NF-κB is negatively associated with Nurr1 to reduce the inflammatory response in Parkinson's disease

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Abstract. Parkinson's disease (PD) is one of the most disabling diseases of the central nervous system, seriously affecting health and quality of life for the elderly. The present study aimed to explore the effects of nuclear receptor subfamily 4 group A member 2 (Nurr1) and nuclear factor-κB (NF-κB) on the progression of Parkinson's disease (PD). Pheochromocytoma (PC12) cells were pretreated with the NF-κB inhibitor quinazoline (QNZ) or transfected with small interfering (si)RNA-NF-κB, followed by the addition of lipopolysaccharide (LPS). After culturing for 24 h, Cell Counting Kit-8 (CCK-8) was utilized to measure cell viability. Next, the expression levels of interleukin (IL)-1β, IL-6 and tumor necrosis factor (TNF)-α were determined using the relevant Enzyme-linked immunosorbent assay kits. Expression levels of p65, tyrosine hydroxylase (TH), α-Synuclein (A-SYN) and Nurr1 were examined by immunofluorescence and western blotting. CCK-8 results showed that the cell viability was significantly reduced in the LPS group than in the control group ($P<0.05$), whereas QNZ and si-NF-κB demonstrated significantly enhanced viability induced by LPS ($P<0.05$). After LPS induction, the levels of IL-1β, IL-6 and TNF-α were significantly elevated when compared with those in the control group ($P<0.05$), whereas QNZ and NF-κB interference partially restored their levels. Additionally, after LPS induction, the expression of p65 and A-SYN was higher, while the expression of TH and Nurr1 was lower. However, QNZ and NF-κB treatment significantly reversed the expression levels induced by LPS ($P<0.05$). Finally, it was observed that NF-κB may be negatively associated with Nurr1. In conclusion, inhibition of NF-κB may reduce the production of inflammatory factors by upregulating Nurr1 and TH and downregulating A-SYN, thus relieving the inflammatory response in PD.

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Introduction

Parkinson's disease (PD), second only to Alzheimer's disease in prevalence, is diagnosed in ~1% of individuals over the age of 65, and is a progressive multi-system neurodegenerative disease closely associated with age (1). A meta-analysis of worldwide data has revealed that the incidence and prevalence of PD increase with age (2). Notably, PD is characterized by the progressive death of dopaminergic neurons in the substantia nigra pars compacta, as well as the widespread presence of the intracellular protein, α-Synuclein (A-SYN) (3). The clinical symptoms of PD mainly include tremors, stiffness, posture instability, bradykinesia, autonomic nerve dysfunction and mental disorders (4). Currently, dopamine-based drugs, such as levodopa, are being utilized in PD therapy to correct dyspraxia (5). However, the long-term use of these drugs can result in a variety of serious side effects, including cardiovascular disease and pneumonia (6). Therefore, it is crucial to comprehensively understand the pathogenesis of PD and develop novel biological therapeutic targets against PD.

Studies have shown that the occurrence and development of PD are related to the environment, genetics, metabolic deficiency, oxidative stress and neuroinflammatory response (2,7,8). Reportedly, inhibition of tyrosine hydroxylase (TH) activity was found to be closely related to the occurrence of PD, and immunohistochemical determination of TH expression has provided an essential tool for visualization and quantification of the damage and loss to dopaminergic neurons in PD models (9,10). Additionally, an *in vitro* study by Salemme *et al* (11) indicated that dihydroasparagusic acid can alleviate neurodegenerative diseases by inhibiting inflammatory and oxidative processes. Another study has shown that compared with healthy subjects, the production of interleukin (IL)-1β, IL-6 and tumor necrosis factor (TNF)-α was significantly enhanced in the peripheral blood of patients with PD (12). Nuclear factor-κB (NF-κB), an important transcription factor, plays an essential role in cell growth and proliferation, regulating the inflammatory response by affecting the expression of its downstream genes,

including TNF-α, IL-6 and IL-1β (13). Furthermore, the NF-κB signaling pathway plays a key physiological role in the central nervous system. Ghosh *et al* (4) reported that the NF-κB essential modifier-binding domain enters the central nervous system, blocks the activation of NF-κB and inhibits the expression of pro-inflammatory factors, thereby improving behavioral functions in PD mice.

A previous study has suggested the interaction between the nuclear receptor subfamily 4 group A member 2 (Nurr1) and NF-κB (14). Nurr1 is a transcription factor belonging to the nuclear steroid hormone receptor superfamily and is involved in numerous biological processes, including cell proliferation, apoptosis and migration (15). McEvoy *et al* (16) elucidated that NF-κB binds to the Nurr1 promoter and stimulates Nurr1 expression in rheumatoid arthritis (RA) synovial cells, thus participating in RA by mediating multiple inflammatory signals. However, the roles of Nurr1 and NF-κB in neuroinflammation associated with PD remain unclear.

Typically, the pheochromocytoma (PC12) cell line is employed as a cell model for neurobiological and neurochemical investigations (17,18). Therefore, in the present study, NF-κB-knockdown PC12 cells were first constructed; alternatively, PC12 cells were first treated with the NF-κB inhibitor, quinazoline (QNZ). Next, the cells were utilized to build an inflammatory cell model using lipopolysaccharide (LPS). Then, mechanisms underlying the role of NF-κB in the inflammatory progression of PD were explored. These findings may help improve our understanding of the inflammatory progression during PD and provide novel therapeutic targets for the amelioration and treatment of PD.

Materials and methods

Cell culture. Highly differentiated rat PC12 cells were obtained from The Cell Bank of Type Culture Collection of The Chinese Academy of Sciences. PC12 cells were cultured in RPMI-1640 medium (Thermo Fisher Scientific, Inc.) supplemented with 10% fetal bovine serum (FBS; Thermo Fisher Scientific, Inc.), 100 kU/l penicillin (Thermo Fisher Scientific, Inc.) and 100 mg/l streptomycin (Thermo Fisher Scientific, Inc.), and incubated in an incubator with 5% carbon dioxide at 37˚C.

Cell transfection. NF-κB-knockdown PC12 cells were constructed using small interfering (si)-NF-κB (Guangzhou RiboBio Co., Ltd.). Cell transfection was performed as previously described (19). The PC12 cells were seeded into 6-well plates ($5x10^5$ cells/well), and then transfected with 50 nM si-negative control (si-NC, forward: UUCUCCGAACGUGUC ACGUTT, reverse: ACGUGACACGUUCGGAGAATT) and 50 nM si-NF-κB [si-NF-κB-1 (forward: GCUUUGACUCAC UCCAUAUTT, reverse: AUAUGGAGUGAGUCAAAGCTT), si-NF-κB-2 (forward: GCACCAAGACCGAAGCAAUTT, reverse: AUUGCUUCGGUCUUGGUGCTT), si-NF-κB-3 (forward: GCAGGUAUUUGACAUACUATT, reverse: UAG UAUGUCAAAUACCUGCTT)] using Lipofectamine® 2000 (Thermo Fisher Scientific, Inc.) according to the manufacturer's instructions. The cells in the control group were cultured in the medium. After culturing for another 24 h, the total RNA of cells was extracted, and the transfection efficiency was evaluated by determining the expression level of *NF-κB* using reverse transcription-quantitative PCR (RT-qPCR) and western blotting.

RT-qPCR. After transfection, total RNA was extracted from the cells using TRIzol® reagent (Thermo Fisher Scientific, Inc.) according to the manufacturer's protocols. Then, total RNA was reverse transcribed into cDNA using the PrimeScript™ II 1st Strand cDNA Synthesis kit (Takara Bio, Inc.), and the temperature protocol used for reverse transcription was 37˚C for 60 min, and 85˚C for 5 sec. SYBR Premix Ex Taq (2X, Thermo Fisher Scientific, Inc.) was used for qPCR, and the thermocycling conditions were as follows: Initial denaturation at 95˚C for 2 min; followed by 40 cycles of denaturation at 95˚C for 15 sec, and 60˚C for 60 sec, and annealing and extension at 95˚C for 15 sec, 60˚C for 60 sec and 95˚C for 15 sec. The relative expression of NF-κB was normalized to the internal reference gene GAPDH (forward: 5'-AGACAGCCG CATCTTCTTGT-3', reverse: 5'-CTTGCCGTGGGTAGAGTC AT-3'), and calculated using the $2^{-\Delta\Delta Cq}$ method (20). The primer sequence of *NF-κB* was as follows: Forward, 5'-ACTATGAGG TCTCTGGGGGGA-3' and reverse, 5'-GAAGCTGAGTTT GCGGAAGG-3'.

Cell viability assay. For all treated PC12 cells, cell viability was measured using the Cell Counting Kit-8 (CCK-8; Biosharp Life Sciences). Highly differentiated PC12 cells were seeded into 96-well plates ($1x10^4$ cells/well), and the cells were randomly divided into the following groups: i) Control group; ii) QNZ groups with different concentrations (3, 5, 7, 9 and 11 nM); iii) LPS group; iv) LPS + QNZ group; v) LPS + si-NC group; and vi) LPS + si-NF-κB group. Except for the control and LPS groups, cells in other groups were first treated with QNZ (Selleck Chemicals) or transfected for 6 h, and then LPS was added at a final concentration of 600 ng/ml. Cells in the control group were treated with phosphate-buffered saline (PBS), whereas cells in the LPS group were first treated with PBS for 6 h, and then with LPS. After 24 h of cell culture, 20 μl CCK-8 reagent was added to cells and incubated at 37˚C for 4 h. Finally, absorbance was measured at 450 nm on a microplate reader.

Enzyme-linked immunosorbent assay (ELISA). In PC12 cells ($5x10^5$ cells/well) that underwent different treatments, the levels of IL-1β, IL-6 and TNF-α were determined using rat IL-1β, IL-6 and TNF-α ELISA assay kits (all purchased from Elabscience, Inc.), respectively, according to the manufacturer's instructions.

Immunofluorescent microscopy of TH, A-SYN and Nurr1 expression. Briefly, PC12 cells were divided into six groups: i) Control; ii) QNZ; iii) LPS; iv) LPS + QNZ; v) LPS + si-NC; and vi) LPS + si-NF-κB. The differentiated PC12 cells ($5x10^5$ cells/well) were plated on glass coverslips, washed with PBS three times, and then fixed with 4% paraformaldehyde for 15 min at room temperature. After washing, fixed cells were permeabilized with 0.5% Triton X-100 (Beyotime Institute of Biotechnology) at room temperature for 20 min, and blocked with 3% bovine serum albumin (Thermo Fisher Scientific, Inc.) in PBS for 10 min at room temperature. Then, anti-TH antibody (1:100; cat. no. 25859-1-AP; ProteinTech Group, Inc.), anti-A-SYN

antibody (1:100; cat. no. 10842-1-AP; ProteinTech Group, Inc.) and anti-Nurr1 antibody (1:100; cat. no. ab41917, Abcam) were added to the coverslips at 4˚C and incubated overnight. After washing, cells were incubated with a Cy3-labeled sheep anti-rabbit IgG secondary antibody (1:100; cat. no. BA1032, Wuhan Boster Biological Technology, Ltd.) at 25˚C for 1 h. After washing, DAPI (two drops) was added to the coverslips and incubated in the dark for 5 min. After washing with PBS, coverslips were mounted in a mounting medium containing DAPI (SouthernBiotech), and then observed under a fluorescence microscope.

Western blotting. In brief, total protein was isolated from different PC12 cell groups using RIPA protein lysis buffer (Beyotime Institute of Biotechnology), and the concentrations were determined using a BCA protein assay kit (Wuhan Boster Biological Technology, Ltd.) in accordance with the manufacturer's protocols. Western blotting was performed as described in a previous study (21). Proteins (20 μg) were separated via SDS-PAGE on a 10% gel, and separated proteins were subsequently transferred to PVDF membranes. After blocking with 5% skimmed milk for 2 h at 37˚C, the membranes were incubated with anti-p65 antibody (1:100; cat. no. 10745-1-AP; ProteinTech Group, Inc.), anti-TH antibody (1:100; cat. no. 25859-1-AP; ProteinTech Group, Inc.), anti-Nurr1 antibody (1:100; cat. no. ab41917, Abcam), anti-A-SYN antibody (1:100; cat. no. 10842-1-AP; ProteinTech Group, Inc.) and anti-β-actin antibody (1:200; cat. no. ab8226, Abcam) at 4˚C overnight. After washing with PBS with 0.05% Tween-20, the membranes were incubated with HRP-labeled goat anti-rabbit IgG (1:500; ProteinTech Group, Inc.) at 37˚C for 2 h. After washing three times, protein bands were visualized with a ECL assay kit (Beyotime Institute of Biotechnology) and chemiluminescence system (Tanon Science and Technology Co., Ltd.). The protein bands were analyzed using ImageJ software (version 6.0; National Institutes of Health).

Statistical analysis. Each experiment was performed in triplicate. Data are presented as the mean ± standard deviation of three independent experiments. GraphPad Prism 5.0 (GraphPad Software, Inc.) was used for statistical analyses. ANOVA followed by Tukey's post hoc test was used to compare the differences among >two groups. P<0.05 was considered to indicate a statistically significant difference.

Results

Optimum concentration of QNZ. To determine the optimum concentration of the NF-κB inhibitor QNZ, different concentrations of QNZ were used to treat PC12 cells. The results revealed that cell viability gradually increased with increasing QNZ concentrations. When the QNZ concentration was 11 nM, the PC12 cell viability was significantly increased by ~28% when compared with the control group (P<0.05; Fig. 1). As cell viability was highest in the 11 nM QNZ group, this concentration was used in subsequent experiments.

Analysis of transfection efficiency. In PC12 cells, the expression of *NF-κB* was determined by RT-qPCR and western blotting to evaluate the cell transfection efficiency. No significant difference in the expression of *NF-κB* was observed between

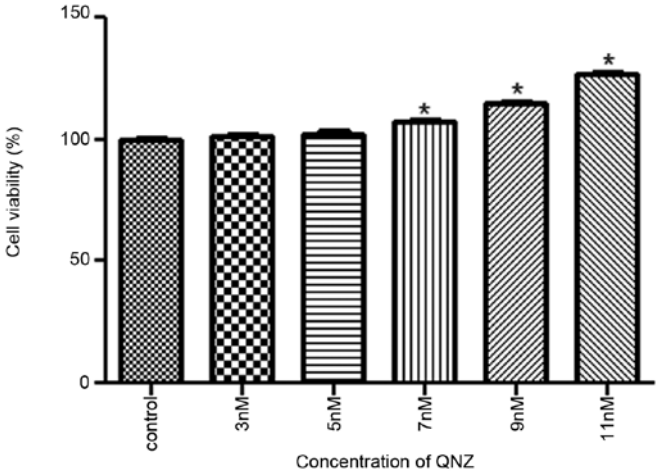

Figure 1. Cell viability of PC12 cells treated with different concentrations of NF-κB inhibitor QNZ. *P<0.05 vs. Control group. QNZ, quinazoline; NF-κB, nuclear factor-κB.

the control and si-NC groups (P>0.05; Fig. 2A). Additionally, the expression of *NF-κB* in the si-NF-κB-1, si-NF-κB-2 and si-NF-κB-3 groups was significantly decreased when compared with that in the control group (P<0.05; Fig. 2A). However, the expression of *NF-κB* in the si-NF-κB-2 group was significantly lower than that in the si-NF-κB-1 and si-NF-κB-3 groups (P<0.05; Fig. 2A). In addition, the expression trend of NF-κB determined by western blotting was similar with that measured using RT-qPCR (Fig. 2B). Based on these findings, si-NF-κB-2 was selected to construct *NF-κB*-knockdown PC12 cells for subsequent experiments.

Cell viability analysis. To investigate the effects of *NF-κB* on inflammatory PC12 cells, LPS was used to induce cellular inflammation, and the CCK-8 assay was used to measure cell viability. Compared with the control group, the cell viability of the QNZ group was significantly increased (P<0.05; Fig. 3), indicating that QNZ could enhance the viability of PC12 cells. After LPS treatment, cell viability was significantly inhibited when compared with the control group (P<0.05; Fig. 3). No significant difference in cell viability was observed between the LPS and LPS + si-NC groups. However, cell viabilities in the LPS + QNZ and LPS + si-NF-κB groups were significantly increased when compared with that in the LPS group (P<0.05; Fig. 3), and the effects of QNZ and *NF-κB* knockdown were similar (P>0.05). These results suggested that *NF-κB* could affect the cell viability of LPS-induced PC12 cells.

Levels of IL-1β, IL-6 and TNF-α in the PC12 cells. To further clarify the effects of *NF-κB* on inflammatory factors, the expression levels of IL-1β, IL-6 and TNF-α were measured in PC12 cells. After LPS induction, IL-1β expression was significantly upregulated when compared with that in the control group (P<0.05; Fig. 4A). No significant difference was observed in IL-1β levels between the LPS and LPS + si-NC groups (P>0.05; Fig. 4A). However, IL-1β expression in the LPS + QNZ and LPS + si-NF-κB groups were 88.39±8.86 and 101.75±4.65 pg/ml, respectively, which were significantly reduced when compared with those in the LPS group (151.67±3.02 pg/ml, P<0.05; Fig. 4A). For IL-6 and TNF-α,

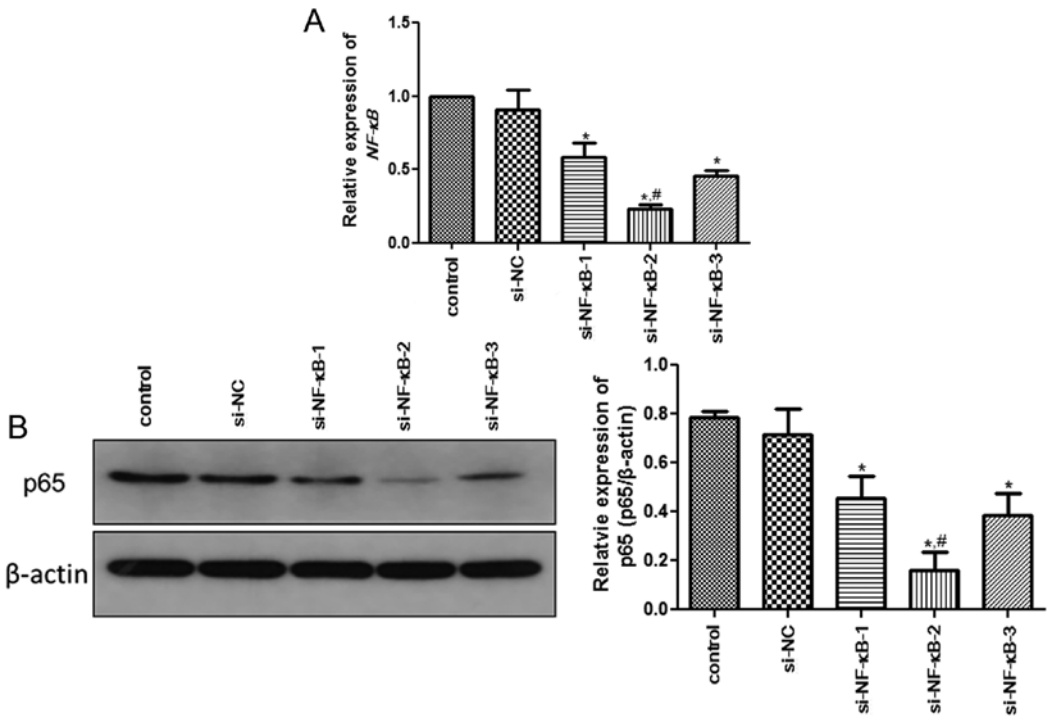

Figure 2. Cell transfection efficiency analysis evaluated by RT-qPCR and western blotting. (A) mRNA and (B) protein expression level of NF-κB in different groups measured by RT-qPCR and western blotting, respectively. *P<0.05 vs. Control group; #P<0.05 vs. si-NF-κB-1 group. RT-qPCR, reverse transcription-quantitative PCR; NF-κB, nuclear factor-κB; si-, small interfering RNA; NC, negative control.

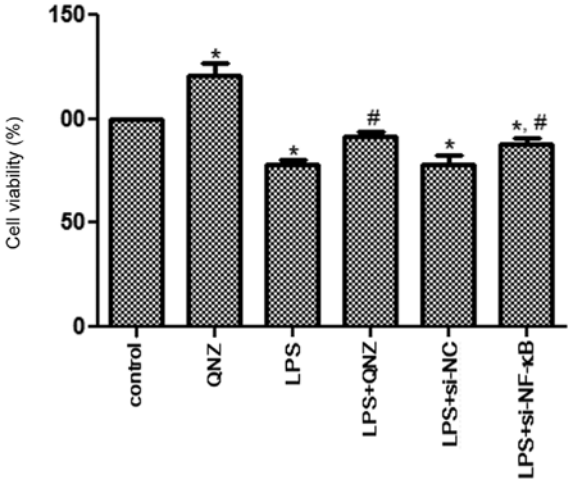

Figure 3. Effects of NF-κB on cell viability of PC12 cells in different groups determined by Cell Counting Kit-8 assay. *P<0.05 vs. Control group; #P<0.05 vs. LPS group. NF-κB, nuclear factor-κB; si-, small interfering RNA; NC, negative control; LPS, lipopolysaccharide; QNZ, quinazoline.

the expression trends were similar to those of IL-1β (Fig. 4B and C). Of note, TNF-α expression in the QNZ group was significantly lower than that in the control group (P<0.05; Fig. 4C), indicating that QNZ may inhibit the generation of TNF-α.

Immunofluorescence analysis. Immunofluorescence was used to examine the relative expression levels of TH, A-SYN and Nurr1. The expression levels of TH and Nurr1 were significantly increased in the QNZ group when compared with the control group (P<0.05), but were significantly decreased in the LPS group (P<0.05; Fig. 5A and C). On pretreating PC12 cells with QNZ and si-NF-κB, the expression levels of TH and Nurr1 in LPS + QNZ and LPS + si-NF-κB groups were significantly increased compared with the LPS group (P<0.05), and QNZ and si-NF-κB restored TH and Nurr1 expression levels to a level similar to that in the control group (P<0.05; Fig. 5A and C). However, the trend of A-SYN expression was the opposite to that of TH and Nurr1 expression levels (Fig. 5B). Compared with the control group, A-SYN expression was significantly reduced in the QNZ group (P<0.05), but significantly elevated in the LPS and LPS + si-NC groups (P<0.05; Fig. 5B). In the LPS + QNZ and LPS + si-NF-κB groups, A-SYN expression levels were significantly lower than that in the LPS group (P<0.05), and were restored to a level similar to that in the control group (P<0.05; Fig. 5B). These results suggested that *NF-κB* could alleviate LPS-induced inflammation in PC12 cells by regulating the expression of TH, A-SYN and Nurr1.

Western blot analysis. The protein expression levels of p65, TH, A-SYN and Nurr1 were detected by western blotting. Protein expression levels of p65 and A-SYN were significantly increased in the LPS group when compared with the control group (P<0.05), whereas p65 and A-SYN expression levels in the LPS + QNZ and LPS + si-NF-κB were significantly reduced (P<0.05; Fig. 6A, B and D). The protein expression levels of TH and Nurr1 were significantly downregulated in the LPS group when compared with the control group; however, these expression levels were upregulated following treatment with QNZ and si-NF-κB (P<0.05; Fig. 6A, C and E).

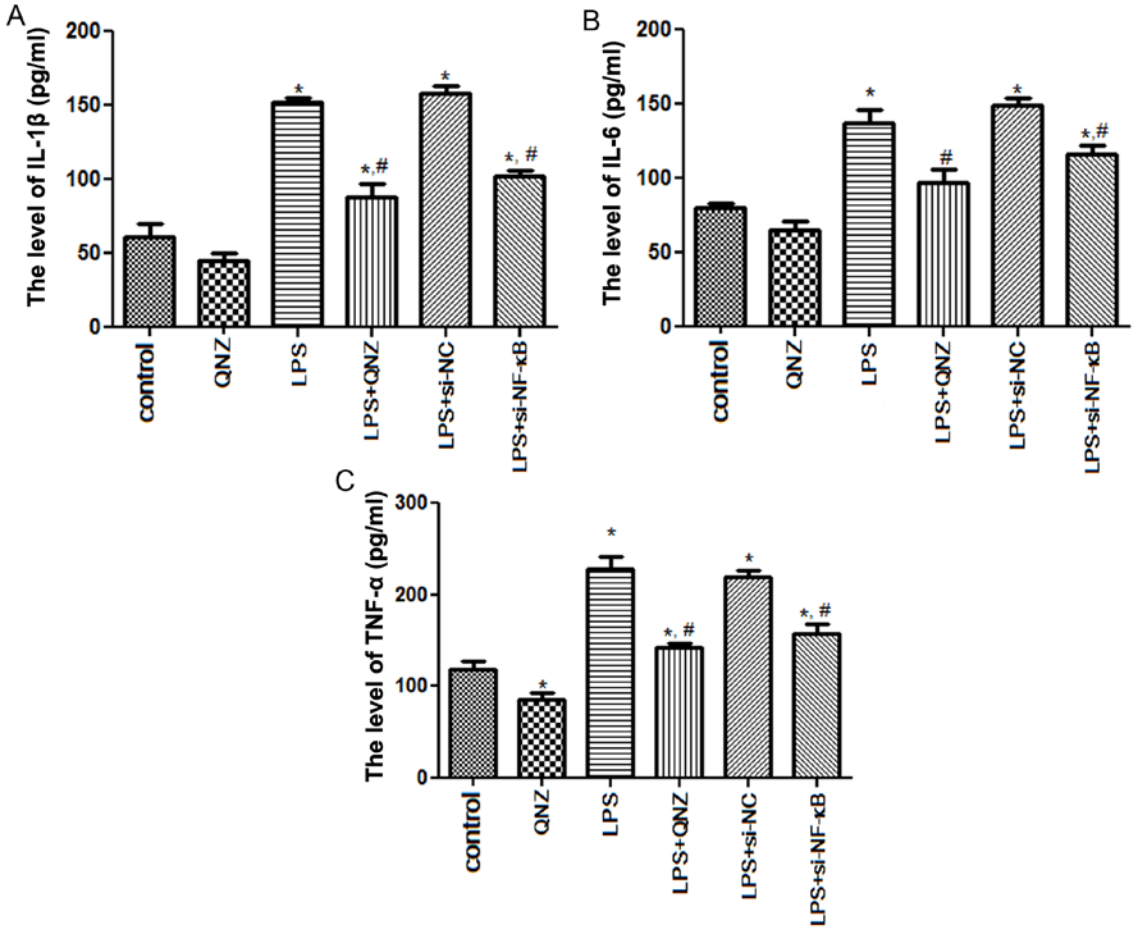

Figure 4. Effects of NF-κB on the related inflammatory cytokines in PC12 cells. Expression levels of (A) IL-1β, (B) IL-6 and (C) TNF-α in PC12 cells following different treatments, as determined via enzyme-linked immunosorbent assay kits. *P<0.05 vs. Control group; #P<0.05 vs. LPS group. IL-1β, interleukin-1β; IL-6, interleukin-6; TNF-α, tumor necrosis factor-α; NF-κB, nuclear factor-κB; si-, small interfering RNA; NC, negative control; LPS, lipopolysaccharide; QNZ, quinazoline.

Discussion

PD is one of the most disabling diseases of the central nervous system, seriously impacting health and day-to-day living in the elderly. A previous study reported the interaction between *NF-κB* and *Nurr1* (14). In the present study, LPS was administered to PC12 cells to construct a PD cellular model, followed by the pretreatment of these cells with an NF-κB inhibitor, QNZ, and si-NF-κB. Based on the CCK-8 assay results, the cell viability of PC12 cells was significantly reduced after LPS induction, whereas pretreatment with QNZ and si-NF-κB significantly enhanced their viability following LPS induction. Based on ELISA, the levels of IL-1β, IL-6 and TNF-α were significantly higher in the inflammatory PC12 cells, and QNZ treatment and NF-κB interference restored these levels, relieving cellular inflammation. Furthermore, immunofluorescence and western blotting revealed that in the LPS group, p65 expression was higher, while Nurr1 expression was lower. On pretreating cells with QNZ and si-NF-κB, p65 expression was downregulated, and Nurr1 expression was upregulated, indicating that p65 may be negatively associated with Nurr1. In terms of TH and A-SYN expression levels, LPS enhanced the expression of A-SYN and suppressed the expression of TH. However, QNZ and NF-κB interference reversed the LPS-induced expression levels of TH and A-SYN.

Previous studies have revealed that neuroinflammation is the primary pathological mechanism of PD and the main target for PD treatment (22,23). It has been reported that *NF-κB*, a transcription factor that regulates the production of pro-inflammatory cytokines, promotes an enhanced inflammatory response, participating in the physiological and pathological processes of several diseases (24,25). IL-1β, IL-6 and TNF-α are pro-inflammatory factors associated with inflammation. In the current study, the levels of IL-1β, IL-6 and TNF-α were increased in LPS-induced PC12 cells, whereas their levels were decreased following NF-κB interference and QNZ treatment. Wei and Shao (26) revealed that nobiletin significantly inhibited the levels of IL-6, IL-1β, TNF-α, matrix metalloproteinase-1 (MMP-1) and MMP-3 in the ectopic endometrium by downregulating NF-κB activity, thus increasing protection against endometriosis. Additionally, another study has shown that pannexin 3 inhibits the inflammatory response induced by TNF-α by suppressing the NF-κB signaling pathway in dental pulp inflammation (27). Based on the aforementioned findings, the present study speculated that activation of NF-κB may be related to the inflammatory response observed in PD, and NF-κB interference may restrict

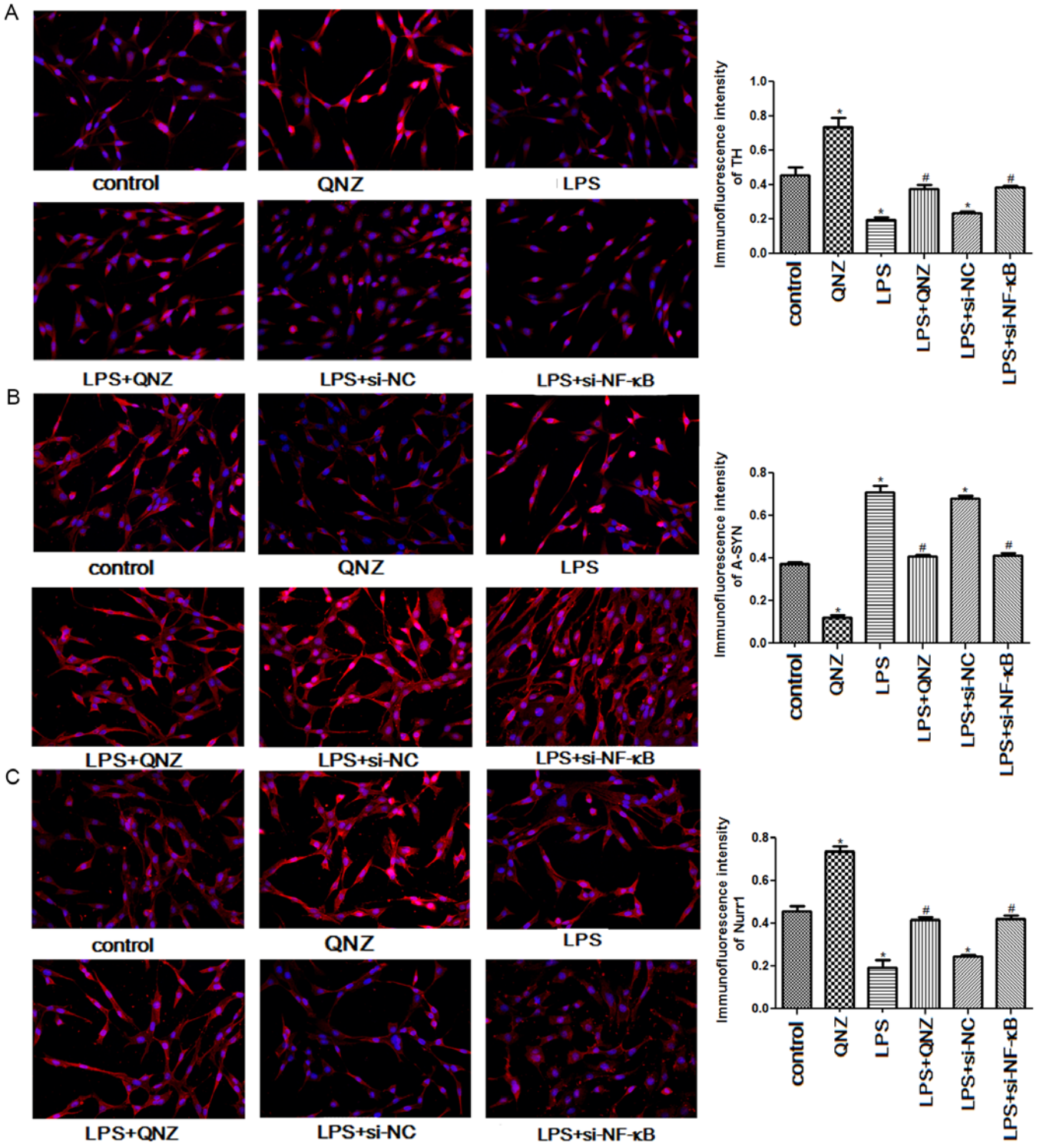

Figure 5. Effects of NF-κB on the expressions of TH, A-SYN, and Nurr1 examined using immunofluorescence at x400 magnification. Relative expression levels of (A) TH, (B) A-SYN and (C) Nurr1. *P<0.05 vs. Control group; #P<0.05 vs. LPS group. TH, tyrosine hydroxylase; A-SYN, α-Synuclein; Nurr1, nuclear receptor subfamily 4 group A member 2; NF-κB, nuclear factor-κB; si-, small interfering RNA; NC, negative control; LPS, lipopolysaccharide; QNZ, quinazoline.

the generation of pro-inflammatory cytokines, including IL-1β, IL-6 and TNF-α, thus downregulating the neuroinflammatory response in PD.

To further elucidate the potential molecular mechanisms of *NF-κB* in PD, immunofluorescence and western blotting were performed. In the present study, LPS enhanced the expression of A-SYN and suppressed the expression of TH, whereas QNZ and NF-κB interference recovered their expression. TH, an enzyme known to catalyze the formation of L-dihydroxyphenylalanine, is a key enzyme of immune reactivity in PD models and is also used to evaluate the efficacy of novel therapeutic agents (28). A-SYN, a rich neuronal protein, is highly abundant in presynaptic nerve terminals and is deemed a neuropathological feature of PD (29). A previous

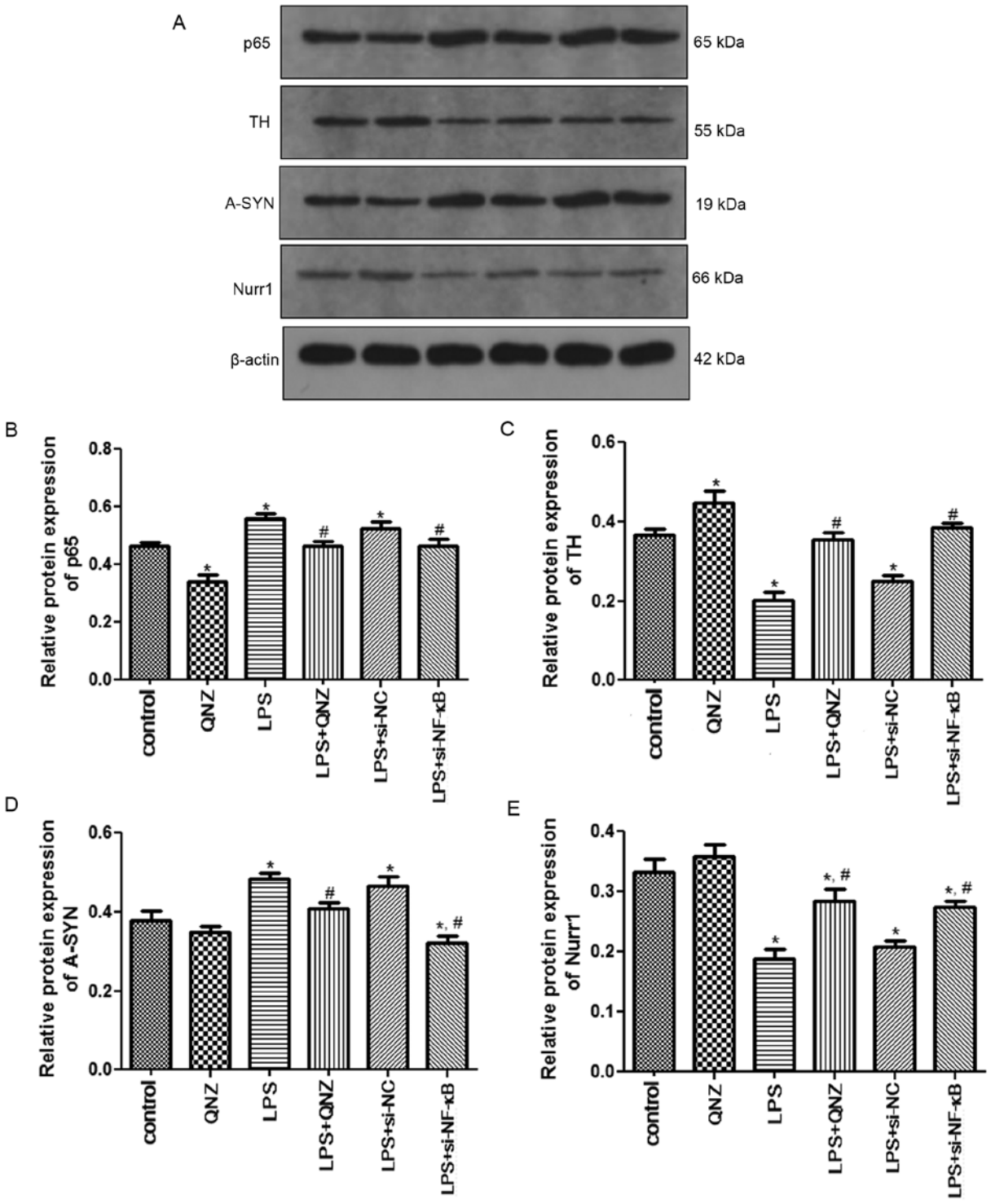

Figure 6. Protein expression levels of p65, TH, A-SYN and Nurr1 determined by western blotting. (A) Representative western blotting images of p65, TH, A-SYN and Nurr1 expression. Semi-quantification of (B) p65, (C) TH, (D) A-SYN and (E) Nurr1 expression. *P<0.05 vs. Control group; #P<0.05 vs. LPS group. TH, tyrosine hydroxylase; A-SYN, α-Synuclein; Nurr1, nuclear receptor subfamily 4 group A member 2; NF-κB, nuclear factor-κB; si-, small interfering RNA; NC, negative control; LPS, lipopolysaccharide; QNZ, quinazoline.

study by Ma *et al* (30) demonstrated that electroacupuncture intervention improved motor function in a PD rat model by upregulating the expression of TH and downregulating the expression of A-SYN. Mani *et al* (31) reported that naringenin exerts a protective role in PD by significantly inhibiting the expression of A-SYN and enhancing TH expression, as well as by mediating the inflammatory response and oxidative stress. Combined with the present results, we assumed that downregulation of NF-κB may have the same effects as those of QNZ and may alleviate the symptoms of PD by regulating the expression of TH and A-SYN.

Moreover, a recent study demonstrated that Nurr1 can inhibit TNF-α production by interacting with NF-κB/p65 and inhibiting its nuclear translocation (32). In the present study,

the results showed that in the LPS group, the expression of p65 was higher, whereas the expression of Nurr1 was lower; however, following pretreatment with QNZ and si-NF-κB, p65 expression was downregulated and Nurr1 expression was upregulated. Nurr1 reportedly plays a crucial role in the development, differentiation and maintenance of dopaminergic neurons (33). Saijo *et al* (34) reported that Nurr1 interacts with NF-κB/p65 promoters and subsequently recruits the REST corepressor complex, thereby resulting in the clearance of NF-κB/p65 and transcriptional repression. Based on the present findings, it was speculated that NF-κB may be negatively associated with Nurr1, and inhibition of NF-κB activation and enhancement of Nurr1 expression may contribute to reduced inflammation in PD.

In conclusion, NF-κB may be negatively associated with Nurr1. Additionally, inhibition of NF-κB reduced the production of inflammatory factors (IL-1β, IL-6 and TNF-α) by upregulating the expression of Nurr1 and TH and downregulating A-SYN expression, thus ameliorating the inflammatory status of PD. These findings may contribute to our understanding of the progression of PD and provide therapeutic targets for the prevention and amelioration of PD.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author upon reasonable request.

Authors' contributions

XY, HG and DW conceived and designed the research. HG and JM was responsible for data acquisition. HG and DW analyzed and interpreted data. SJ performed statistical analysis. HG wrote the original draft preparation. XY, HG and DW reviewed and edited the manuscript. XY supervised the study. HG, DW and XY confirm the authenticity of all the raw data. All authors have read and approved the final version of the manuscript.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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