypothesis that IL-1β and hypoxia would have a synergetic effect in promoting ECM degradation. To better understand the underlying mechanisms, we also attempted to examine the effects of IL-1β on the expressions of MMPs, ADAMTS, Agg and Col II in human NP cells under hypoxia conditions, determining whether the IL-1β effects were modulated by activating the nuclear factor kappa B (NF-κB) signaling pathway. To the best of our knowledge, this work was the first to evaluate the physiological and pathophysiological roles of IL-1β, hypoxia, and NF-κB pathway in human IVD homeostasis. Our results may provide novel information on NF-κB-mediated IVD metabolism in humans.

Patients and Methods

Human NP Cell Isolation and Culture

Ethical approval for human patients was obtained from the Medical Ethics Committee of Shanghai General People's Hospital (2015KY017). All patients and their next of kin signed an informed consent form allowing the researchers to use IVD tissues obtained during the patients' spinal surgery. All the donor patients underwent spinal fusion surgery that required their intervertebral discs to be resected. The patients aged between 19 and 45 years old with the mean age of 30.52 years. Based on the Pfirrmann grading scale[31], all the disc tissues selected for the present study were non-degenerative IVD (Pfirrmann < grade III). Of note, all the IVD tissues used in the present study would have been discarded as medical waste and would not be used in any purposes in the clinical treatment. The NP tissues obtained were washed thrice with phosphate-buffered saline (PBS; Gibco, Grand Island, NY, USA), minced into small fragments, and digested in a solution of 0.25% trypsin (Gibco, Grand Island, NY, USA) and 0.2% type II collagenase (Gibco, Grand Island, NY, USA). They were then placed in PBS in a gyratory shaker at 37°C for 3 h. The cells were filtered through a 70-μm mesh filter (BD Biosciences, Franklin Lakes, NY, USA), and cultured in 100-mm culture dish with the growth medium (Dulbecco's Modified Eagle's Medium and Ham's F-12 Nutrient Mixture [DMEM-F12; Gibco, Grand Island, NY, USA], 20% fetal bovine serum [FBS; Gibco, Grand Island, NY, USA], 50 U/mL penicillin, 50 μg/mL streptomycin (Gibco, Grand Island, NY, USA) in an incubator with 5% CO_2, 21% O_2, and 75% N_2. The cells were passaged by trypsin treatment at approximately 80% confluence, and sub-cultured in 60-mm culture dish at a cell density of 2.5×10^5 cells/dish. All the experiments in the present study used first or second generation of cells. For the hypoxic experiments, the NP cells were serum starved and placed in a hypoxic incubator with an atmosphere of 1% O_2, 5% CO_2 and 94% N_2. For each experiment, the parallel cultures were grown in serum-free medium under normoxic conditions in a 5% CO_2 incubator.

Cell Viability Assay

The cell viability was determined using the Cell Counting Kit-8 assay (CCK-8, Dojindo Laboratories, Kumamoto, Japan), according to the manufacturer's instructions. Briefly, the NP cells were grown in 96-well plates (5000 cells/well) without treatment. Next, the cells were incubated with a vehicle control (DMSO; Sigma-Aldrich, St. Louis, MO, USA) or 5 different concentrations of recombinant human IL-1β (0~100 ng/mL, Peprotech, London, UK). After a 48-h incubation, 10 ul of the WST-8 reagent [2-(2-methoxy-4-nitrophenyl)-3-(4-nitrophenyl)-5-(2,4-disulfonyl)-2H-tetrazolium] was added to each well, and the cells were incubated at 37°C for 2 h. The absorbance of each well was measured at 450 nm using a Bio-Rad 680 microplate reader (Bio-Rad, Hercules, CA, USA). Each experiment was performed in triplicate. Cell viability was calculated based on the optical density of experimental sample/optical density of control (×100%), and the cell viability in the hypoxic or (hypoxic and IL-1β) treated cells was calculated relative to the control. At least five independent repeats of this experiment were performed.

Cell Treatment

The NP cells obtained were grown to confluence in 60-mm culture dishes, serum starvation overnight to synchronize the cell cycles and then stimulated with 10 ng/mL of recombinant human IL-1β under normoxic or hypoxic conditions for 24 h, the NF-κB activation and catabolic response was examined. Our preliminary CCK-8 experiments showed that BAY11-7082 (Sigma-Aldrich, St. Louis, MO, USA) at concentrations of less than 6.25 uM was not toxic to the NP cells[29]. In the next experiment, the cells were pretreated with BAY11-7082 (2.5 and 5.0 uM) under a hypoxic environment for 1 h and then with IL-1β (10 ng/mL) and BAY11-7082 for 48 h. The cells were harvested for subsequent gene and protein expression analyses.

Gene Expression Analysis

Total RNA was extracted from the NP cells using TRIzol reagent (Invitrogen, Carlsbad, CA, USA), according to the manufacturer's instructions. The mRNA level was analyzed with Real-time polymerase chain reaction (PCR) using the ABI 7500 Fast Real-time PCR system (Applied Biosystems, Foster City, CA, USA). cDNA was then reverse transcribed (Thermo Fisher, Waltham, MA, USA), according to the manufacturer's instructions. Real-time PCR reactions were done in triplicate in 96-well plates using a SYBR Premix Ex Taq Kit (TaKaRa, Dalian, China) in a final volume of 20 uL. All primers (Table I) were designed based on published coding sequences and obtained from Sangon (Shanghai, China). The cycle threshold values were obtained, and data were normalized to β-actin expression using the $2^{-\Delta\Delta Ct}$ method[32].

Western Blotting Analysis

The cultured NP cells were lysed in RIPA buffer and the nuclear lysates were generated with Thermo Nuclear Extraction Kit (Thermo Fisher, Waltham, MA, USA), supplemented with phosphatase inhibitors and protease inhibitor cocktail (Biotech. Well, Shanghai, China), according to the manufacturers' instructions. The lysates were centrifuged at 12,000 g for 20 min. The protein concentrations were determined using the BCA protein assay (Beyotime, Shanghai, China), and equivalent amounts of total protein (40 ug) were

Table I. Sequences primers used in the present study.

Gene	Primer sequence
Aggrencan	Forward: 5'-TGAAACCACCTCTGCATTCCA-3' Reverse: 5'- GACGCCTCGCCTTCTTGAA-3'
MMP-3	Forward: 5'- GCTGTTTTTGAAGAATTTGGGTTC-3' Reverse: 5'- GCACAGGCAGGAGAAAACGA-3'
MMP-13	Forward: 5'- CCAGGCATCACCATTCAAG-3' Reverse: 5'- ATCATCTTCATCACCACCACTG-3'
ADAMTS-4	Forward: 5'- ACTGGTGGTGGCAGATGACA-3' Reverse: 5'- TCACTGTTAGCAGGTAGCGCTTT-3'
ADAMTS-5	Forward: 5'- GCTTCTATCGGGGCACAGT-3' Reverse: 5'- CAGCAGTGGCTTTAGGGTGTAG-3'
ACTIN	Forward: 5'- AGCGAGCATCCCCCAAAGTT-3' Reverse: 5'- GGGCACGAAGGCTCATCATT-3'

separated by electrophoresis on sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and transferred onto polyvinylidene difluoride (PVDF) membranes. After being blocked in Tris-buffered saline and Tween-20 (TBST) with 5% milk-powder for 2 h, the membranes were incubated with specific antibody for MMP-3, MMP-13, and p65 (Epitomics, Burlingame, CA, USA) and antibodies for ADAMTS-4, ADAMTS-5 and Agg (Abcam, Cambridge, MA, USA) at 4°C with gentle shaking overnight. After washing, the membranes were incubated with the respective secondary antibody (Jackson, MS, USA) at the appropriate concentration for 2 h at room temperature. Immuno-blotting was finally done with enhanced chemiluminescence (ECL) reagents (Amersham Biosciences, Roosendaal, The Netherlands).

Lentivirus Infection

Lentivirus (8×10^8 TU/mL) packaging of green fluorescent protein (GFP) LV-shp65 and negative control (NC) was constructed by Genechem (Shanghai, China). The cells (1×10^3) were plated into 96-well plates 48 h before the LV-shp65 at various volumes and negative control were added to determine the best MOI value at which concentrations have no virus toxicity effect on the cells. The GFP gene expression was observed at 96 h after infection, under a fluorescence microscope, and the infected cells were collected for subsequent silencing treatments. The cells were harvested at 5 days after viral transduction for protein extraction. The experiment was repeated three times.

Statistical Analysis

The SPSS 17.0 software (SPSS, Inc., Chicago, IL, USA) was used for statistical calculations. The data were represented as means ± standard deviation. Student's *t*-test was used for comparisons between 2 different groups. One-way analysis of variance and a post-hoc Tukey HSD test (S-N-K method) were used for comparisons among 3 or more groups. $p < 0.05$ was considered statistically significant.

Results

IL-1β Decreases NP Cellular Viability Under Hypoxic Conditions

To evaluate the functional effects of IL-1β on NP cellular viability in normoxia or hypoxia, we first examined the effects of IL-1β and hypoxia on NP cellular viability. As shown in Figure 1, the NP cells gradually became spindle shape with the cytoplasm being less abundant, refractivity was reduced and the rate of growth was also decreased. The NP cells were also treated with various concentrations of IL-1β in serum-containing medium for 24 h under normoxic or hypoxic conditions, and the cell viability was assessed using the CCK-8 viability assay. As shown in Figure 2, hypoxia increased the cell viability by approximately 1.075-fold, compared to the normal group. The treatment with 5-100 ng/mL of IL-1β significantly decreased the cell viability in a IL-1β concentration-dependent manner in a hypoxic environment, compared to the cells without IL-1β treatment.

IL-1β Enhances NF-κB Activity in NP Cells Under Hypoxic Conditions and Regulates Catabolic Effects

Since overexpressed MMPs and ADAMTS play important roles in ECM degradation of the IVD, we investigated the effects of IL-1β on the expression MMPs and ADAMTS in the NP cells under hypoxia. The expression of MMP-3, MMP-13, ADAMTS-4, and ADAMTS-5 were analyzed by qRT-PCR and compared among the IL-1β-treated and untreated cells under hypoxic or normoxic conditions, respectively. The results showed that, compared to the untreated cells, the expressions of MMP-3, MMP-13, ADAMTS-4, and ADAMTS-5 were upregulated in IL-1β-treated NP cells under both normoxic and hypoxic conditions, but the effect under hypoxic conditions was greater (Figure 3). Western blotting analyses showed that the protein levels of MMP-3, MMP-13, ADAMTS-4, and ADAMTS-5 were upregulated in IL-1β-treated NP cells under both normoxic and hypoxic conditions, compared to the untreated cells (Figure 4A). Considering that some preliminary data showed that IL-1β increased the expression of MMPs and ADAMTS by activating the NF-κB pathway in other types of cells, we next determined whether the upregulation of MMP-3, MMP-13, ADAMTS-4, and ADAMTS-5 in NP cells under hypoxic conditions by IL-1β was attributable to increased NF-κB activity. The NP cells were cultured under normoxic or hypoxic conditions with or without 10 ng/mL of IL-1β for 24 h. Based

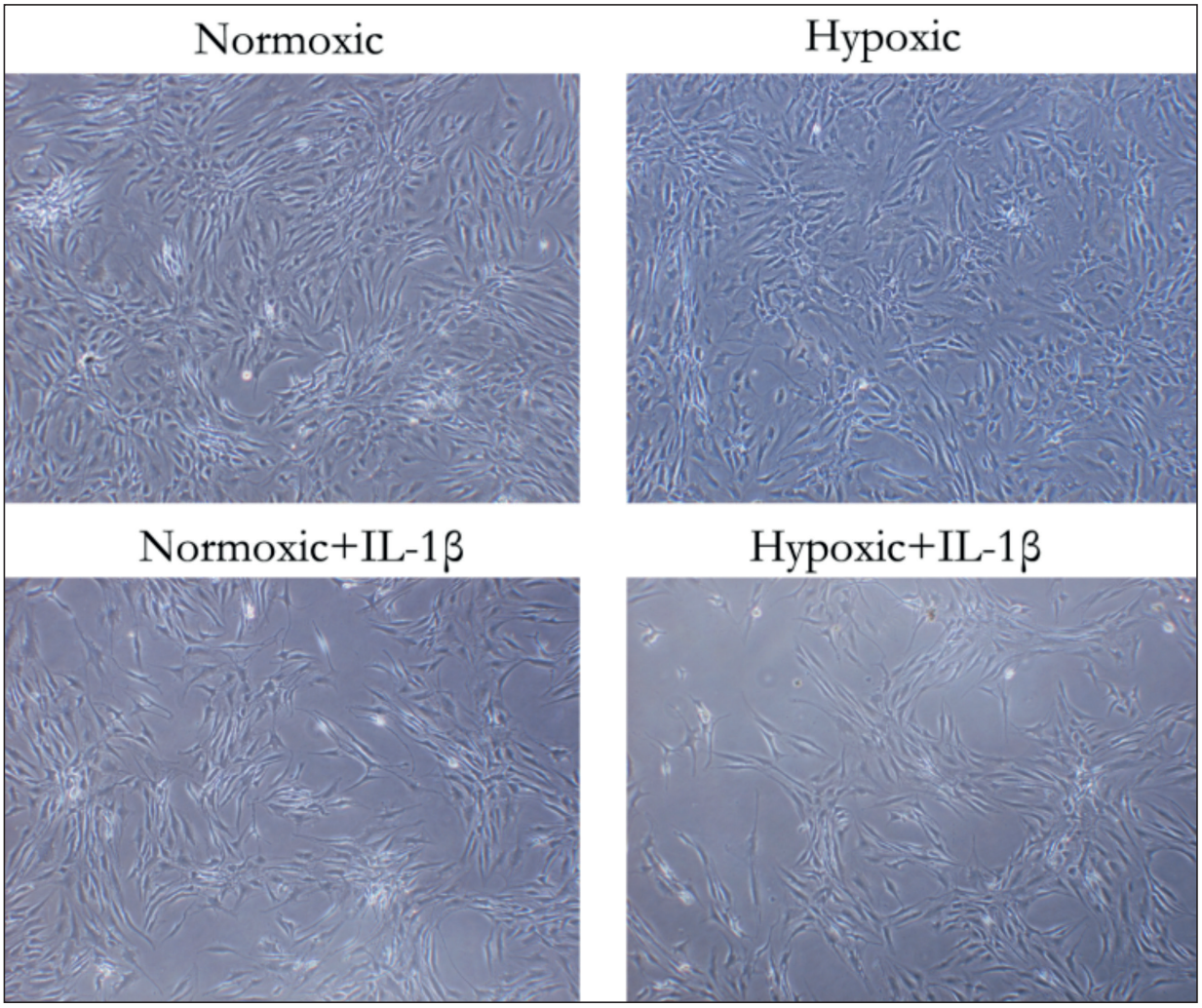

Figure 1. Combined effects of IL-1β and hypoxia on NP cellular viability. The NP cells were cultured under normoxic or hypoxic conditions with or without IL-1β (10 ng/ml) for 72 h. Cell images were observed under an inverted microscope (magnification ×100).

on Western blotting analyses, we found that both the expression and activity of NF-κB were increased in hypoxia-treated NP cells, compared to that treated with IL-1β under normoxic conditions and that untreated under hypoxic conditions (Figure 4B).

IL-1β Pp-Regulates the Expressions Of MMP-3, MMP-13, ADAMTS-4, and ADAMTS-5 by Activating NF-κb Pathway in Hypoxic Environment

Having established that IL-1β resulted in elevated NF-κB activity in the NP cells under hypoxic conditions, we next investigated whether inhibition of NF-κB could attenuate the observed effects. We used both pharmacological and molecular means to inhibit NF-

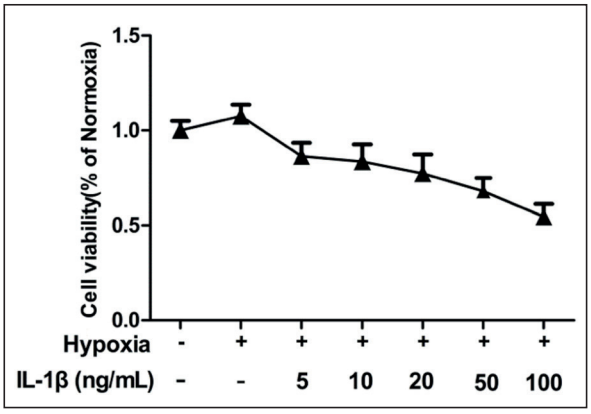

Figure 2. Dose-dependence of IL-1β effects on cell viability. NP cells were incubated with the indicated concentration of IL-1β in serum-containing medium for 48 h and the cell viability was measured using the CCK-8 assay.

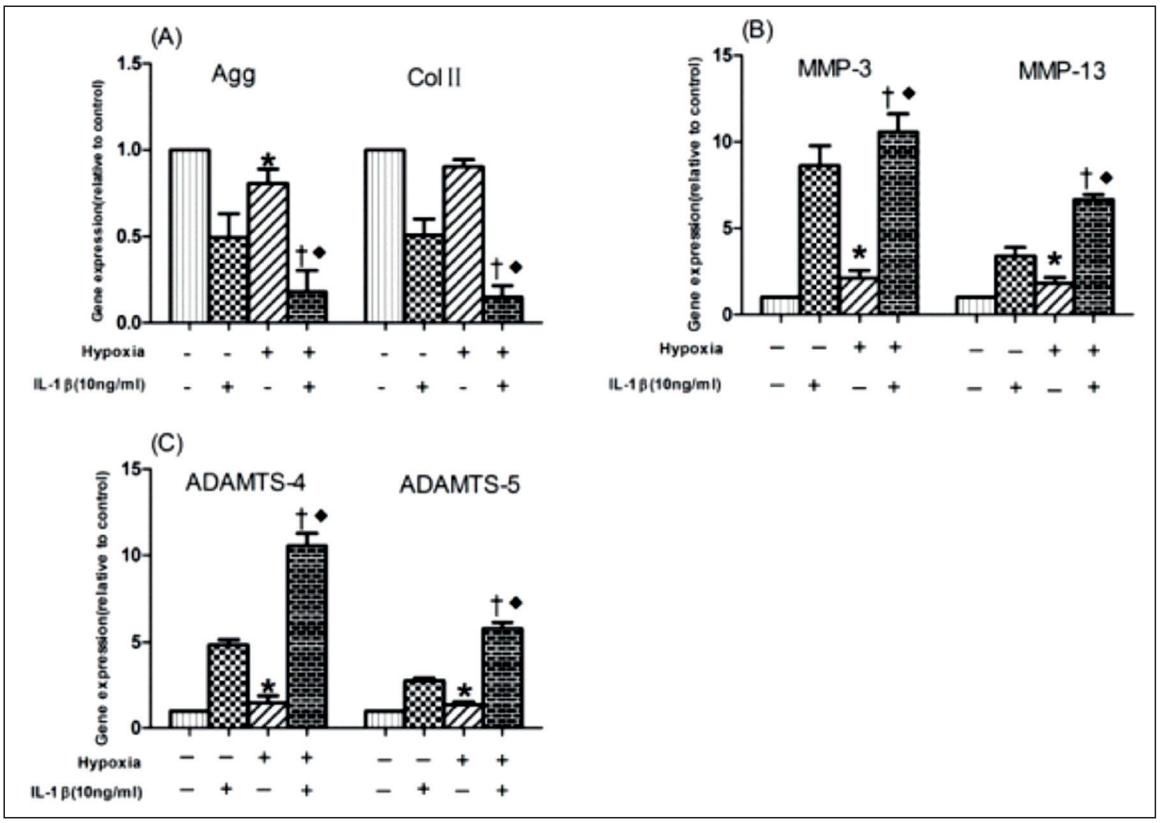

Figure 3. Effects of IL-1β on gene expression in NP cells. The cells were treated with or without IL-1β (10 ng/ml) for 24 h under hypoxic or normoxic conditions. The data were normalized by β -ACTIN levels and are expressed as ratios to the control cells. Control cell value = 1. ***A,*** The data for matrix component genes. ***B, C,*** The change of catabolic gene expression; One-way analysis of variance and Tukey HSD test was used (n = 6 samples). *$p < 0.05$ *vs.* he normoxia group. †$p < 0.05$ *vs.* hypoxia group, ♦$p < 0.05$ *vs.* IL-1β-treated normoxia group.

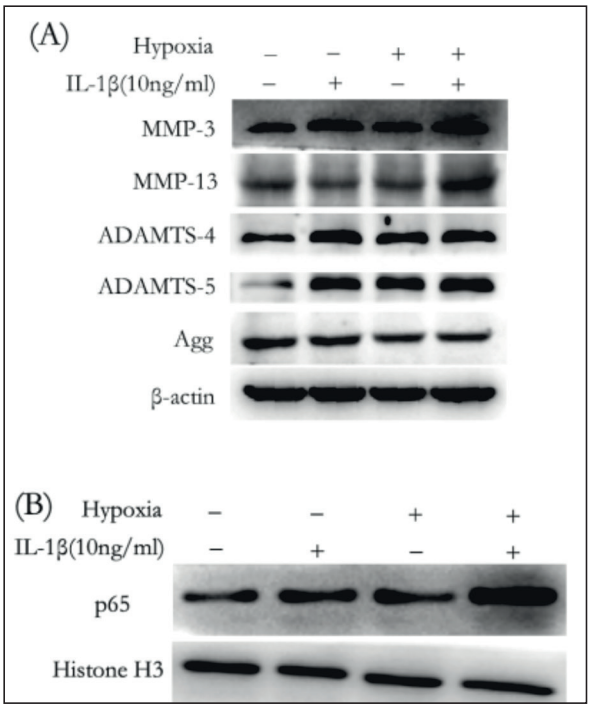

Figure 4. Effects of IL-1β on proiein expressions in NP cells. ***(A)*** MMP-3, MMP-13, ADAMTS-4 andADAMTS-5 were detected by whole cell extract Western blotting from human NP cells with or without IL-1β (10 ng/ml) for 24 h under hypoxic or normoxic conditions. ***(B)*** NF-κB activity was determined by Western blotting on nuclear extracts. Nucleoprotein was extracted from human NP cells treated without or with IL-1β (10 ng/ml) under hypoxic or normoxic conditions for 24 h. Shown is a representative blotting from three independent experiments with similar results from all three.

κB before the NP cells were incubated under hypoxic conditions and stimulated with IL-1β, and the effects were compared to control-treated cells without NF-κB inhibition. The siRNA used in the present study successfully down-regulated the p65 subunit of NF-κB. We also used the commercially available NF-κB inhibitor BAY11-7082 to pharmacologically block NF-κB activity. Our results indicated that, under hypoxic conditions, inhibition of NF-κB activity by either siRNA or BAY11-7082 for 24 h resulted in a dramatic decrease in mRNA and protein levels of MMP-3, MMP-13, ADAMTS-4, and ADAMTS-5 as determined by qRT-PCR and Western blotting analyses (Figures 5-7). These data demonstrated that IL-1β resulted in activation of the NF-κB pathway, which stimulated MMP-3, MMP-13, ADAMTS-4, and ADAMTS-5 production, leading to an increased ECM degradation.

Discussion

We demonstrated that, under hypoxic conditions, IL-1β promoted the ECM degradation in the NP cells, possibly via NF-κB-induced overexpression of MMP-3, MMP-13, ADAMTS-4, and ADAMTS-5. Our results might shed new insights into the progression triggered by the synergetic effect of IL-1β and hypoxia, which results in the loss of disc matrix macromolecular components, major Agg and Col II. To confirm the combined effect of IL-1β and hypoxia in ECM degradation in the NP cells, we first examined the cellular viability changes of NP cells under normoxia or hypoxia with or without IL-1β stimulation. Our findings showed that IL-1β combined with hypoxia decreased the cellular viability as compared to that without IL-1β in the hypoxic or normoxic environment. Increasing evidence indicates that MMPs and ADAMTS

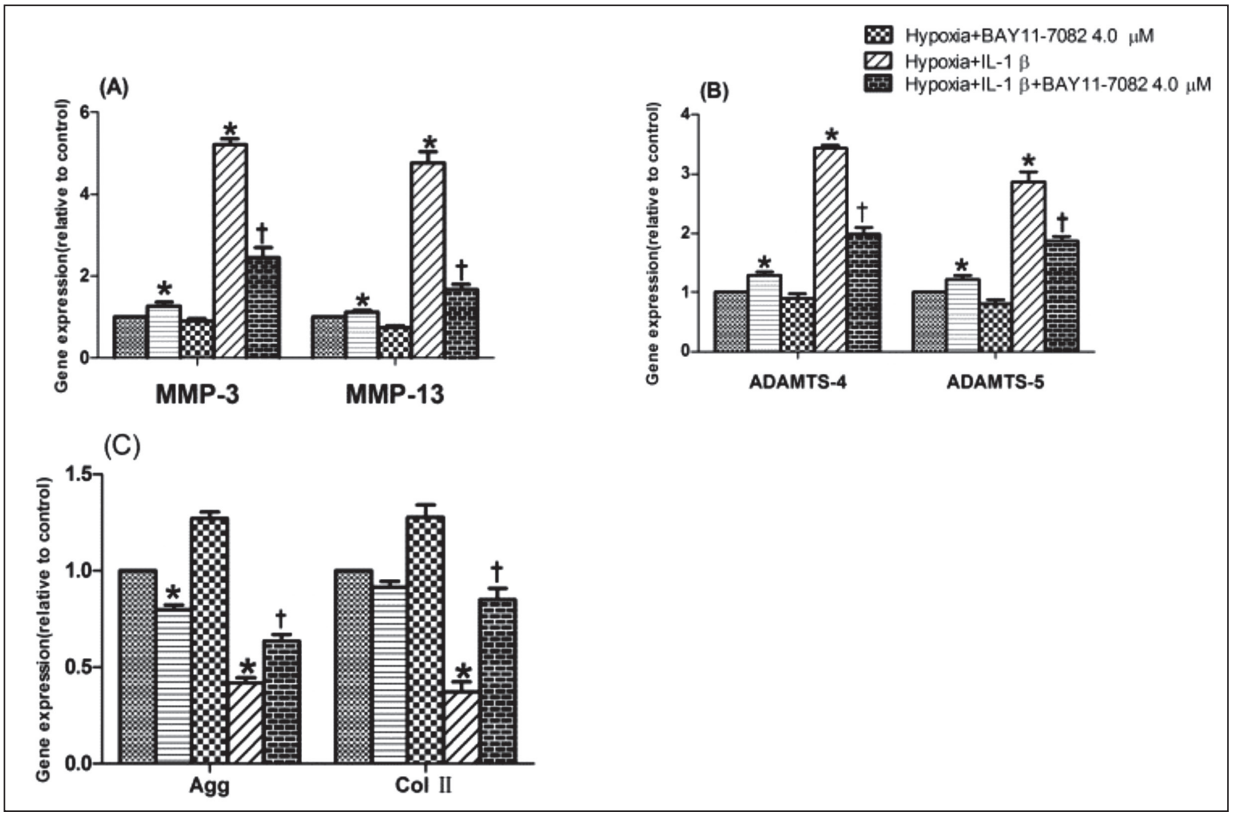

Figure 5. Effects of IL-1β inhibition on gene expression detected by qRT-PCR in NP cells. Before treating with IL-1β (10 ng/ml) for 24 h under hypoxic conditions, NP cells were pretreated with NF-κBp65 inhibitor BAY11-7082 (4.0 μM) for 24 h. MMP-3, MMP-13, ADAMTS-4, and ADAMTS-5 expression was detected by qRT-PCR. Histograms show means ± standard deviation of the mRNA levels relative to β-actin in six independent experiments performed in quadruplicate. *$p < 0.05$ *vs.* Normal; †$p < 0.05$ *vs.* hypoxia+BAY11-7082.

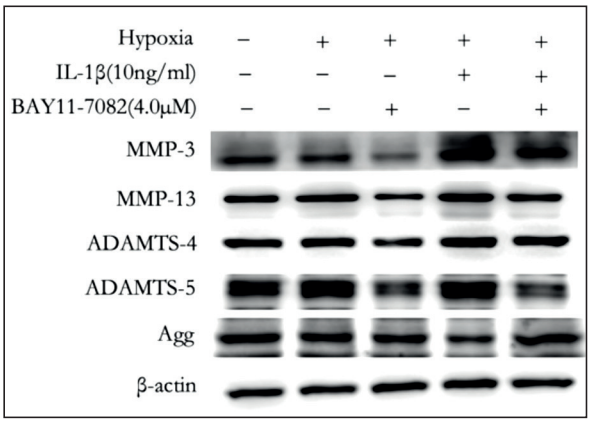

Figure 6. Effects of IL-1β inhibition on gene expression detected by western blotting in NP cells. Before treating with IL-1β (10 ng/ml) for 24 h under hypoxic conditions, NP cells were pretreated with NF-κBp65 inhibitor BAY11-7082 (4.0 μM) for 24 h. MMP-3, MMP-13, ADAMTS-4, and ADAMTS-5 expression was detected by Western blotting.

play important roles in the ECM degradation of the IVD, especially MMP-3, MMP-9, MMP-13, ADAMTS-4, and ADAMTS-5[33-35]. We investigated whether IL-1β could affect MMPs and ADAMTS expression under hypoxic conditions. The results showed that IL-1β upregulated the expression of MMP-3, MMP-13, ADAMTS-4, and ADAMTS-5 at both the transcriptional levels and protein level. In addition to hypoxia, pro-inflammatory cytokines can elevate MMPs and ADAMTS gene expression during IDD under normoxia. Hypoxia regulates the effects of IL-1β on MMPs and ADAMTS expression differently from normoxia[36]. Our results showed that the ECM degradation effect induced by IL-1β on the NP cells under hypoxia might be through the upregulation of MMP-3, MMP-13, ADAMTS-4, and ADAMTS-5 expression. This partly supported our findings that synergistic effects of hypoxia and pro-inflammatory cytokines contributed to an exacerbation of the expression of MMPs and ADAMTS. It is reported that chronic activation of NF-κB is a major mediator of many degenerative diseases. Our preliminary researches demonstrated that there was a significant increase in the proportion of cells with activated NF-κB in the degenerative disc[37]. MMP-1, MMP-2, MMP-3, MMP-13, ADAMTS-4, and ADAMTS-5 have been identified as NF-κB target genes in IVD cells[38,39]. We investigated whether IL-1β significantly activated the NF-κB signaling pathway in the NP cells

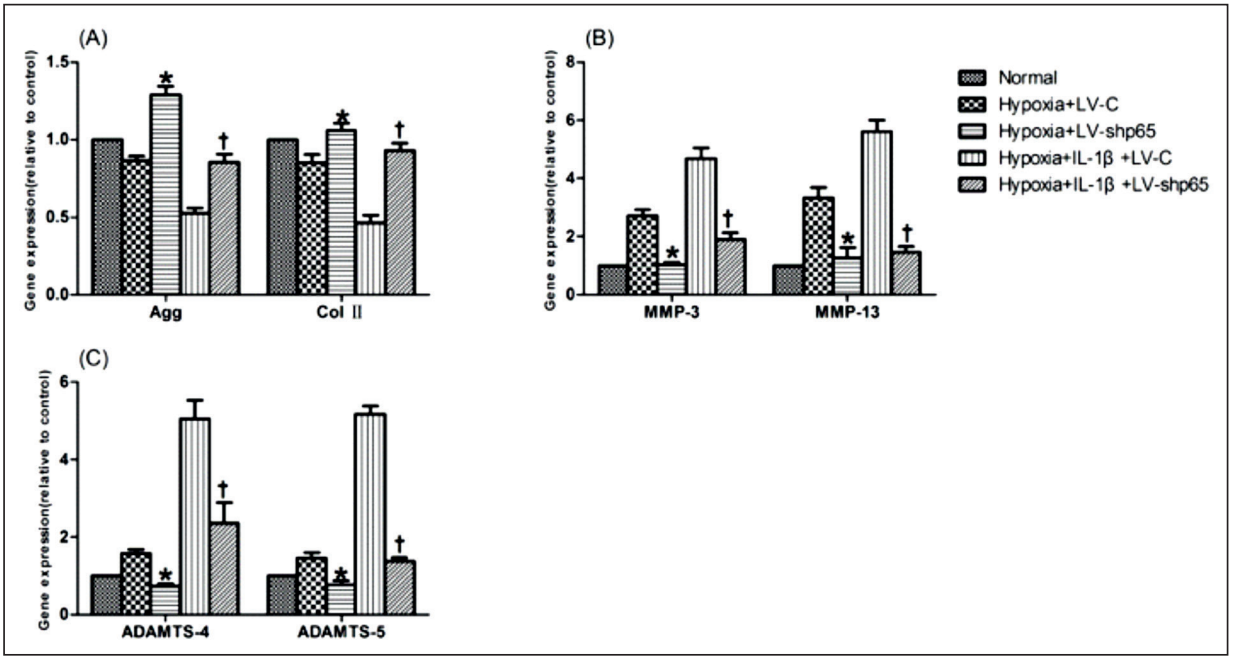

Figure 7. Effects of IL-1β inhibition by SiRNA on gene expression in NP cells. The NP cells were transfected with NF-κBp65 siRNA or control siRNA for 48 h, and then stimulated with 10 ng/ml of IL-1β for 24 h under hypoxia conditions. The expression levels of MMP-3, MMP-13, ADAMTS-4, and ADAMTS-5 was determined by qRT-PCR. Histograms show means ± standard deviation of the mRNA levels relative to β-actin in six independent experiments performed in quadruplicate. *$p <$ 0.05 *vs.* Hypoxia +LV-C; †$p < 0.05$ *vs.* Hypoxia +LV-shp65.

under hypoxic conditions. Our results showed that IL-1β increased activity of NF-κB under hypoxia, suggesting that pro-inflammatory cytokines such as IL-1β may exacerbate catabolic effects of the NP cells through the NF-κB signaling pathway under hypoxia. In recent years, clear evidence showed that when treatment with IL-1β under normoxia, NF-κB is activated in NP cells, leading to Agg and Col II loss and production of multiple matrix-degrading enzymes[40,41]. Therefore, we examined whether IL-1β exacerbated the ECM degradation in the NP cells via the NF-κB signaling pathway under hypoxia. In the present study, we used both molecular and pharmacological means to inhibit NF-κB, which resulted in remarkable decreases in the expressions of MMP-3, MMP-13, ADAMTS-4 and ADAMTS-5, compared with the control cells without NF-κB inhibition. The effects of NF-κB inhibition were also confirmed at the protein level. This effect of IL-1β on the expression of MMPs and ADAMTS of NP cells under hypoxic was effectively reduced by NF-κB inhibitors, suggesting that the upregulation of MMPs and ADAMTS expression by IL-1β might be through the activation of an NF-κB signal pathway in hypoxia. This is important because pro-inflammatory cytokines, even at low concentrations, can have a synergistic effect with hypoxia, enhancing or maintaining a high level of gene expression induced by pro-inflammatory cytokines. These results demonstrated that NF-κB pathway might play a critical role in the regulation of MMPs and ADAMTS expression in human NP cells under hypoxia and inflammatory environment. We speculated that the loss of Agg could be due to the action of NF-κB pathway, which could play a key role in IVD degradation. For patients with IDD, this could be a new treatment to protect against, decrease or even reverse IDD. Such a new approach would help to decrease IDD patients' suffering and the economic burden associated with IDD-induced low backache. Further studies will be required to develop this strategy for clinical application.

Conclusions

We demonstrated that the overexpression of MMPs and ADAMTS mediated by the NF-κB pathway promoted ECM degradation in the NP cells, in the presence of both IL-1β and hypoxia. The synergetic effect of IL-1β and hypoxia might contribute to promoting the activation of NF-κB pathway, thus inducing expression of MMPs and ADAMTS. NF-κB inhibition could mitigate IL-1β-induced upregulation of MMPs and ADAMTS, and consequently reverse the degradation of disc matrix macromolecules Agg and collagen II. Our study suggested a critical mechanism underlying the effect of combining IL-1β with hypoxia on promoting ECM degradation in the NP cells, providing important information that may be helpful in future development of potential therapy for IDD.

Conflict of Interest

The Authors declare that they have no conflict of interests.

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