

Insulin-like growth factor 1 promotes neurological functional recovery after spinal cord injury through inhibition of autophagy via the PI3K/Akt/mTOR signaling pathway

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Abstract. Spinal cord injury (SCI) is a serious trauma; however, the mechanisms underlying the role of insulin-like growth factor 1 (IGF-1) in autophagy following SCI remain to be elucidated. The present study aimed to investigate the therapeutic effect of IGF-1 on SCI and to determine whether IGF-1 regulates autophagy via the PI3K/Akt/mTOR signaling pathway. SH-SY5Y neuroblastoma cells were assigned to the H₂O₂, IGF-1 and control groups to investigate subsequent neuron injury *in vitro*. An MTT assay was performed to evaluate cell survival. In addition, Sprague-Dawley rats were randomly assigned to SCI, SCI + IGF-1 and sham groups, and Basso-Beatlie-Bresnahan scores were assessed to determine rat neurological function. Western blotting was used to analyze the autophagy level and the activation of the PI3K/Akt/mTOR signaling pathway. Cell survival was increased significantly in the IGF-1 group compared with the control group *in vitro* (P<0.05). Furthermore, neurological function was improved in the SCI + IGF-1 group compared with the control group *in vivo* (P<0.05). The western blotting results further demonstrated that LC3II/LC3I expression was increased in the IGF-1 group compared with the sham group *in vivo* and compared with the control group *in vitro* (both P<0.05). In the SCI + IGF-1 group, the expression levels of PI3K, phosphorylated (p)-Akt and p-mTOR were higher compared with those in the sham and SCI groups *in vivo* (P<0.05). Moreover, in the IGF-1 group, the expression levels of p-Akt and p-mTOR were higher compared with the control and the H₂O₂ groups *in vitro* (P<0.05). Collectively, the

results of the present study suggested that IGF-1 promoted functional recovery in rats following SCI through neuroprotective effects. Furthermore, the underlying mechanism may involve activation of the PI3K/Akt/mTOR signaling pathway, followed by inhibition of autophagy. However, further investigation into the association between IGF-1-regulated autophagy and the activation of different subtypes of PI3K is required.

Introduction

Spinal cord injury (SCI) has been considered one of the most severe types of central nervous system (CNS) injury that directly or indirectly results in impairment of motor, sensory and other functions (1). However, there is currently a lack of effective therapeutic methods for the treatment of SCI, including medicine or surgery (2,3). SCI is characterized by a disruption in the arrangement, type and number of cells at the injury sites (4). Thus, therapeutic targets should focus on the retention of neural cells at the injury site (5). Although it has been reported previously that scaffolds with neural stem cells improved rat locomotor function, the first choice of therapeutics in a clinical setting is to promote cell survival or generate additional neural cells (6).

Insulin-like growth factors (IGFs) serve a crucial role in the survival and integrity of neurons that promote recovery in several disease models, such as hypoxia/ischemia brain injury, multiple sclerosis and Alzheimer's disease (7,8). IGF-1 regulates a number of important neurophysiological activities, including neurogenesis, cytoplasmic synthesis and complex cognitive function (9,10). Moreover, IGF-1 is a major growth factor for primary neural cells in the CNS (11). The results of a previous study demonstrated that, together, IGF-1 and matrix molecule osteopontin facilitate robust axon regeneration via the PI3K pathway following optic nerve crush (12).

It has previously been reported that IGF-1 expression is decreased in patients with SCI (13), and that high IGF-1 expression levels in patients with SCI may contribute to improved functional recovery (14). Additionally, IGF-1 with osteopontin promoted mouse hind limb function following SCI with T10 lateral hemisection (15). Furthermore, IGF promoted the survival of oligodendrocytes in the spinal cord, and rats with SCI exhibited a quicker neurological recovery (16).

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The results of previous studies have demonstrated that autophagy is one of the key processes involved in cell survival, differentiation and homeostasis (17,18). Autophagy serves an important role in the intracellular catabolic mechanism underlying the recycling of damaged organelles and senescent proteins following SCI (19,20). Previous studies have further confirmed that dysfunction of autophagy increased neuronal apoptosis following SCI, indicating that autophagy may be involved in SCI (21,22). A number of signaling pathways are involved in autophagy, including the key PI3K/Akt/mTOR signaling pathway (23). Moreover, IGF-1 exerts its function via the PI3K/Akt/mTOR signaling pathway (24). Although it has previously been reported that IGF-1 exhibited therapeutic effects on neurological trauma, few studies have investigated the role and mechanism underlying IGF-1 in the treatment of SCI (25).

The present study aimed to investigate the neuroprotective and autophagic effects of IGF-1 on SCI, and to determine the role of the PI3K/Akt/mTOR signaling pathway in this process. Investigation into the role of IGF-1 in SCI may lead to the discovery of novel treatment options for SCI and further clinical applications of IGF-1.

Materials and methods

SH-SY5Y cell cultures. SH-SY5Y is a human derived neuroblastoma cell line that is often used to establish neuronal function and differentiation models *in vitro* (26). SH-SY5Y cells (cat. no. CL0278) were obtained from Hunan Fenghui Biotechnology Co., Ltd., and were evaluated by the short tandem repeat method. Cells were cultured in DMEM/F12 (Cytiva) supplemented with 10% FBS (Zhejiang Tianhang Biotechnology, Co., Ltd.), 1% penicillin-streptomycin (Cytiva) and 2 mM L-Glutamine (Beyotime Institute of Biotechnology) at 37°C and 5% CO₂ (27,28). Trypsin (Thermo Fisher Scientific, Inc.) was prepared for cell separation.

SH-SY5Y cell injury model and IGF-1 interference. Oxidative stress is one of the most important mechanisms underlying SCI, and H₂O₂ is used for simulating oxidative stress in cells *in vitro* (29). There were three groups, H₂O₂ group, IGF-1 group and control group. Following culture of the three groups of SH-SY5Y cells (5×10⁴/ml) in 96-well plates for 12 h, supernatants were removed. In the control group, SH-SY5Y cells were incubated in the aforementioned neural media (DMEM/F12 with FBS, penicillin-streptomycin and L-Glutamine) for 6, 12 and 24 h at 37°C and 5% CO₂. In the H₂O₂ group, cells were incubated in neuronal media supplemented with 200 µmol/l H₂O₂ for 6, 12 and 24 h at 37°C and 5% CO₂ (28). In the IGF-1 group, cells were incubated in neuronal media supplemented with 200 µmol/l H₂O₂ and 100 ng/ml IGF-1 (Sigma-Aldrich; Merck KGaA) for 6, 12 and 24 h at 37°C and 5% CO₂ (30,31). This concentration of IGF-1 was selected using preliminary tests (data not shown). At 6 h following cell injury, cells were washed with PBS twice and collected for western blot analysis, and at 6, 12 and 24 h, cell samples were used for the MTT assays.

MTT assay. An MTT assay was performed to examine cell survival following injury and IGF-1 interference. Cell culture medium was removed from each well of the 96-well-plate as

mentioned, and cells were incubated for 4 h at 37°C in the CO₂ (5%) incubator, followed by the addition of 20 µl MTT reagent (5 mg/ml) for a further incubation at 37°C for 4 h. Following the MTT incubation, 150 µl 20% DMSO was added to each well to remove the purple formazan crystals. The plate was subsequently shaken for 10 min for solubilization. The absorbance was measured using a microplate spectrophotometer at a wavelength of 490 nm. The final optical density was calculated using the average of six wells.

Animal care and groups. In total, 24 healthy adult male Sprague-Dawley rats (age, 8-12 weeks; weight, 240-280 g) were purchased from Shanghai Jieyi Biotechnology, Co., Ltd., and used for the SCI model generation. All rats were housed in a specific-pathogen-free laboratory under a 12-h light/dark cycle and a stable temperature of 23-25°C. All animals were housed individually and had free access to standard food and water. All experimental procedures were performed in accordance with the Guiding Opinions on the Ethical Treatments of Laboratory Animals published by the Ministry of Science and Technology of the People's Republic of China in 2006 (32). All animal protocols were approved by the Animal Ethics Committee of Capital Medical University of China (approval no. PYZ2017082).

The rats were assigned into sham (n=8), SCI (n=8) and SCI + IGF-1 (n=8) groups using a random numbers table. In total, five rats were randomly selected from each group for Basso-Beatlie-Bresnahan (BBB) score evaluation, and the remaining three rats in each group were sacrificed for western blot analysis at 1 day after SCI modeling.

SCI model and IGF-1 treatment. Rats in the SCI and SCI + IGF-1 groups received an SCI operation, which was conducted as previously described (33,34). Briefly, after anesthetization with 4% sodium pentobarbital [50 mg/kg; intraperitoneal (i.p.) injection], rats were placed on a heating pad to maintain body temperature. The spine at T9-T11 was exposed from the dorsal side and a laminectomy was performed. A 10-g rod was freely dropped from a height of 25 mm, which was controlled by an NYU Impactor II (New York University Medical Center, New York, NY, USA), to impact the T10 segment of the spinal cord; wagging tail reflex and lower limb spasms were observed. Laminectomy was performed on the rats in the sham group without SCI (35). The wound was sutured layer by layer. Manual bladder emptying was performed and an i.p. injection of 20 U/kg penicillin was administered once daily until bladder function was re-established.

Rats were administered an i.p. injection of 1 ml IGF-1 (50 g/kg; dissolved in 0.9% NaCl; Sigma-Aldrich; Merck KGaA) <5 min after SCI operation (36,37). This concentration was selected in preliminary tests (data not shown). This was repeated once daily in the SCI + IGF-1 group until animals were sacrificed (36). At the same time, an i.p. injection of 1 ml 0.9% NaCl was administered to rats in the sham and SCI groups. All the procedures were performed by the same unblinded investigator.

Neurological function assessment. The neurological function of the rats was assessed using the BBB rating scale, which was described in our previous studies (33,34). The BBB score ranged from 0 (complete paralysis) to 21 (healthy) (38). In total, two blinded, independent examiners evaluated the BBB score

before the operation and again at 1 day, and 1, 2, 3 and 4 weeks following SCI. The average score was used for subsequent analysis. After 4 weeks, an i.p. injection of 300 mg/kg sodium pentobarbital was used to sacrifice the rats.

Collection of the specimens. Based on the results of previous studies and our previous research, autophagy is altered and increases quickly following SCI, reaching a peak on day 1 (20,34). On day 1 after the initial treatment of IGF-1, rats were anesthetized with 4% sodium pentobarbital (50 mg/kg; i.p. injection) followed by an intracardiac perfusion with normal saline (0.9% NaCl). After SCI, the injury area expanded and the injury margin was more difficult to clearly distinguish (39). Thus, a 10-mm segment of spinal cord tissue centered at the injury site was removed. An i.p. injection of 300 mg/kg sodium pentobarbital was subsequently used to sacrifice the rats. The spinal cord tissue was immediately frozen in liquid nitrogen and stored at -80°C .

Western blot analysis. Protein determination was performed by western blot analysis. Each specimen was handled individually for the following procedures. RIPA lysis buffer was used for purification of total proteins from the spinal cord specimens. The 10-mm spinal cord tissue and each group of the SH-SY5Y cell samples were dissolved in ice-cold RIPA lysis buffer containing PMSF (Beyotime Institute of Biotechnology). Protein concentration was determined by BCA kit (Beyotime Institute of Biotechnology). Homogenates of spinal cord specimens and cell samples were centrifuged at $13,500 \times g$ for 30 min at 4°C to collect the supernatant. A total of $50 \mu\text{g}$ protein per lane was separated by SDS-PAGE on 15% gel (80 V for 30 min; followed by 120 V for 90 min) and transferred onto a PVDF membrane (Beyotime Institute of Biotechnology). The membranes were blocked with 5% non-fat milk in TBS with 0.1% Tween-20 for 2 h at 25°C . Subsequently, the membranes were incubated with the following antibodies: GAPDH (1:5,000; cat. no. ab8245; Abcam), anti-LC3I/II (1:1,500; cat. no. ab52768; Abcam), anti-PI3K (1:1,000; cat. no. ab191606; Abcam), anti-Akt (1:1,000; cat. no. ab8805; Abcam), anti-phosphorylated (p)-Akt (1:1,000; cat. no. ab81283; Abcam), anti-mTOR (1:1,000; cat. no. ab134903; Abcam) and anti-p-mTOR (1:1,000; cat. no. ab137133; Abcam) at 4°C overnight. Subsequently, the membranes were washed three times with TBST (0.2% Tween-20) and incubated with HRP-conjugated IgG secondary antibody (1:5,000; cat. no. ab97051; Abcam) at 25°C for 1 h. Following secondary incubation, the membranes were washed again, and proteins were visualized using ECL solution (Beyotime Institute of Biotechnology). Images were captured using a ChemiDoc-ItTM TS2 Imager (Analytik Jena GmbH). ImageJ software version 2 (National Institutes of Health) was used to measure the density of the scanned protein bands, with GAPDH used for normalization. All the procedures were performed by the same blinded investigator.

Statistical analysis. Experimental data are presented as the mean \pm SD, and all data were analyzed using SPSS software version 23.0 (IBM Corp.). Comparison of BBB score among three groups was performed using a non-parametric Kruskal-Wallis test followed by Dunn's post hoc test. Additional comparisons among three groups were performed

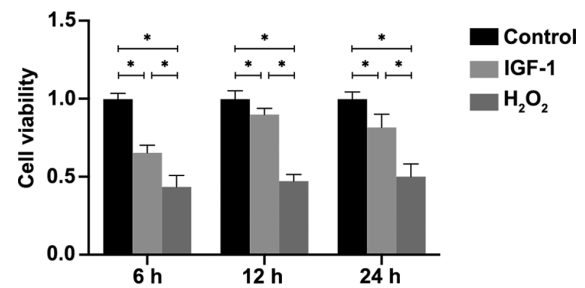


Figure 1. Assessment of cell viability using an MTT assay *in vitro*. Cell viability of the IGF-1 group is significantly increased compared with the H₂O₂ group at 6, 12 and 24 h. * $P < 0.05$. IGF-1, insulin-like growth factor 1.

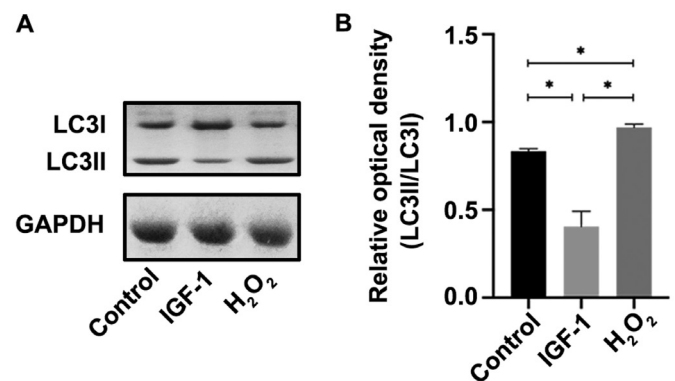


Figure 2. Western blot analysis of autophagy-related proteins *in vitro*. H₂O₂-induced (6 h) SH-SY5Y cell SCI models were treated with or without IGF-1. (A) Representative western blotting results of LC3I and LC3II expression and (B) semi-quantification of the LC3II/LC3I ratio in each group. Data are expressed as the mean \pm SD. Experiments were performed in triplicate. $n = 3$ per group. * $P < 0.05$. IGF-1, insulin-like growth factor 1.

using a one-way ANOVA followed by a least significant difference post hoc test. $P < 0.05$ was considered to indicate a statistically significant difference.

Results

IGF-1 promotes cell survival *in vitro*. To simulate the micro-circulation in SCI, SH-SY5Y cells were exposed to $200 \mu\text{mol/l}$ H₂O₂ for 6, 12 and 24 h to establish a neural cell injury model *in vitro*. The MTT assay results indicated that the cell viability was significantly decreased in the H₂O₂ and IGF-1 groups compared with the control group at 6, 12 and 24 h (all $P < 0.05$; Fig. 1). Furthermore, viability was significantly higher in the IGF-1 group compared with the H₂O₂ group ($P < 0.05$; Fig. 1).

IGF-1 activates the PI3K/Akt/mTOR signaling pathway to reduce autophagy *in vitro*. The expression levels of autophagy-associated proteins were measured to evaluate the effect of IGF-1 on autophagy and to identify the mechanisms underlying SH-SY5Y cells following exposure to H₂O₂ and co-treatment of IGF-1 for 6 h. The results demonstrated that LC3II/LC3I expression was significantly increased in the H₂O₂ group compared with the control ($P < 0.05$), whereas it was significantly decreased in the IGF-1 group compared with the H₂O₂ and control groups (both $P < 0.05$; Fig. 2A and B) at 6 h after cells injury.

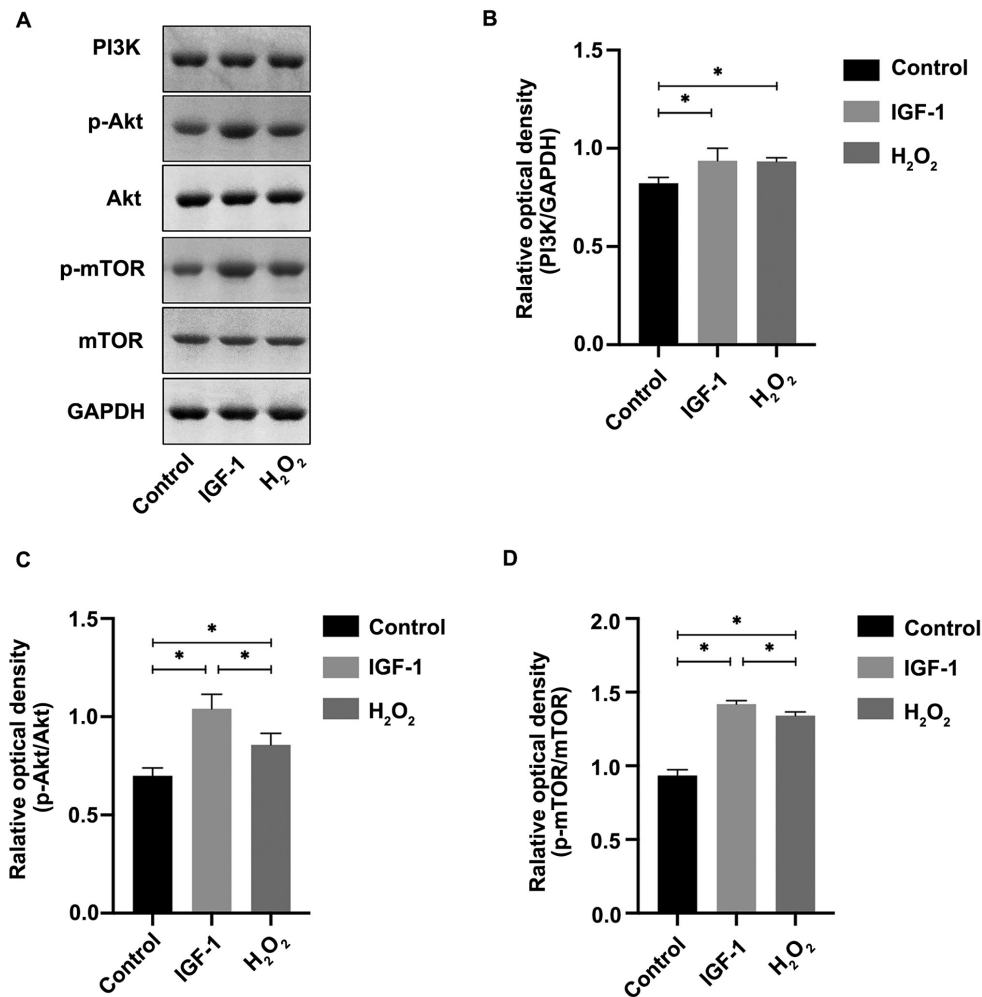


Figure 3. Western blot analysis of the effects of IGF-1 on the PI3K/Akt/mTOR signaling pathway *in vitro*. H₂O₂-induced (6 h) SH-SY5Y cell SCI models were treated with or without IGF-1. (A) Representative western blotting images and semi-quantification of (B) PI3K, (C) p-Akt/Akt and (D) p-mTOR/mTOR expression in each group. Data are expressed as the mean \pm SD. Experiments were performed in triplicate. * $P < 0.05$. IGF-1, insulin-like growth factor 1; p-, phosphorylated.

To study the role of the PI3K/Akt/mTOR signaling pathway in IGF-1-induced inhibition of autophagy, activation of the pathway was determined using western blotting. As presented in Fig. 3, PI3K expression was increased significantly in the IGF-1 group compared with the control ($P < 0.05$). In the same trend as PI3K expression, p-Akt/Akt and p-mTOR/mTOR expression was increased in the IGF-1 group compared with both the H₂O₂ and control groups ($P < 0.05$).

Overall, these data suggested that IGF-1 may activate the PI3K/Akt/mTOR signaling pathway, which resulted in down-regulation of autophagy.

Neurological functional recovery. In the SCI model rats, lower hindlimb function was evaluated using the BBB scale 1 day before SCI, and at 1 day and 1, 2, 3 and 4 weeks after SCI. Following SCI, the locomotor function score of rats was zero in the SCI + IGF-1 and SCI groups at 1 day after SCI, which gradually improved over time in these groups (Fig. 4). Although there was no significant difference between the SCI + IGF-1 and SCI groups at 1 day and 1 week ($P > 0.05$), rats in the SCI + IGF-1 group exhibited significantly improved neurological functional recovery compared with the SCI group at 2, 3 and 4 weeks ($P < 0.05$; Fig. 4).

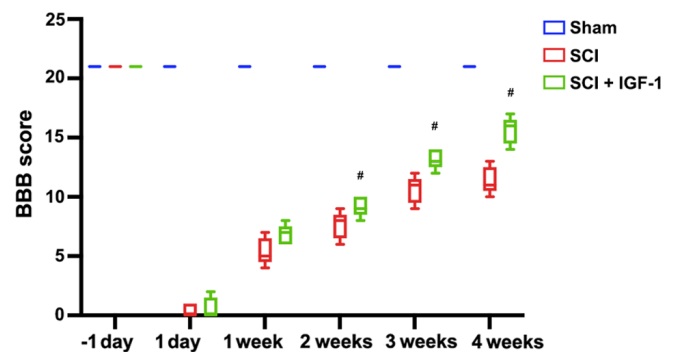


Figure 4. Effects of IGF-1 on locomotor functional recovery in rats after SCI. The IGF-1 group received IGF-1 treatment (50 μ g/kg) daily following SCI, whereas an intraperitoneal injection of 0.9% NaCl was administered to the SCI and sham groups. BBB scores are represented as the median and inter-quartile range; $n = 5$ rats/group. BBB scores were evaluated at -1 day before SCI and at 1 day and 1, 2, 3 and 4 weeks after SCI. * $P < 0.05$ vs. SCI group. BBB, Basso-Beatlie-Bresnahan; IGF-1, insulin-like growth factor 1; SCI, spinal cord injury.

IGF-1 inhibits autophagy via the PI3K/Akt/mTOR signaling pathway following SCI in vivo. LC3II and LC3I expression levels were evaluated by western blot analysis on day 1 after

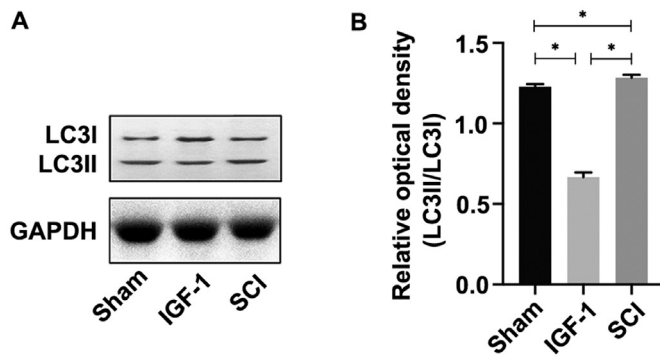


Figure 5. Western blot analysis of the expression levels of autophagy-related proteins *in vivo*. Protein expression was examined in injured spinal cords were at 1 day after SCI with or without IGF-1 treatment. (A) Protein expression levels of LC3I and LC3II and (B) semi-quantification of the LC3II/LC3I ratio in each group. Data are expressed as the mean \pm SD; n=3 rats/group. *P<0.05. IGF-1, insulin-like growth factor 1; SCI, spinal cord injury.

SCI. The expression level of LC3II/LC3I was significantly lower in the SCI + IGF-1 sham group compared with sham group, whereas it was significantly higher in the SCI group compared with the sham group (P<0.05; Fig. 5).

To evaluate the activation of the PI3K/Akt/mTOR signaling pathway, the relative expression level of PI3K and the ratios of p-Akt/Akt and p-mTOR/mTOR expression were analyzed. Following SCI, the relative expression levels of PI3K, p-Akt and p-mTOR were higher compared with the expression levels in the sham group (all P<0.05). However, the relative expression levels of p-Akt/Akt and p-mTOR/mTOR in the SCI + IGF-1 group were significantly higher compared with the expression levels in the untreated SCI group (all P<0.05; Fig. 6).

Discussion

IGF-1 is an inherent molecule of the insulin family that has numerous physiological functions in the human body, such as regulating growth, insulin sensitivity and cardiovascular activity (40). Moreover, IGF-1 regulates various neurological activities, such as neuronal survival, energy metabolism and plasticity (41). In addition, IGF-1 is a potent growth factor for different types of nerve cells (42). A higher expression level of IGF-1 in patients with SCI may improve functional recovery (14).

A significant decrease in serum IGF-1 levels was observed in patients with SCI compared with physiological range (13,43), and Ferbert *et al* (43) reported that patients with higher IGF-1 levels exhibited improved neurological recovery at 12 weeks following SCI. The results of another study indicated that the IGF-1 concentration in the plasma and injured spinal cord of SD rats was significantly decreased compared with the control group, and the administration of exogenous IGF-1 reduced cell damage after SCI (44). The results of the present study are consistent with these previous data; the neurological function of SCI rats receiving treatment with IGF-1 recovered to a greater extent compared with untreated SCI rats. However, the role of IGF-1 and the underlying mechanisms are yet to be fully elucidated.

It has been reported that autophagy serves an important role in the process of cell death (45,46). The present study was conducted to examine the inhibitory effect of IGF-1 on autophagy as a therapy for SCI and aimed to elucidate the potential underlying mechanisms. The results of the present study demonstrated that IGF-1 promoted functional recovery in SCI rats and increased the number of surviving neural cells following injury *in vitro*. *In vivo* and *in vitro* results demonstrated that IGF-1 inhibited autophagy, with higher expression levels of p-Akt and p-mTOR. These findings indicated that IGF-1 may inhibit autophagy via the PI3K/Akt/mTOR signaling pathway.

However, the detailed mechanisms underlying autophagy in cell death and survival remain a matter of debate (47-50). Autophagy involves a series of complex processes, such as the delivery of cytoplasmic cargo sequestered inside double-membrane vesicles to the lysosome, which is a dynamic equilibrium and changes rapidly from one stage to the other. The process is hard to monitored in real-time accurately. The intensity, timing and regulating mechanisms underlying autophagy may have protective effects or may aggravate damage in cells (20,51). At present, it is recognized that autophagy is increased following SCI (52,53). However, it has been demonstrated by various studies that the same interference may lead to different outcomes (18). On the one hand, a number of studies reported that the therapeutic effects of various methods, like rapamycin, simvastatin, lithium, were based on increased autophagy, which protected a larger number of neural cells from death (20,34,54,55). On the other hand, the results of previous studies have indicated that increased autophagy was the cause of neural cell death, as it has been observed that inhibition of autophagy promoted neurological functional recovery (20,56,57).

The present study demonstrated that IGF-1 treatment improved neurological function following SCI. IGF-1 is involved in neural cell proliferation, clearance of abnormal cells, inflammation, myelination, neurogenesis and plasticity (24,58-61). Moreover, the results of the present study demonstrated that IGF-1 may improve neurological function via the PI3K/Akt/mTOR signaling pathway and inhibition of autophagy.

In addition to the aforementioned functions of IGF-1, it has been reported that antagonism of the IGF-1 receptor inhibits rather than induces autophagy (62). A potential factor that may affect the function of the IGF-1 signaling pathway in autophagy includes the existence of different types of PI3K proteins, which function in different manners (63). The PI3K family comprises kinases that are key to the regulation of autophagy, and they are divided into three classes. Class I PI3K proteins have been largely described and activate the Akt/mTOR signaling pathway that inhibits autophagy (64). Class II PI3K proteins are involved in a wide variety of biological activities. For example, PI3K-C2a participates in glucose transportation, secretion of insulin, release of neurosecretory granules, endocytosis and contraction of muscle cells; PI3K-C2b is active in cell migration and potassium channel activation; and both PI3K-C2a and PI3K-C2b are involved in cell proliferation and survival (65). Moreover, PI3K-C2 is involved in autophagy (66). It has previously been reported that both the Class II and Class III PI3K proteins enhance

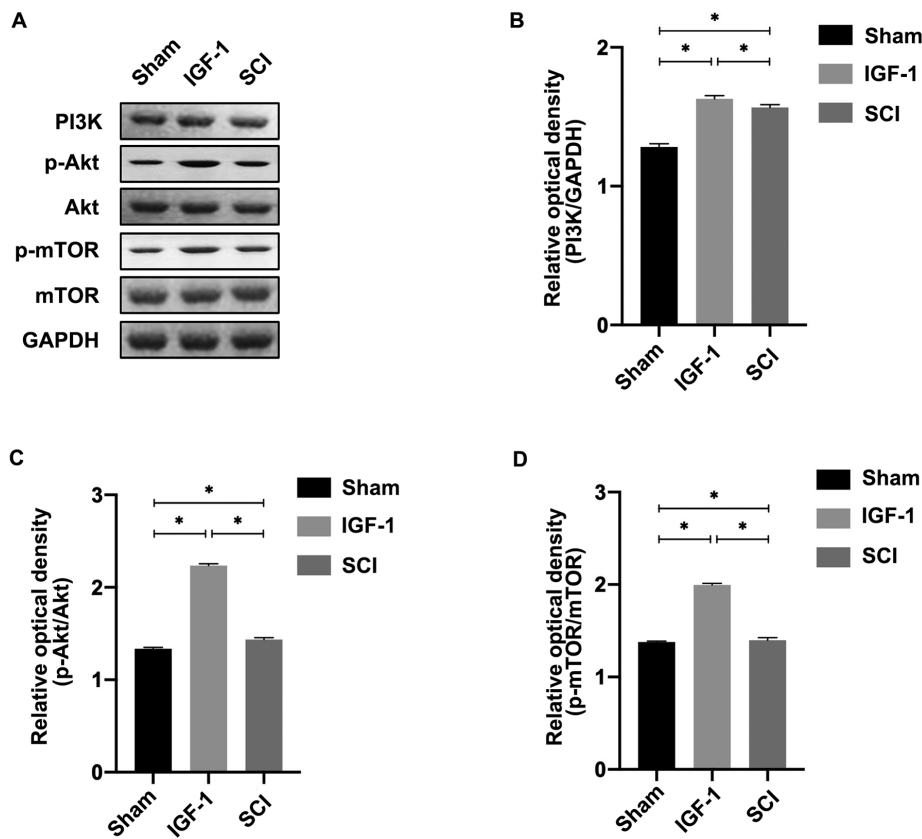


Figure 6. Western blot analysis of the effects of IGF-1 on the PI3K/Akt/mTOR signaling pathway *in vivo*. Protein expression was examined in injured spinal cords at 1 day after SCI with or without IGF-1 treatment. (A) Western blotting results of the PI3K, Akt, p-Akt, mTOR and p-mTOR protein expression levels. (B) Relative protein expression levels of PI3K. (C) Ratio of p-Akt and Akt protein expression levels. (D) Ratio of p-mTOR and mTOR protein expression levels. Data are expressed as the mean \pm SD; n=3 rats/group. *P<0.05. IGF-1, insulin-like growth factor 1; p-, phosphorylated; SCI, spinal cord injury.

autophagy (67). In addition, Class I proteins contribute to the formation of phosphatidylinositol-3-phosphate (PI3P), which is the key secondary messenger in autophagy and is further affected and regulated by the Class II and Class III PI3Ks (68).

Different types of cells, such as neurons, astrocytes and oligodendrocytes in the spinal cord produce different responses to harmful stimuli and varying activation of the same signaling pathway, including autophagy. Neurons would suffer apoptosis after damage, while the astrocytes would proliferate and secrete neurotrophic factors (69). The results of a previous study demonstrated that the expression of p-Akt was increased at 6 h after contusion rat SCI in neurons, reaching a peak at 3 days, before returning to baseline. However, the increase in the expression of p-Akt was initiated at 3 days and continued until 7 days after SCI in astrocytes (70). The characteristics of autophagy vary amongst different neural cells involved in SCI. A higher level of autophagy was found in neurons at 3 h following SCI (26), whereas Hou *et al* (71) observed an increased level of autophagy in astrocytes at 3 days after SCI. Moreover, no increase of autophagy was observed in astrocytes at 4 days after SCI with enhanced autophagy, but the glial scar was reduced at 2 weeks after SCI (72,73). The results of a previous study revealed that changes in neuronal autophagy are associated with the neuronal subtype, and neurons from the spinal cord may be affected more easily by the inhibition of autophagic flux compared with brain neurons (53). Previous studies have also demonstrated that the accumulation of autophagosomes in oligodendrocytes was observed

at later timepoints compared with neurons, suggesting that the reaction to damage is not the same between these two cells (21,74,75). Thus, it was suggested that autophagy is a dynamic process, which exerts positive or negative effects in early or late stages (20,76). Fang *et al* (76) reported that neurological function was improved when autophagy was stimulated at an early stage after SCI, compared with poor neurological function when autophagy was inhibited at the early stage. However, neurological function was not improved when autophagy was stimulated at a late stage compared with improved neurological function when autophagy was inhibited at the same stage following SCI.

IGF-1 has been applied in the clinical setting for the treatment of growth failure in pediatric patients with severe primary IGF-1 deficiency and in patients with growth hormone (GH) gene deletion who have developed neutralizing antibodies to GH (77,78). IGF-1 is safe and positively effects body composition, and IGF-1 treatment for SCI in the clinical setting is easily accessible, which has been approved by The Food and Drug Administration (FDA) (34). Based on previous studies and the results of the present study, IGF-1 has potential as a clinical treatment of SCI. However, determining the optimal timing for IGF-1 treatment requires further investigation.

The present study is the first to report the effect of IGF-1 on autophagy in treating SCI involving the PI3K/Akt/mTOR signaling pathway. However, the current study presents preliminary evidence lacking deep exploration, thus, further investigation is required. In future studies, the duration of

the BBB scoring experiment will be extended to 2 months. Additionally, inhibitors of PI3K, Akt, mTOR and the IGF-1 receptor will be used to confirm the impact of IGF-1 and mechanisms underlying this protein in the PI3K/Akt/mTOR signaling pathway. Although the BBB scale is widely used to evaluate neurological function, it is a subjective tool. More objective techniques involve measurement of the neurological regeneration, including immunofluorescence staining of regenerated nerve fibers, examination of the myelinated axon density, electrophysiological tests and diffusion tensor imaging (33,79).

In conclusion, the present study demonstrated that IGF-1 promoted functional recovery in rats after SCI and exerted neuroprotective effects. Furthermore, the IGF-1-induced inhibition of autophagy may be associated with activation of the PI3K/Akt/mTOR signaling pathway. However, whether the regulation of autophagy by IGF-1 is associated with the activation of different PI3K signaling pathways in different neural cells at varying injury stages remains to be elucidated and requires further investigation.

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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 on reasonable request.

Authors' contributions

DZha and YY conceived the project. DZha, DZhu, CL and SD performed the animal and cell experiments. DZha, YY and JZ integrated and analyzed the experimental data. DZha, YY and BL conceived and designed the study. DZha drafted the manuscript. JZ, LW, SM and WC performed the statistical analysis. BL and DZha confirm the authenticity of all the raw data. All authors have read and approved the final manuscript.

Ethics approval and consent to participate

All experimental procedures were performed in accordance with the Guidelines for the Care and Use of Laboratory Animals (Institute for Laboratory Animal Research, National Academy of Science). The protocols were approved by the Animal Ethics Committee of Capital Medical University of China (Beijing, China; approval no. PYZ2017082).

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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