The PI3K/Akt/FOXO3a pathway regulates regeneration following spinal cord injury in adult rats through TNF-α and p27^{kip1} expression

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Abstract. The aim of the present study was to elucidate the expression and role of the phosphatidylinositol 3-kinase (PI3K)/Akt/forkhead box O3 (FOXO3a) pathway in the regeneration of the spinal cord following spinal cord injury (SCI), and its regulatory effect on tumor necrosis factor (TNF)-α and cyclin-dependent kinase inhibitor 1B (p27^{kip1}) expression. Firstly, in a Sprague-Dawley rat model of SCI, western blot analysis revealed that the protein levels of PI3K, phosphorylated Akt and FOXO3a were markedly inhibited compared with those in the sham control group. *In vitro* experiments were also conducted, in which primary dissociated cultures of rat dorsal spinal cord cells were induced with lipopolysaccharide (LPS; 4 μg/ml). The downregulation of PI3K using LY294002 markedly suppressed cell viability, reduced the protein levels of FOXO3a and p27^{kip1}, and increased TNF-α protein production in the LPS-induced spinal cord cells. In addition, when the LPS-induced spinal cord cells were infected with FOXO3a adenoviral vectors, the overexpression of FOXO3 markedly promoted cell proliferation, activated p27^{kip1} protein levels and inhibited TNF-α protein production in the spinal cord cells. These results suggest that the PI3K/Akt/FOXO3a pathway regulates regeneration following SCI in adult rats via its modulatory effects on TNF-α and p27^{kip1} expression.

Introduction

Spinal cord injury (SCI) is a serious trauma of the nervous system (1). It may cause paralysis and sphincter disturbance, resulting in patients becoming bedridden long-term, and may even be life-threatening, which is a heavy burden to society and the patient's family. The morbidity of SCI is ~20-40 per million population, and there are predicted to be ~2,500,000 individuals affected by SCI, with an annual growth in incidence of >100,000 (2). The expense of nursing and treatment is very high due to the lack of effective treatments and rehabilitation measures (3). The morbidity of SCI in Beijing in 2002 was reported to be 50 per million population, which is much higher than the mean morbidity worldwide (4). The frequency of earthquakes, mining, construction and traffic accidents in China is high, which is likely to result in a higher morbidity compared with that in other countries. Due to the consistent and rapid development of the economy in China, SCI has become a clear threat to health and quality of life.

The phosphatidylinositol 3-kinase (PI3K)/Akt signal transduction pathway affects cell proliferation, differentiation, migration and apoptosis (5). Recent studies have demonstrated that this pathway is associated with a variety of cerebral injuries (5,6). The PI3K/Akt pathway has been observed to have a regulatory effect of the α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptor in the central nervous system (6). In the optic nerve cells and cerebellar tracts of mice, it has been observed that oligodendrocytes are dynamically regulated through the PI3K/Akt pathway and AMPA receptor-mediated Ca^{2+}-signaling (7).

Fibroblast growth factor 2 (FGF-2) is a multi-functional growth factor that is extensively distributed in various tissues of the body and promotes cell proliferation, differentiation, migration and angiogenesis (8). FGF-2 has been demonstrated to be an important endogenous cardioprotective protein (9). Previous studies have indicated that the protective effects of exogenous FGF-2 are associated with a signal transduction pathway comprising fibroblast growth factor receptor 1 (FGFR1), activated protein kinase C (PKC) and mitogen-activated protein kinase (MAPK) (10). Activation of the FGFR1 tyrosine kinase induces activation of the phospholipase C-PKC, Ras-MAPK and PI3K/Akt signaling pathways; the PI3K/Akt pathway is independent of PKC, PKA and MAPK (11). It participates in the regulation of the physiological or pathological processes of nerve cells (12). In addition, it serves a crucial role in cell proliferation,

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differentiation and apoptosis (12). Studies have shown that in nerve cells, retinal photoreceptor cells and human umbilical vein endothelial cells, FGF-2 antagonizes the cell apoptosis induced by oxidative stress through activation of the PI3K/Akt signaling pathway. Forkhead box O3 (FOXO3a) has been demonstrated to be an essential transcription factor downstream of the PI3K/Akt signal pathway (11,12). It is vital in the antagonism of oxidative stress-induced apoptosis (13). The aim of the present study was to investigate the expression and role of PI3K/Akt/ FOXO3a in the regeneration of the spinal cord following SCI in adult rats, and explore the underlying mechanism.

Materials and methods

SCI surgery. All surgical interventions were carried out in accordance with the Guide for the Care and Use of Laboratory Animals of PLA General Hospital and were approved by the ethics committee of Chinese PLA General Hospital (Beijing, China). Male Sprague-Dawley rats (n=24; age, 6-7 weeks, weight, 200-230 g) were purchased from Beijing Vital River Laboratory Animal Technology Co., Ltd. and housed at 22-23˚C, 55-60% humidity, under a light/dark cycle (8:00-20:00), with free access to food and water. The rats were deeply anesthetized with 350 mg/kg chloral hydrate intraperitoneally. All rats were randomly assigned to control, SCI-1 day, SCI-2 day and SCI-3 day (n=6 rat/group) and 18 rats underwent SCI surgery. Dorsal laminectomy at the level of the ninth thoracic vertebra was conducted and the spinal cord was contused by dropping a rod 2.0 mm in diameter. Following this, the overlying muscles and skin were closed in layers with 4-0 silk sutures and staples.

Hematoxylin and eosin (H&E) staining. The experimental rats (n=6) were sacrificed on days 1, 2 and 3 following SCI, and spinal cord tissue was extracted and fixed in 10% neutral formalin for 3 days. The spinal cord tissue was sliced into 1-cm sections, which were dehydrated using a graded alcohol series. The tissue samples were then sliced into 20-μm longitudinal sections. The sections were stained with hematoxylin for 5 min, then washed with water for 10 min, treated with ethanol for 10 sec to remove excess stain, and washed with water for 10 min. Finally, they were stained with eosin for 5 min and washed with water for 10 min. The stained sections were dehydrated through a graded alcohol series, permeabilized with xylene, and mounted with neutral resin. Imaging was performed using a confocal microscope (LSM 700: Zeiss AG, Oberkochen, Germany) with ImageJ 3.0 software (National Institutes of Health, Bethesda, MD, USA).

Western blotting. Spinal cord tissue samples and cultured cells were each homogenized in RIPA assay (Beyotime Institute of Biotechnology, Jiangsu, China) and then centrifuged at 10,000 x g for 20 min at 4˚C. The protein content of the supernatant was measured using a bicinchoninic acid assay (Beyotime Institute of Biotechnology). Samples containing 100 μg protein were subjected to 10-12% sodium dodecyl sulfate-polyacrylamide gel electrophoresis and then transferred to a polyvinylidene difluoride membrane (EMD Millipore, Billerica, MA, USA). The membrane was blocked in TBS with Tween 20 with 5% non-fat milk for 1 h at 37˚C prior to incubation with the primary antibodies anti-PI3K (sc-7174; 1:500), anti-p-Akt (sc-7985-R; 1:2,000), anti-FOXO3a (12829; 1:3,000), anti-cyclin-dependent kinase inhibitor 1B ($p27^{kip1}$; sc-756; 1:2,000) and anti-β-actin (sc-10731; 1:5,000) (all from Santa Cruz Biotechnology, Inc., Dallas, TX, USA) at 37˚C for 1 h. The membrane was then incubated with the appropriate horseradish peroxidase-conjugated secondary antibody (sc-2004; 1:5,000; Santa Cruz Biotechnology, Inc.) at 37˚C for 1 h and visualized using an enhanced chemiluminescence system (Pierce; Thermo Fisher Scientific, Inc., Waltham, MA, USA) and quantified using ImageJ 6.0 software.

Enzyme-linked immunosorbent assay. Tumor necrosis factor (TNF)-α was quantified in Sprague-Dawley rat cell cultures using a mouse TNF-α Enzyme-Linked Immunosorbent assay kit (RTA00; R&D Systems, Inc., Minneapolis, MN, USA) according to the manufacturer's protocol. The lower limit of detection of the kit was <20 pg/ml.

Spinal cord cell culture and downregulation of PI3K. Primary dissociated cell cultures were prepared from the dorsal spinal cords of Sprague-Dawley rats. Collagenase 0.25% (Sigma-Aldrich; Merck KGaA, Darmstadt, Germany) was used to digest the spinal cords for 10-20 min, and the obtained cells were then cultured with Dulbecco's modified Eagle's medium (DMEM; Sigma-Aldrich; Merck KGaA) containing 5% fetal bovine serum for 1 week. The spinal cord cells were stimulated with lipopolysaccharide (LPS; 4 μg/ml; Beyotime Institute of Biotechnology) for 2 h without PI3K inhibitor (with negative plasmid), and then treated with 10 μmol/l PI3K inhibitor (LY294002; Beyotime Institute of Biotechnology) for 1, 2 and 3 days.

Measurement of the regeneration of spinal cord cells. In the LPS-challenged cells following 1, 2 and 3 days of treatment with LY294002, the effect of PI3K inhibition on the viability of the spinal cord cells was determined using a colorimetric 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. In this assay, 50 μl MTT solution (1 mg/ml final concentration) was added to the cells in each well of a 96-well plate and the plate was incubated for 4 h at 37˚C. The formazan was removed by the addition of 100 μl dimethylsulfoxide and the optical density was measured with a microplate reader (Multiskan; Labsystems Diagnostics Oy, Helsinki, Finland) at 540 nm.

Overexpression of FOXO3a using an adenoviral vectors. A FOXO3a adenoviral vector was designed by and purchased from Takara Biotechnology Co., Ltd. (Dalian, China). The primers of the FOXO3a adenoviral vector were: Sense, 5'-CCC GGTGCGTGCCTATCAGGGGC-3' and antisense, 5'-CCG ACTTCTCGTCCCCTCG-3'. Spinal cord cells induced by LPS (4 μg/ml) for 2 h were infected with FOXO3a adenoviral vectors (100 nM) in DMEM/F12 for 8 h. The medium was replaced with fresh complete culture medium prior to subsequent analysis.

Statistical analysis. Results are presented as the mean ± standard error of the mean using SPSS 17.0 (experiments were repeated in triplicate). One-way analysis of variance followed

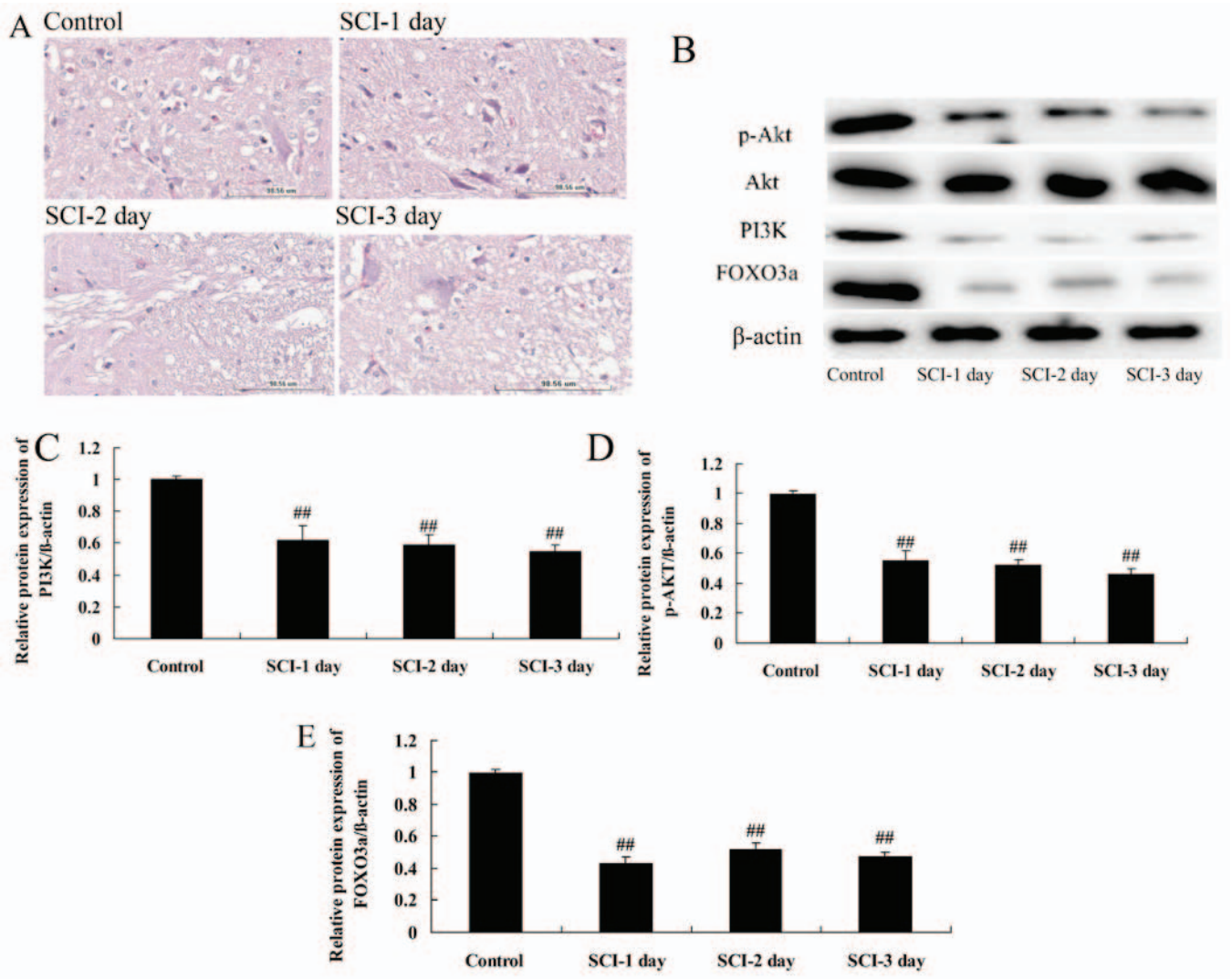

Figure 1. Spinal cord histology and the expression of PI3K, Akt, p-Akt and FOXO3a proteins in a rat model of SCI. (A) Hematoxylin and eosin-stained spinal cord tissue 1, 2 and 3 days following SCI compared with the control. Scale bar, 98.56 μm. (B) Western blots of PI3K, Akt, p-Akt and FOXO3a and quantitative analysis of (C) PI3K, (D) p-Akt and (E) FOXO3a protein expression levels in the SCI model rats. $^{\#\#}$P<0.01 vs. the control group. PI3K, phosphatidylinositol 3-kinase; p, phosphorylated; FOXO3a, forkhead box O3; SCI, spinal cord injury.

by Turkey's post hoc multiple comparison tests were used for statistical analysis. P<0.05 was considered to indicate a statistically significant difference.

Results

Histological examination of the spinal cord and the expression of PI3K and Akt proteins in a rat model of SCI. H&E staining of the spinal cord tissue (Fig. 1A) revealed a complete and clear structure in the control group, with no cavities and densely arranged nerve fibers. However, the nerve fibers were loosely arranged and appeared shorter and less numerous at 1, 2 and 3 days following SCI compared with those in the control group.

To investigate the involvement of the PI3K/Akt signaling pathway in the tissue damage associated with SCI, the PI3K and p-Akt protein levels in the spinal cord tissue were determined in the rats following SCI using western blotting. The protein levels of PI3K and p-Akt were significantly suppressed in the rats 1, 2 and 3 days following SCI compared with the respective expression levels in the control group (Fig. 1B-D). However, Akt protein expression was not changes in the rats 1, 2 and 3 days following SCI compared with the respective expression levels in the control group (Fig. 1B-D). This suggests that the PI3K/Akt signaling pathway is inhibited following SCI.

FOXO3a protein levels in a rat model of SCI. Whether FOXO3a participates in the PI3K/Akt signaling pathway in the rat model of SCI was investigated by measuring the protein levels of FOXO3a using western blotting. The FOXO3a protein levels at 1, 2 and 3 days following SCI were significantly lower compared with those in the control group (Fig. 1B and E). This suggests that the PI3K/Akt/FOXO3a signaling pathway was inhibited following SCI in the rat model.

PI3K inhibition reduces PI3K and p-Akt protein levels in spinal cord cells in vitro. The effects of a PI3K inhibitor on the levels of various proteins in LPS-induced spinal cord cells were investigated using western blotting (Fig. 2). The results demonstrated

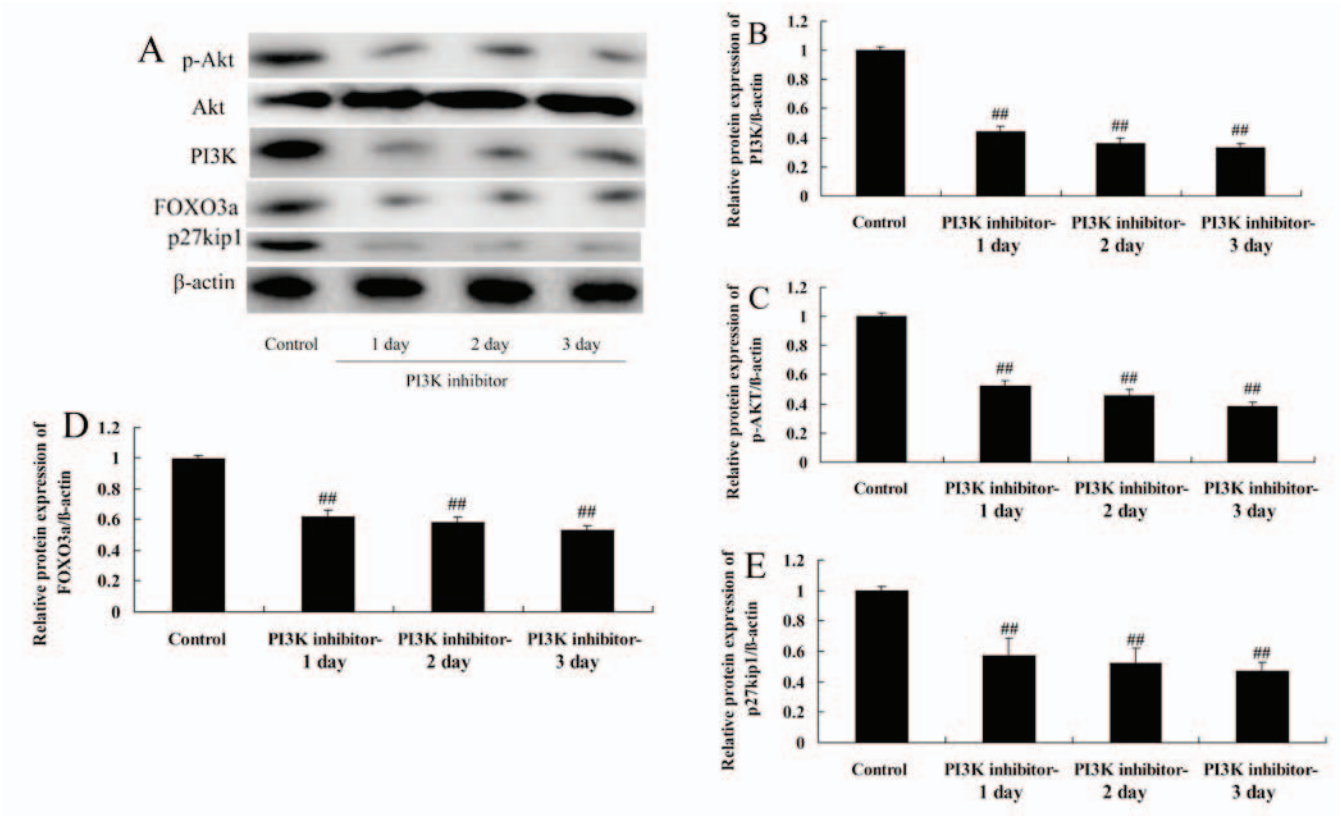

Figure 2. Effect of PI3K inhibition on PI3K, Akt, p-Akt, FOXO3a and $p27^{kip1}$ protein levels in spinal cord cells. Control cells were treated with LPS (4 μg/ml) for 2 h, and cells in the PI3K inhibitor-1, 2 and 3 day groups were treated with LPS (4 μg/ml) for 2 h and then with 10 μmol/l LY294002 for 1, 2 and 3 days. (A) Western blotting of PI3K, Akt, p-Akt, FOXO3a and $p27^{kip1}$, and quantitative analysis of (B) PI3K, (C) p-Akt, (D) FOXO3a and (E) $p27^{kip1}$ protein levels. $^{##}$P<0.01 vs. the control group. PI3K, phosphatidylinositol 3-kinase; p, phosphorylated; FOXO3a, forkhead box O3; $p27^{kip1}$, anti-cyclin-dependent kinase inhibitor 1B; LPS, lipopolysaccharide.

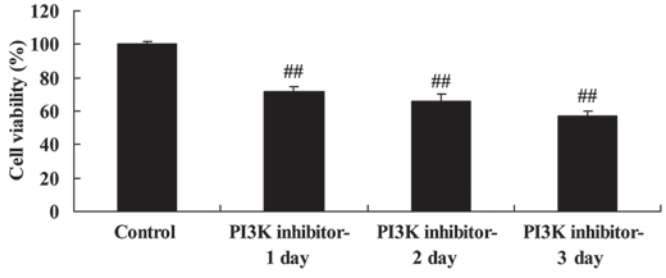

Figure 3. Inhibition of PI3K reduces the viability of LPS-induced spinal cord cells. $^{##}$P<0.01 vs. the control group. PI3K, phosphatidylinositol 3-kinase; LPS, lipopolysaccharide.

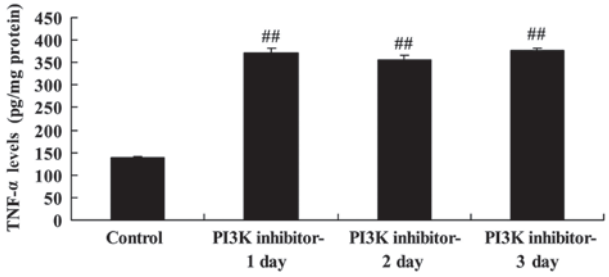

Figure 4. Inhibition of PI3K increases the TNF-α levels of LPS-induced spinal cord cells. $^{##}$P<0.01 vs. the control group. PI3K, phosphatidylinositol 3-kinase; TNF-α, tumor necrosis factor-α; LPS, lipopolysaccharide.

that the protein levels of PI3K and p-Akt were significantly suppressed by treatment with a PI3K inhibitor for 1, 2 or 3 days compared with those in the control group (Fig. 2A-C), confirming that the PI3K/Akt pathway was downregulated.

Downregulation of PI3K inhibits the regeneration of spinal cord cells. The effect of PI3K/Akt pathway inhibition on the viability of the LPS-induced spinal cord cells was investigated using an MTT assay. The PI3K inhibitor significantly inhibited the viability of the LPS-induced spinal cord cells compared with that of the control group following 1, 2 and 3 days of treatment (Fig. 3). This suggests that the downregulation of PI3K reduced cell viability in this *in vitro* SCI model.

Downregulation of PI3K reduces FOXO3a protein levels in spinal cord cells. To further investigate the role of inhibition of the PI3K/Akt pathway in LPS-induced spinal cord cell damage, FOXO3a protein levels were determined in the LPS-induced spinal cord cells using western blotting. The inhibition of PI3K significantly reduced FOXO3a protein levels in the LPS-induced spinal cord cells, compared with those in the control group (Fig. 2A and D). This suggests that FOXO3a may participate in the PI3K/Akt signaling pathway in SCI.

Downregulation of PI3K increases tumor necrosis factor (TNF)-α levels in spinal cord cells. The effect of PI3K inhibition on TNF-α activity was evaluated to assess its involvement

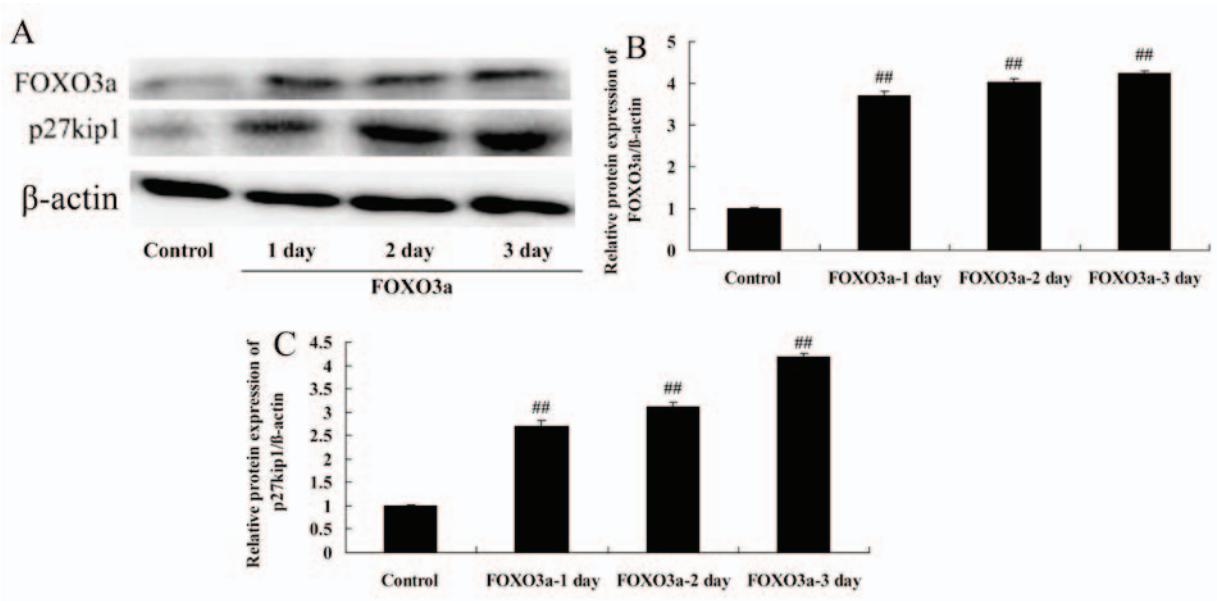

Figure 5. Effect of FOXO3a overexpression on FOXO3a and $p27^{kip1}$ proteins in LPS-induced spinal cord cells. (A) Western blotting of FOXO3a and $p27^{kip1}$, and quantitative analysis of (B) FOXO3a and (C) $p27^{kip1}$ protein expression. $^{##}P<0.01$ vs. the control group. FOXO3a, forkhead box O3; $p27^{kip1}$, anti-cyclin-dependent kinase inhibitor 1B; LPS, lipopolysaccharide.

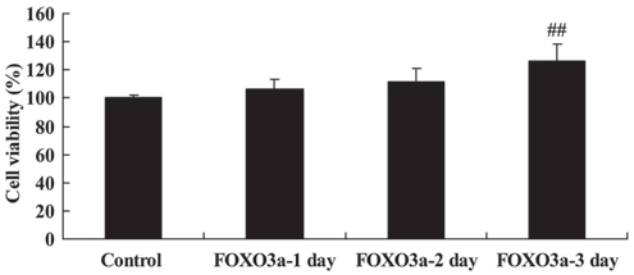

Figure 6. Effect of FOXO3a overexpression on the viability of LPS-induced spinal cord cells. Control cells were treated with LPS (4 μg/ml) for 2 h and then transfected with negative plasmid, and cells in the FOXO3a-1, 2 and 3 day groups were treated with LPS (4 μg/ml) for 2 h and then transfected with FOXO3a plasmid for 1, 2 and 3 days. $^{##}P<0.01$ vs. the control group. FOXO3a, forkhead box O3; LPS, lipopolysaccharide.

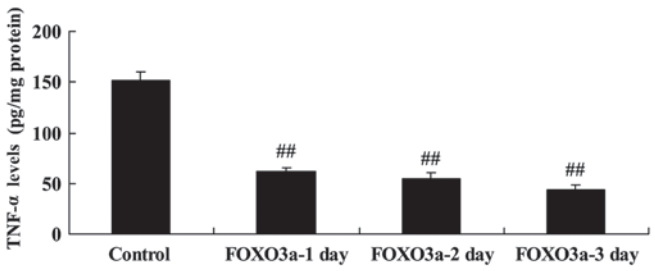

Figure 7. Overexpression of FOXO3a reduces the TNF-α protein levels of spinal cord cells. $^{##}P<0.01$ vs. the control group. FOXO3a, forkhead box O3; TNF-α, tumor necrosis factor-α.

in the pathogenic mechanism of SCI. TNF-α levels in the LPS-induced spinal cord cells were significantly increased in the PI3K inhibitor group on days 1, 2 and 3 compared with those in the control group (Fig. 4). This indicates that the PI3K/Akt signaling pathway affected TNF-α levels in this *in vitro* SCI model, and may have inflammatory effects.

Inhibition of PI3K reduces $p27^{kip1}$ protein levels in spinal cord cells. The effect of PI3K inhibition on the $p27^{kip1}$ protein levels of LPS-induced spinal cord cells was explored using western blotting. In the cells, a significant reduction in $p27^{kip1}$ protein levels was observed on days 1, 2 and 3 in the cells treated with PI3K inhibitor compared with the control group (Fig. 2A and E). This demonstrates that the PI3K/Akt signaling pathway affected $p27^{kip1}$ expression levels in this *in vitro* SCI model.

Overexpression of FOXO3a improves the regeneration of spinal cord cells. The mechanism of PI3K/Akt signaling in the progression of SCI was further examined via the overexpression of FOXO3a. The effects of FOXO3a overexpression on the levels of various proteins in LPS-induced spinal cells were evaluated using western blotting (Fig. 5). The results confirmed that transfection with a FOXO3a vector significantly increased the FOXO3a protein levels in the LPS-induced spinal cord cells compared with those in the control group (Fig. 5A and B). Furthermore, the overexpression of FOXO3a significantly increased the viability of the LPS-induced spinal cord cells compared with that of the control group at 3 days after transfection (Fig. 6). This indicates that FOXO3a has a potential role in the regulation of SCI progression.

Overexpression of FOXO3a reduces TNF-α levels in spinal cord cells. The effect of FOXO3a overexpression on TNF-α levels in LPS-induced spinal cord cells was evaluated. The overexpression of FOXO3a significantly reduced TNF-α levels in the LPS-induced spinal cord cells on days 1, 2 and 3 compared with those in the control group (Fig. 7). These results suggest that the PI3K/Akt/FOXO3a signaling pathway affects TNF-α levels in SCI.

Overexpression of FOXO3a increases the $p27^{kip1}$ protein levels of spinal cord cells. The effect of the overexpression of FOXO3a on $p27^{kip1}$ protein levels in LPS-induced

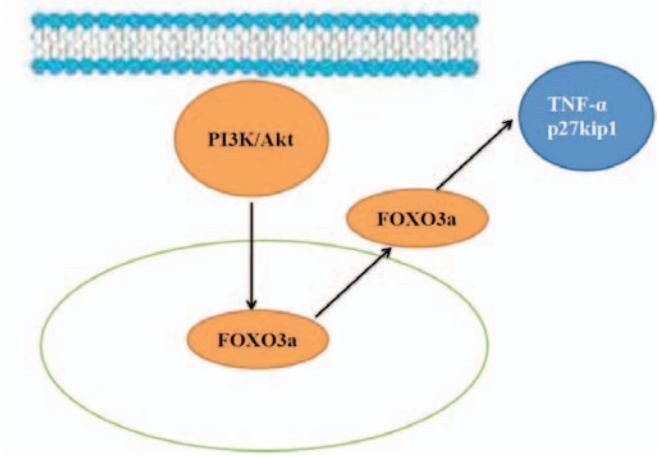

Figure 8. The PI3K/Akt/FOXO3a pathway regulates regeneration following spinal cord injury in adult rats through TNF-α and $p27^{kip1}$ expression. PI3K, phosphatidylinositol 3-kinase; FOXO3a, forkhead box O3; TNF-α, tumor necrosis factor-α; $p27^{kip1}$, anti-cyclin-dependent kinase inhibitor 1B.

spinal cord cells was investigated. The overexpression of FOXO3a significantly increased the $p27^{kip1}$ protein levels of the LPS-induced spinal cord cells compared with those in the control group (Fig. 5A and C). This indicates that the PI3K/Akt/FOXO3a signaling pathway affected $p27^{kip1}$ in this SCI model.

Discussion

The PI3K/Akt/FOXO3a pathway has been demonstrated to serve an important role in the apoptosis of rat neurons induced by hypoxia and ischemia (7). The present study verified that FOXO3a is regulated via PI3K/Akt. In a previous study involving a mouse model of cardiac ischemia/reperfusion, the PI3K/Akt/FOXO3a signaling pathway was demonstrated to mediate the cardioprotective effects of bromelain and sodium sulfonate (14). It has been speculated that FGF-2 promotes the phosphorylation of FOXO3a, inhibits its nuclear translocation and reduces the expression of pro-apoptotic proteins, thereby inhibiting the oxidative stress-induced apoptosis of nerve cells (11). The present study provides novel evidence that the protein levels of PI3K, p-Akt and FOXO3a were significantly suppressed in an adult rat model of SCI.

SCI involves nervous system damage, and generally leads to severe motor and sensory dysfunction (4). SCI includes primary damage and sequential damage caused by primary factors. The primary injury is an instantaneous and irreversible event, while the sequential injury arises on the basis of the primary injury and may potentially be attenuated (15). The extensive infiltration of inflammatory cells and generation of inflammatory cytokines following SCI are the principal factors leading to sequential spinal cord injury. Since sequential injury of the spinal cord can be inhibited, the treatment of SCI is focused on relieving or preventing the sequential injury, and is an essential objective of the early treatment of SCI (16).

TNF-α is an inflammatory cytokine that serves major roles in inflammatory reactions and immune regulation (17). Studies have shown that following SCI, TNF-α rapidly and consistently participates in the sequential spinal cord injury process as an injury factor (16,17). It is a cytokine that increases following SCI, upregulates the generation of other cytokines, and induces the generation of arachidonic acid, lipid peroxides and oxygen free radicals that may damage cytomembranes and increase the vascular permeability of blood (18). Such characteristics may be associated with the mechanism of formation of edema following SCI (18). In the present study, it was observed that the inhibition of PI3K significantly increased the TNF-α levels in the LPS-induced spinal cord cells, which may be the cause of the reduction in cell viability that was observed.

Cell apoptosis has multiple upstream pathways, including the death receptor-mediated, mitochondrial and endoplasmic reticulum pathways (19). The PI3K/Akt pathway is important for controlling cell apoptosis and promoting proliferation through affecting the activation state of various downstream effector molecules (20). In the present study, the inhibition of PI3K significantly reduced the protein levels of FOXO3a, PI3K, p-Akt and $p27^{kip1}$ in the LPS-induced spinal cord cells. The PI3K/Akt/FOXO3a pathway has been demonstrated to be an essential pathway in the control of cell apoptosis (21). In this pathway, when PI3K/Akt is activated, the phosphorylation of FOXO3a is stimulated and FOXO3a transfers from the cell nucleus to the cytoplasm, which reduces the expression of Bcl-2-like protein 11 (Bim) and thus inhibits cell apoptosis; however, if the PI3K/Akt signaling pathway is inhibited, FOXO3a is dephosphorylated and transfers to the nucleus from the cytoplasm, thereby increasing Bim expression and initiating cell apoptosis (22). Studies have indicated that in Alzheimer's disease, β amyloid protein inhibits the PI3K/Akt signaling pathway which increases the nuclear transfer of FOXO3a protein, thereby increasing the production of the pro-apoptotic protein Bim and inducing nerve cell apoptosis (22,23). In the present study, the overexpression of FOXO3a significantly increased the cell viability and reduced the TNF-α activity of LPS-induced spinal cord cells. This is consistent with a previous study in which Pun *et al* (24) indicated that the sirtuin 1-FOXO3a axis regulates LPS-induced TNF-α expression and serves a crucial role in globular adiponectin-induced autophagy in macrophages.

The FOXO3a protein, as a transcription factor associated with cell cycle regulation and apoptosis induction, serves a vital role in cell proliferation, cell apoptosis, cell cycle arrest, cell senescence, cell differentiation, tumor angiogenesis, DNA rehabilitation and oxidative stress, mainly through regulating the expression of downstream genes (25). FOXO3a downstream proteins include apoptosis-mediating proteins such as Bim, TNF-related apoptosis-inducing ligand, death receptor (DR)4 and DR5; cell cycle regulators including cyclin-dependent kinase inhibitor 1 and $p27^{kip1}$; ataxia-telangiectasia mutated, which controls rehabilitation from DNA injury; and manganese superoxide dismutase, which protects against anti-oxidative stress (25). $p27^{kip1}$ is a negative regulator of the cell cycle, the expression level of which changes during different processes of the cell cycle (22). It serves a vital role in the regulation of cell cycle arrest and thus inhibits cell proliferation. It has been reported that in addition to regulating the cell cycle and cell proliferation, $p27^{kip1}$ also regulates cell migration, apoptosis and autophagy (22). Furthermore, $p27^{kip1}$ has been demonstrated to regulate the cell cycle, proliferation

and migration of vascular smooth muscle cells, atherosclerosis and the formation of neointima (23). The overexpression of FOXO3a significantly increased the protein levels of $p27^{kip1}$ in the LPS-induced spinal cord cells in the present study, indicating that FOXO3a affects $p27^{kip1}$ in SCI. This is consistent with a study by Pramod and Shivakumar (26), which reported that $p27^{kip1}$ was activated via a FOXO3a-dependent mechanism during cardiac fibroblast growth.

In conclusion, the results of the present study suggest that the PI3K/Akt/FOXO3a signaling pathway regulates regeneration of the spinal cord following SCI in adult rats through its effects on TNF-α and $p27^{kip1}$ expression (Fig. 8). Furthermore, regulation of the PI3K/Akt/FOXO3a pathway is potentially an important mechanism that could be targeted in the treatment of SCI and may provide a novel therapeutic strategy for clinical application.

Competing interests

The authors declare that they have no competing interests.

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