Research Study

SIRT1 Inhibits the Catabolic Effect of IL-1β Through TLR2/SIRT1/NF-κB Pathway in Human Degenerative Nucleus Pulposus Cells

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Background: Low back pain (LBP), one of the most prominent problems worldwide, lacks effective disease-modifying medical therapy. Intervertebral disc degeneration (IVDD) is a major cause of LBP, and proinflammatory cytokines are the key factors involved in the development of IVDD-induced back pain. Sirtuin 1 (SIRT1) is implicated in the molecular control of aging and immune responses in various diseases; however, its effect on IVDD is not clearly understood.

Objective: To investigate the effects of SIRT1 on proinflammatory stress and signal transduction pathways induced by interleukin-1β (IL-1β) in human degenerative nucleus pulposus (NP) cells.

Study Design: Research study.

Setting: Chongqing Key Laboratory of Ophthalmology.

Methods: Anti-apoptotic and anti-catabolic effects of SIRT1 on IL-1β were investigated using a three-dimensional cell culture model of prestimulated human NP cells transfected with a lentiviral vector overexpressing SIRT1 or a small-interfering RNA (siRNA) against the gene encoding SIRT1. In addition, molecular mechanisms underlying the association of SIRT1 with Toll-like receptor-2 (TLR2) and nuclear factor kappa B (NF-κB) were investigated.

Results: Our results indicated that decreased SIRT1 expression was associated with IVDD. Direct regulation of SIRT1 expression did not affect the synthesis of extracellular matrix (ECM). However, SIRT1 overexpression mediated by the lentiviral vector suppressed IL-1β-induced ECM degradation and cell apoptosis. In contrast, knockdown of the gene encoding SIRT1 by the siRNA increased MMP expression and cell apoptosis induced by IL-1β. Furthermore, SIRT1 deacetylated RelA/p65 to inhibit the nuclear translocation of NF-κB, thus inhibiting inflammation. On the other hand, IL-1β downregulated SIRT1 expression by activating TLR2. However, inhibition of TLR2 expression by an siRNA did not inhibit IL-1β-induced nuclear translocation of NF-κB.

Limitations: Limitations of this study were the in vitro study design and lack of in vivo validation of the observed effects of SIRT1 on IVDD.

Conclusions: Our results indicated that SIRT1 exerted anti-inflammatory effects against IL-1β-mediated degeneration of NP cells through the TLR2/SIRT1/NF-κB pathway, suggesting that it could be used as a potential candidate for treating IVDD-mediated back pain.

Key words: SIRT1, TLR2, NF-κB, intervertebral disc degeneration

Pain Physician 2016; 19:E215-E226

Low back pain (LBP) is the sixth leading cause of disability worldwide. The number of people affected with LBP is increasing, indicating that it has become a severe socioeconomic problem (1). Preventive and therapeutic management of LBP is difficult because its veritable cause remains unclear. However, some studies have established a correlation between LBP and intervertebral disc degeneration (IVDD) (2-4). A normal IVD contains highly viscous cartilaginous nucleus pulposus (NP) surrounded by fibrous annulus and cartilage endplates. Radiographic analysis of NP tissue from patients with IVDD shows obvious changes (5), so it has been investigated most extensively. A healthy NP tissue is gel like, highly hydrated, and rich in proteoglycans. However, degenerated NP tissue shows increased extracellular matrix (ECM) degradation and cell apoptosis, which results in the loss of its capacity to withstand physical stress (6).

Many studies have shown that proinflammatory cytokines such as interleukin-1β (IL-1β) stimulate the expression of matrix-degrading enzymes and induce apoptosis, leading to the loss of collagen and proteoglycans and concomitant reduction in water content of the NP tissue (7,8). Toll-like receptors (TLRs) play a beneficial immunological role in inflammatory diseases involving the cartilage (9). Particularly, TLR2 is suggested to mediate catabolic and inflammatory processes in human IVD cells (10). Further, a recent study showed that NF-κB is involved in catabolic processes responsible for the pathophysiology of IVDD (11). Therefore, we hypothesized that the TLR2/NF-κB pathway played an important role in the inflammatory pathogenesis of IVDD.

Sirtuin 1 (SIRT1) is a member of the mammalian sirtuin family and is an NAD+-dependent class III histone deacetylase. A recent study showed that SIRT1 exerted comprehensive therapeutic effects on some age-related diseases (12). SIRT1 expression was decreased in chondrocytes derived from the cartilage of patients with osteoarthritis compared with those derived from age-matched normal ones (13,14). SIRT1 overexpression inhibited chondrocyte apoptosis and stimulated ECM synthesis in vitro (15). Our previous study also showed that SIRT1 inhibited the apoptosis of degenerative human NP cells after stimulation by resveratrol, a natural activator of SIRT1 (16). However, resveratrol is not a specific activator of SIRT1 and may also activate other sirtuin enzymes, thus exerting other biological effects. To examine the exact mechanism of SIRT1 on IVDD, we transfected NP cells with a lentiviral vector overexpress-

ing SIRT1 and a small-interfering RNA (siRNA) for silencing SIRT1 to target-regulate SIRT1 expression in the present study.

Molecular coordination between SIRT1 and the TLR2/NF-κB pathway has not been reported in human NP cells to date. Therefore, the present study also determined whether SIRT1 exerted anabolic and/or anti-inflammatory effects on degenerative NP cells and whether these effects were mediated by the TLR2/NF-κB pathway induced by IL-1β.

Methods

NP Tissue Sampling and Processing

Degenerative and normal NP tissues were obtained from 7 patients with lumbar disc herniation (LDH; 4 women and 3 men; average age, 35.86 ± 4.18 years) and 5 age-matched control patients with lumbar vertebral fracture (LVF) who did not have a documented medical history of LBP (2 women and 3 men; average age, 33.60 ± 5.23 years), respectively. Written informed consent was obtained from all the tissue donors before the surgery. This study was approved by the Ethics Committee of Chongqing Medical University. Degree of IVDD was assessed according to Pfirrmann classification by using preoperative MRI scans (5). IVDD grades of tissue samples from patients in the degenerative group were grade III (3 samples), grade IV (3 samples), and grade V (one sample) and those of tissue samples from patients in the control group were grade I (2 samples) and grade II (3 samples).

NP tissues from 10 donors (5 donors from each group) were first used for performing immunohistochemical (IHC) analysis and Western blotting. NP cells from patients in the degenerative group were isolated by enzymatic digestion and were cultured into a monolayer by incubating in DMEM/F-12 medium (HyClone, USA) supplemented with 10% (V/V) FCS (Gibco, USA), 100 μU/mL penicillin, and 100 μg/mL streptomycin, as described previously (17). To maintain the NP phenotype, the cells were cultured in a three-dimensional (3D) cell culture model by encapsulating them in alginate beads after the first passage.

IHC and TUNEL Staining

NP tissues were embedded in paraffin and were cut serially into 5-μm-thick sections for IHC and TUNEL staining. IHC staining was performed using streptavidin–peroxidase IHC kit (Boster, China), according to the manufacturer's directions. Primary antibodies against SIRT1

(1:200 dilution; Abcam, UK) and MMP-3 and MMP-13 (1:50 dilution; Santa Cruz Biotechnology, USA) were used to stain the respective proteins, and IgG was used as a negative control. The sections were then incubated with 3-3'-diaminobenzidine to detect immunoreactivity. DNA strand breaks in dead cells were enzymatically labeled using TUNEL assay kit (Roche, Switzerland). Stained cells in 3 different areas were counted under a microscope.

Alginate Culture and Transfection

After the first passage, NP cells were encapsulated in sodium alginate beads (Sigma, USA) at a density of 1 × 106 cells/mL, with the final alginate concentration being 1.2% (W/V). Sodium alginate was polymerized using 100 mM CaCl2 (Sigma, USA). For analyzing gene and protein expression, encapsulated cells were released by treating the capsules with 55 mmol/L sodium citrate to dissolve the alginate microspheres. NP cells were cultured in the alginate beads for 2 weeks, after which they were incubated with or without 10 ng/mL IL-1β for 24 hours.

Because a preliminary test showed low transfection efficiency of cells after alginate culture, NP cells were transfected with the lentiviral vector or siRNAs in a monolayer culture before encapsulation in alginate beads. A recombinant lentiviral vector overexpressing SIRT1–EGFP at a titer of 109 TU/mL was constructed by GeneChem Co., Ltd (Shanghai, China) and was synthesized using the following primers: forward primer 5'-GAGGATCCCCGGGTACCGGTCGCCACCATGGCGGAC-GAGGCGGCCCTC-3' and reverse primer 5'- TCACCATG-GTGGCGACCGGTGATTTGTTTGATGGATAGTTCATG-3'. For transfection, NP cells were plated in 6-well plates at a density of 1 × 105 cells/well. After 24 hours, the cells were transfected with 5 μL of the lentiviral vector by using polybrene (8 μg/mL). At 72 hours after transfection, the cells harboring the recombinant vector were selected using 5 μg/mL puromycin and were encapsulated in alginate beads.

Double-stranded siRNAs against genes encoding SIRT1, NF-κB, and TLR2 and scrambled siRNA were purchased from Santa Cruz Biotechnology (USA). Cells were transfected with 30 nM of the indicated siRNAs in the monolayer culture by using PepMute siRNA Transfection Reagent (SignaGen, USA), according to the manufacturer's protocol, after which they were encapsulated in alginate beads.

Real-time PCR

Total RNA was extracted from the cells by using RNAiso PLUS (Takara, Japan), according to the manufacturer's instructions. Next, 1 μg of mRNA was reverse transcribed to cDNA. The cDNA samples were amplified by performing real-time PCR in ABI Prism 7500 (ABI, USA) by using SYBR® Green Real-Time PCR Master Mix (Takara, Japan). Relative expression levels of the indicated genes were calculated using 2-ΔΔCt method. Primers for the amplification of genes encoding SIRT1, aggrecan, collagen II (COL2A1), and GAPDH were synthesized by Takara and are listed in Table 1.

Western Blotting and Immunoprecipitation Assay

Tissues or cells were lysed on ice by using RIPA lysis buffer or nuclear and cytoplasmic protein extraction kit supplemented with PMSF (Beyotime, China). Samples containing 50 μg proteins were electrophoresed by performing SDS-PAGE on 8%~12% gel, and separated proteins were transferred onto a PVDF membrane. The membrane was incubated overnight at 4°C with antibodies against SIRT1 (1:2000 dilution; Abcam, USA), aggrecan, COL2A1 (1:500 dilution; Santa Cruz Biotechnology, USA), p65, acetyl-p65 (Lys310), TLR2 (1:1000 dilution; Cell Signaling, USA), MMP-3, MMP-13 (1:500 dilution; Santa Cruz Biotechnology, USA), and β-actin (1:8000 dilution; Beyotime, China). The membrane was then washed and incubated with HRP-conjugated secondary antibody at 37°C for one hour. After washing the membrane, the blotted proteins were visualized using ECL Plus Reagent (Beyotime, China) and Chemi-Doc XRS+ Imaging System (Bio-Rad, USA).

The relationship between SIRT1 and NF-κB was determined by immunoprecipitation assay using protein A/G Plus agarose (Santa Cruz Biotechnology), according

Table 1. *Primer sequence for target gene*

Gene	Primer Sequence
GAPDH	Forward: 5'-GCACCGTCAAGGCTGAGAAC-3' Reverse: 5'-TGGTGAAGACGCCAGTGGA-3'
MMP-3	Forward: 5' -ATTCCATGGAGCCA GGCTTTC-3' Reverse: 5' -CATTTGGGTCAAACTC CAACTGTG-3'
MMP-13	Forward:5'-TTGATGATGATGAAACCTGGACAAG-3' Reverse: 5'-TTGCCGGTGTAGGTGTAGATAGGAA-3'
Collagen type II	Forward:5'-CAGGTGAACCTGGACGAGAG-3' Reverse: 5'-CCCACAGCACCAGTCTCAC-3'
Aggrecan	Forward: 5'-CTACCAGTGGATCGGCCTGAA-3' Reverse: 5'-CGTGCCAGATCATCACCACA-3'
SIRT1	Forward: 5'-GCCTCACATGCAAGCTCTAGTGAC-3' Reverse: 5'-TTCGAGGATCTGTGCCAATCATAA-3'

to a standard protocol. Briefly, anti-SIRT1 antibody was added to 50 μg of the protein sample, incubating for one hour at 37°C. Protein-A/G Plus agarose beads were then added to the sample, incubating overnight in a shaker at 4°C. The next day, beads were resuspended in lysis buffer, and proteins were eluted from the beads by using sodium dodecyl sulfate sample buffer. The proteins were then analyzed by performing Western blotting with anti-p65 antibody.

Detection of Apoptosis by Flow Cytometry

Apoptosis was detected by using Annexin V/PI apoptosis detection kit (Keygen, China). In all, 1 × 104 cells were harvested and were suspended in binding buffer supplemented with 5 μL Annexin-PE and 5 μL PI. The cells were gently vortexed and incubated in the dark for 15 minutes at room temperature. Next, 1 × ending buffer was added to the cells, and flow cytometry was performed immediately. Apoptotic rate was expressed as the sum of the percentage of early (Annexin V+/PI-) and late apoptotic cells (Annexin V+/PI+).

Fluorescence Immunocytochemistry

Fluorescence immunocytochemistry was used to detect the nuclear translocation of p65. Briefly, cells seeded in 24-well plates were fixed with 4% paraformaldehyde, washed, blocked, and incubated overnight with anti-p65 antibody at 4°C. Then, the cells were washed and incubated with an FITC-labeled secondary antibody. The cells were then counterstained with DAPI and were visualized under a fluorescence microscope at 550 nm.

Statistical Analysis

All measurements were performed in triplicate, and results are expressed as mean ± standard deviation. Differences between the groups were analyzed using one-way analysis of variance (ANOVA), with Tukey's post test for multiple groups. All statistical analyses were performed using GraphPad Prism Version 5.0. $P < 0.05$ was considered statistically significant.

Results

Basal Expression of SIRT1

IHC staining of untreated degenerative and normal NP tissues from age-matched patients with LDH and LVF, respectively, showed that SIRT1 was expressed in all the tissues. However, SIRT1-positive cells were significantly lower in tissues from patients in the degenerative group (Fig. 1B), which was further confirmed by Western blotting analysis (Fig. 1F). Expression of COL2A1 and aggrecan, which are the most abundant proteins in the ECM of NP tissues, also decreased (Fig. 1F), while that of MMP-3 and MMP-13 increased (Fig. 1C, 1D) in degenerative NP tissues. In addition, it had increased proportion of TUNEL-positive cells (Fig. 1E), which was consistent with the degradation of ECM observed in these tissues. These results indicated that SIRT1 expression was associated with the degeneration of NP tissue. Therefore, we hypothesized that SIRT1 may play an important role in the homeostasis of NP cells.

SIRT1 Inhibits IL-1β-induced ECM Degradation

To directly ascertain the role of SIRT1 in ECM metabolism, we used a lentiviral vector to promote SIRT1 expression in the presence or absence of IL-1β. Western blotting and qPCR confirmed that transfection of cells with the lentiviral vector overexpressing SIRT1 increased SIRT1 expression compared with that in control cells. However, SIRT1 overexpression did not induce changes in COL2A1 and aggrecan expression (Fig. 2A, 2B). This result suggested that SIRT1 did not directly regulate ECM metabolism in NP cells. Further, SIRT1 overexpression significantly increased COL2A1 and aggrecan expression which were downregulated by IL-1β treatment (Fig. 2C, 2D), suggesting that SIRT1 could suppress ECM degradation induced by IL-1β. Because upregulation and activation of MMP expression is associated with the loss of NP tissue matrix, we investigated the effect of SIRT1 on MMP expression. As expected, IL-1β treatment significantly upregulated MMP-3 and MMP-13 expression and knockdown of SIRT1 by the siRNA promoted IL-1β-induced MMP-3 and MMP-13 expression (Fig. 2E, 2F, 2G, 2H). These results indicated that SIRT1 exerted anti-inflammatory effects against ECM degradation by decreasing MMP expression.

SIRT1 Inhibits IL-1β-induced Apoptosis

To clarify the effect of SIRT1 on IL-1β-induced apoptosis of NP cells, we performed flow cytometric analysis by using Annexin V/PI. The results of flow cytometric analysis showed that apoptosis of NP cells significantly increased after IL-1β treatment. However, SIRT1 overexpression inhibited IL-1β-induced apoptosis of NP cells. In contrast, protective effects of SIRT1 against cell apoptosis were attenuated after the knockdown of endogenous SIRT1 by the siRNA (Fig. 3).
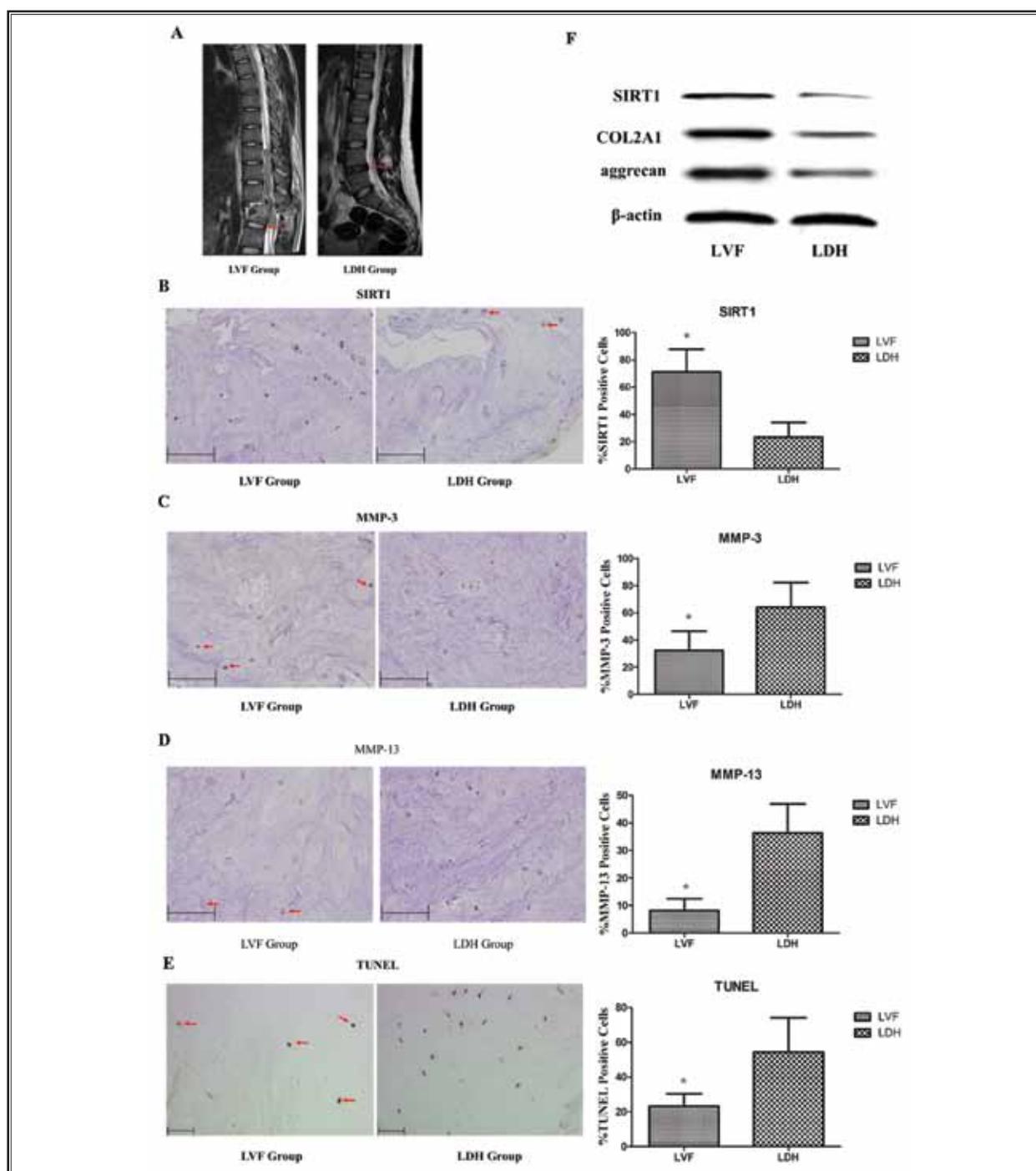
Fig. 1. *Analysis of SIRT1 different expression in NP tissues from age-matched normal and degenerative group. A, representative lumbar MRI of one patient with LVF aged 33 years old and the other with LDH aged 35 years old were classified according to Pfirrmann's grading system. The red arrows represented the grade II disc in normal group and grade III disc in degenerative group. B, C, and D, SIRT1 (B), MMP-3 (C), and MMP-13 (D) proteins distribution were determined by immunohistochemical staining in NP tissues. E, the percentages of apoptotic cells were determined by TUNEL staining in NP tissues. F, SIRT1, COL2A1 and aggrecan protein expression level were determined by Western blotting in NP tissues. Three independent experiments were performed, and data of analysis represents the mean ± SD. *P < 0.05. Bars = 100µm.*
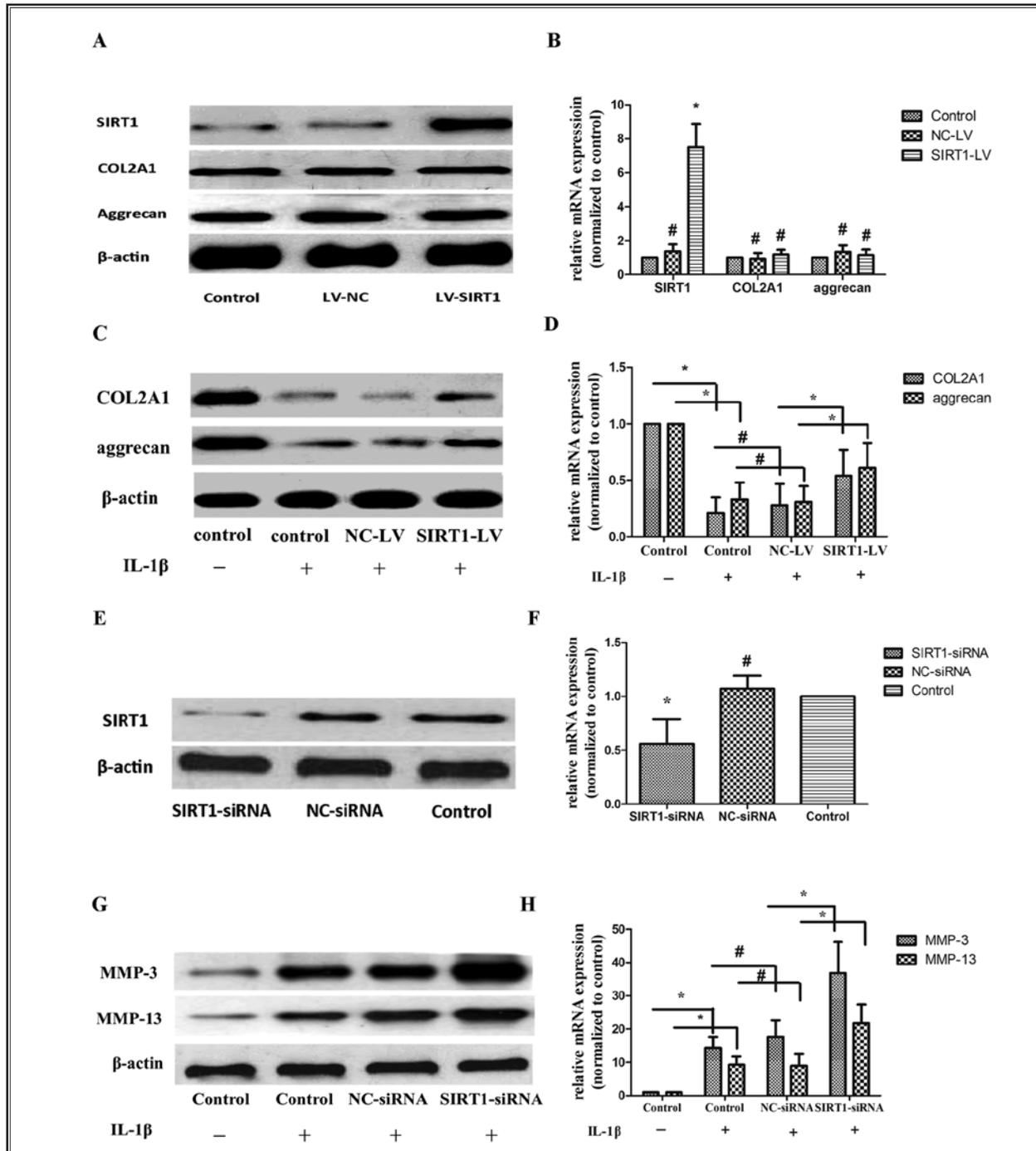
Fig. 2. *SIRT1 inhibits the IL-1β mediated catabolic effect in NP cells. A, NP cells were transfected with lentivirus overexpression SIRT1or negative control. Transfected cells were further alginate cultured for 2 weeks, SIRT1, COL2A1, and aggrecan protein expressions were assessed by Western blotting. B, total RNA was isolated and real-time PCR was perfomed using SIRT1, COL2A1, and aggrecan primer sets. C, D, Lentiviral transfected NP cells were further untreated or treated with IL-1β (10ng/mL), COL2A1, and aggrecan expressions were determined by Western blotting and real-time PCR. E, F, NP cells were transfected with negative control siRNA (NC-siRNA) or SIRT1-specific siRNA. The silencing effect were determined by Western blotting and real-time PCR. G, H, MMP-3, MMP-13 protein, and mRNA levels were quantified by Western bolt and real-time PCR in siRNA transfected cell with or without IL-1β treatment. Relative gene and protein expression levels are normalized against control. Data represents mean ± SD, *P < 0.05 and #P > 0.05 compared with control.*
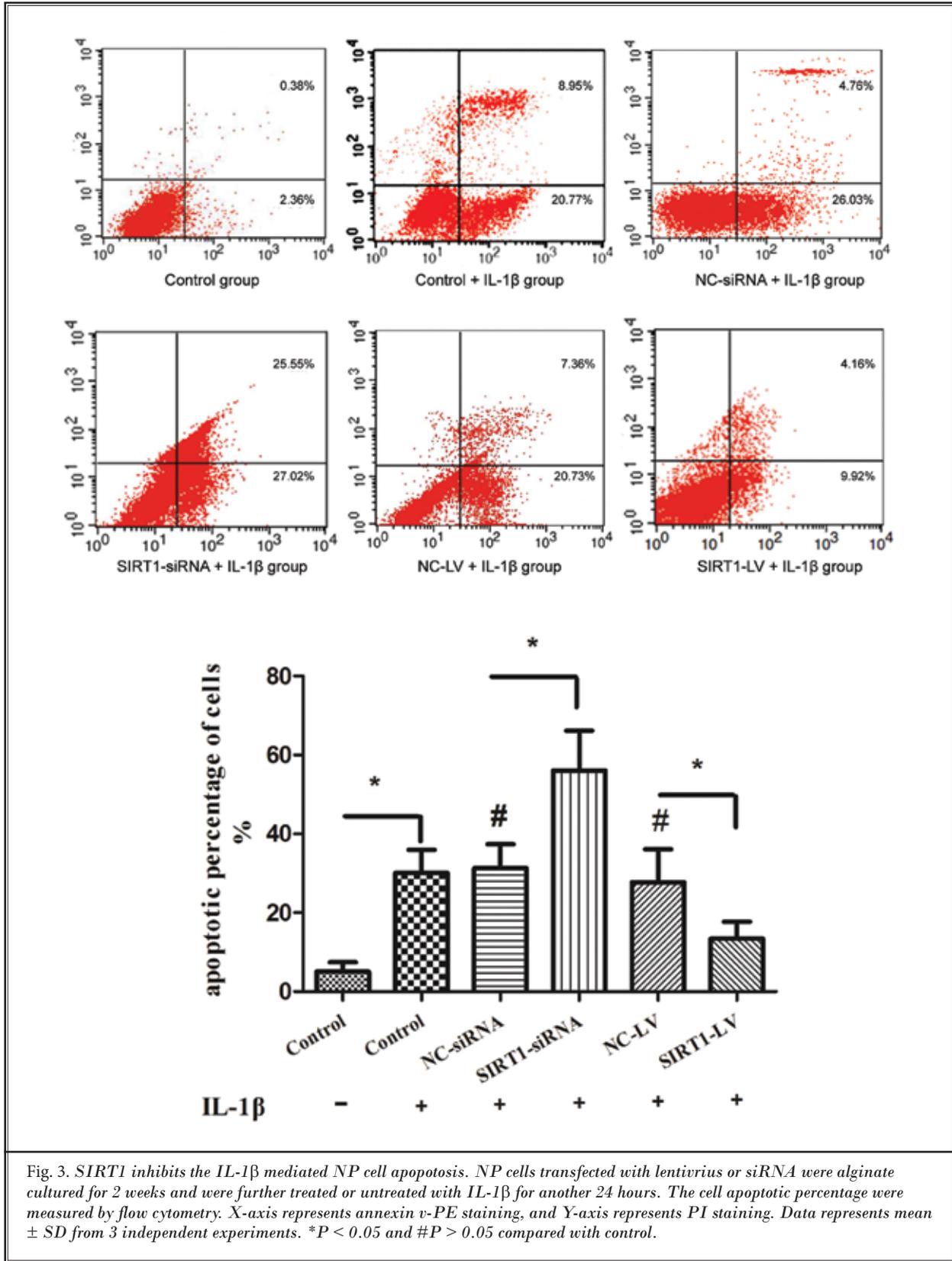
Fig. 3. *SIRT1 inhibits the IL-1β mediated NP cell apopotosis. NP cells transfected with lentivrius or siRNA were alginate cultured for 2 weeks and were further treated or untreated with IL-1β for another 24 hours. The cell apoptotic percentage were measured by flow cytometry. X-axis represents annexin v-PE staining, and Y-axis represents PI staining. Data represents mean ± SD from 3 independent experiments. *P < 0.05 and #P > 0.05 compared with control.*

TLR2 Inhibits SIRT1 Expression after IL-1β Stimulation

Because TLR2 is the main mediator of IL-1β response in NP cells, we examined whether TLR2 influenced the activation of NF-κB subunit p65 after IL-1β treatment. IL-1β treatment significantly increased TLR2 and nucleoprotein p65 expression and downregulated SIRT1 expression (Fig. 4A). Western blotting showed no changes in p65 expression after TLR2 silencing (Fig. 4A). Immunocytochemical analysis confirmed that IL-1β-induced nuclear translocation of p65 was not attenuated after TLR2 silencing (Fig. 4E), however, SIRT1 expression increased (Fig. 4A). These results indicated that TLR2 did not directly result in the downstream activation of NF-κB, despite inhibiting SIRT1 expression after IL-1β stimulation.
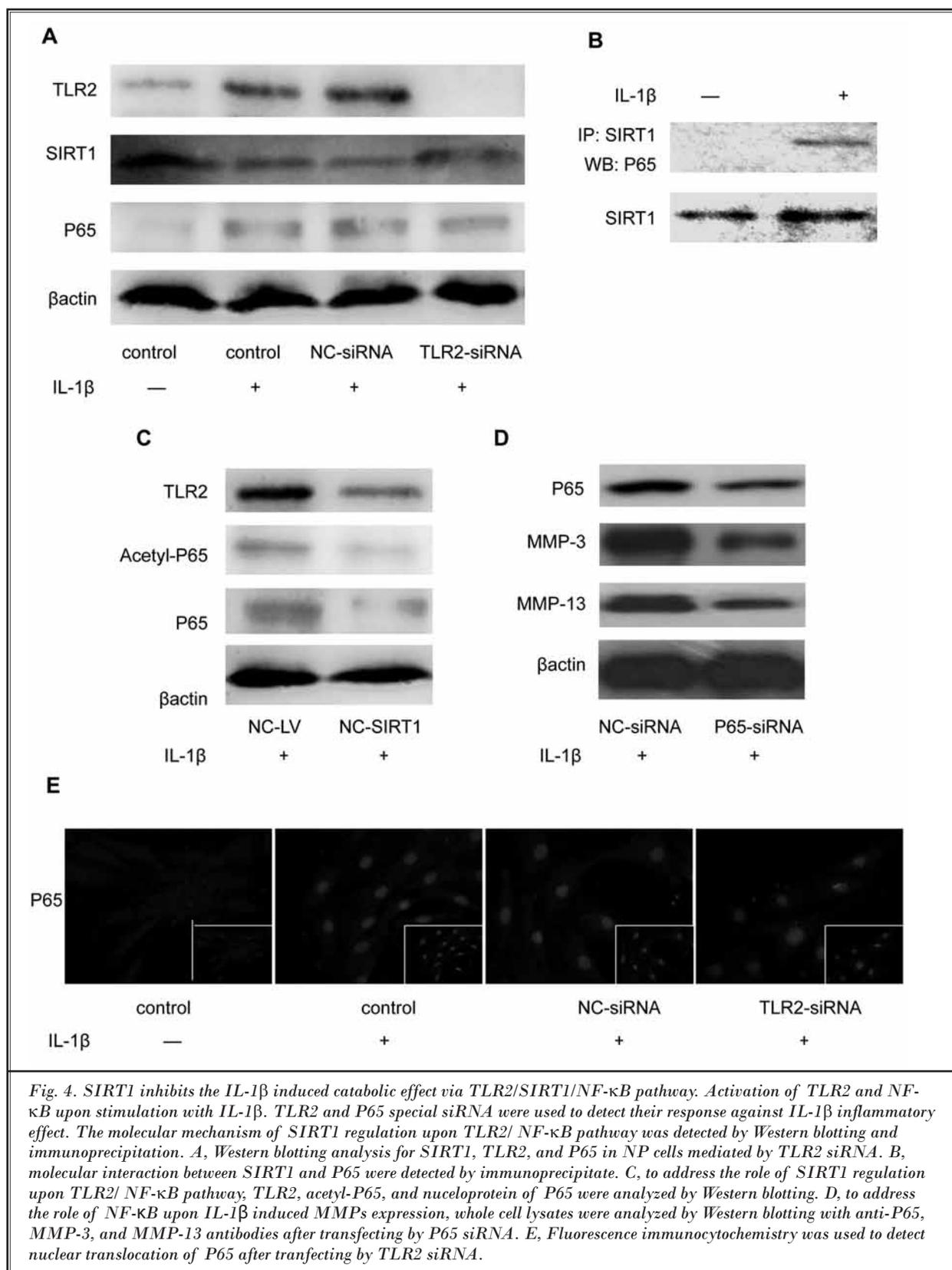
SIRT1 Inhibits NF-κB Activation after IL-1β Stimulation

Because inflammatory responses in IVDs are regulated by NF-κB, we transfected NP cells with siRNA against p65 to verify the role of NF-κB in IL-1β-induced catabolic effects. We observed that knockdown of p65 blocked IL-1β-induced MMP-3 and MMP-13 expression (Fig. 4D), indicating that NF-κB inhibition exerted an anti-inflammatory effect against IL-1β stimulation. We then investigated whether the anti-inflammatory effect of SIRT1 was mediated by the NF-κB pathway. IL-β treatment significantly increased the nuclear translocation and acetylation of p65, which was blocked by SIRT1 overexpression. Further, SIRT1 overexpression markedly reduced IL-1β-induced TLR2 activation. We then examined the interaction between SIRT1 and NF-κB by performing immunoprecipitation assay. We immunoprecipitated whole cell lysates by using anti-SIRT1 antibody and performed Western blotting with anti-p65 antibody to determine the presence of p65 (Fig. 4B). Results of immunoprecipitation assay and Western blotting suggested that SIRT1 suppressed NF-κB activity by directly regulating its subunit p65.
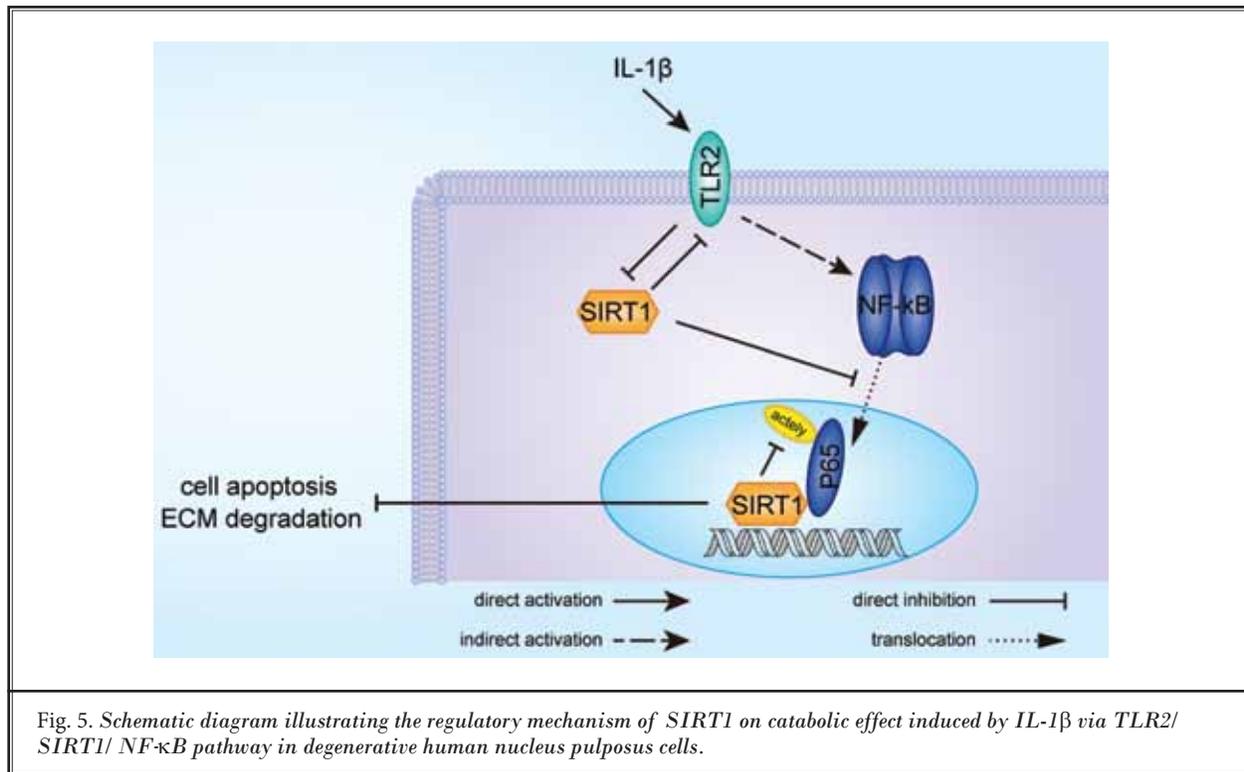
Discussion

In the present study, we showed for the first time that SIRT1 inhibited the catabolic response to IL-1β in degenerative NP cells through the TLR2/SIRT1/NF-κB pathway. Our previous studies have reported the different expression status of SIRT1 in IVDs (16), but have neglected the age factor, which is a major risk factor for the development of IVDD (18). To determine whether pathological changes in IVDD were caused by sponta-

neous degeneration or simply aging, we used an age-matched control group. It is difficult to obtain healthy human NP tissues in clinical practice even by using postmortem samples, because IVDD might occur at a very early age (19). Therefore, NP tissues from patients with LVF, as determined by the examination of magnetic resonance imaging (MRI) scans and clinical manifestation, were used as control tissues in this study. Our results showed that SIRT1 expression decreased significantly in degenerative NP tissues compared with that in age-matched control tissues. Several studies have indicated that SIRT1 plays a protective role in IVDD (16,20,21). However, Zhang et al (17) showed that SIRT1 treatment by using a recombinant human SIRT1 significantly reduced the mRNA expression of genes encoding COL2A1, aggrecan, and SOX9. To confirm the role of SIRT1 in cell homeostasis, degenerative NP cells cultured in 3D alginate beads were transfected with a lentiviral vector overexpressing SIRT1. Interestingly, our results showed that SIRT1 overexpression alone had little effect on the basal expression of COL2A1 and aggrecan. The reason for slight inconsistency between our result and Zhang's may be because they performed cells in vitro monolayer culture, which may have caused NP cells to lose their phenotype during culture process (22). Our result suggested that SIRT1 did not directly trigger ECM synthesis in the 3D culture of NP cells.

Several investigations have characterized the upregulation of inflammatory factors during IVDD progression. Of these inflammatory factors, IL-1β contributes to IVDD by upregulating catabolic enzymes, inhibiting normal ECM production, and increasing cell apoptosis (8,23,24). Our results showed that MMP-3 and MMP-13 expression increased and COL2A1 and aggrecan expression decreased after IL-1β stimulation, which is in agreement with the results of previous studies. Several studies indicate that resveratrol increases the deacetylase activity of SIRT1, which is known to exert anti-inflammatory effects (25,26). However, results of a recent study challenges the overall use of resveratrol as a pharmacological tool for activating SIRT1 (27). Therefore, to date limited studies, we are the first to target regulate the SIRT1 expression transduced by lentivirus and siRNA in NP cells. Our results showed that SIRT1 was directly involved in IL-1β-induced catabolism. SIRT1 overexpression mediated by lentiviral vector reversed the decrease in COL2A1 and aggrecan expression induced by IL-1β. In contrast, the anti-catabolic effect of inhibition of MMP-3 and MMP-13 expression disappeared after knockdown of SIRT1 by the siRNA. Apop-

Fig. 4. SIRT1 inhibits the IL-1β induced catabolic effect via TLR2/SIRT1/NF-κB pathway. Activation of TLR2 and NF-κB upon stimulation with IL-1β. TLR2 and P65 special siRNA were used to detect their response against IL-1β inflammatory effect. The molecular mechanism of SIRT1 regulation upon TLR2/ NF-κB pathway was detected by Western blotting and immunoprecipitation. A, Western blotting analysis for SIRT1, TLR2, and P65 in NP cells mediated by TLR2 siRNA. B, molecular interaction between SIRT1 and P65 were detected by immunoprecipitate. C, to address the role of SIRT1 regulation upon TLR2/ NF-κB pathway, TLR2, acetyl-P65, and nuceloprotein of P65 were analyzed by Western blotting. D, to address the role of NF-κB upon IL-1β induced MMPs expression, whole cell lysates were analyzed by Western blotting with anti-P65, MMP-3, and MMP-13 antibodies after transfecting by P65 siRNA. E, Fluorescence immunocytochemistry was used to detect nuclear translocation of P65 after tranfecting by TLR2 siRNA.

Fig. 5. *Schematic diagram illustrating the regulatory mechanism of SIRT1 on catabolic effect induced by IL-1β via TLR2/ SIRT1/ NF-κB pathway in degenerative human nucleus pulposus cells.*

tosis is another crucial process involved in IVDD (28). The TUNEL assay performed in this study confirmed that the ratio of apoptotic cells in NP tissues from patients in the degenerative group was higher than that in normal tissues. Flow cytometric analysis further showed that SIRT1 attenuated apoptosis induced by IL-1β.

Previous studies have suggested that TLR pathways regulate many inflammatory and catabolic responses in IVDs (10,29,30). Our results also confirmed that IL-1β induced TLR2 expression and NF-κB activation. However, Western blotting and immunofluorescence analysis showed that nuclear translocation of p65 was not directly mediated by the TLR2 pathway. Furthermore, our results suggested that SIRT1 played an intermediary role between IL-1β and TLR2. We found that IL-1β inhibited SIRT1 expression but SIRT1 overexpression downregulated TLR2 expression induced by IL-1β. NF-κB is a major downstream target of the IL-1β pathway (31). Our results showed that NF-κB mediated IL-1β-induced expression of MMPs. To determine the mechanism underlying the anti-catabolic effects of SIRT1 in NP cells, results of Western blotting showed that SIRT1 decreased the nuclear translocation of p65 and deacetylation of Lys310 residue of p65. The molecular

interaction between SIRT1 and p65 was confirmed by performing coimmunoprecipitation assay. These results suggested that the TLR2/SIRT1/NF-κB pathway was critical for the anti-inflammatory and cytoprotective effects of SIRT1 against IL-1β in human degenerative NP cells.

Conclusion

In summary, we found that SIRT1 expression was decreased in degenerative NP tissues compared with that in age-matched control tissues. Further, regulation of SIRT1 expression by using a lentiviral vector or an siRNA showed that SIRT1 exerted protective effects against ECM degradation and apoptosis induced by IL-1β in degenerative NP cells. In addition, our results showed that the TLR2/SIRT1/NF-κB pathway was involved in regulating IL-1β-induced catabolic effect. Overall, our results suggested that SIRT1 could be used as a novel therapeutic candidate for treating IVDD-induced back pain.

Acknowledgments

References

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