Interference of interleukin-1β mediated by lentivirus promotes functional recovery of spinal cord contusion injury in rats via the PI3K/AKT signaling pathway

Yi-li Wang (✉ 2807383079@qq.com)
Chengdu Medical College

Xi Hu
Chengdu Medical College

Qin-xuan Li
Chengdu Medical College

Li-xin Zhang
Yunnan Academy of Forestry

Qing-jie Xia
Sichuan University

Nan Liang
Chengdu Medical College

Wei-hua Liu
Chengdu Medical College

Xiao Zhang
Chengdu Medical College

Research

Keywords: Spinal cord contusion, motor function, IL-1β, AKT1, signal pathway

DOI: https://doi.org/10.21203/rs.3.rs-33767/v1

License: This work is licensed under a Creative Commons Attribution 4.0 International License. Read Full License
### Abstract

**Background:** Spinal cord contusion (SCC) results in a series of pathophysiologic consequences such as edema, apoptosis, and inflammation. However, inflammation may also be beneficial for the recovery of motor function after SCC, but the underlying mechanisms remain incompletely elucidated. Interleukin-1 beta (IL-1β) is a pro-inflammatory factor that has synergistic effects with other inflammatory factors to aggravate spinal cord injury. Inflammatory factors have been found to activate the serine/threonine-specific protein kinase, protein kinase B (AKT) and to inhibit cell survival, but it is not clear whether inflammation upregulates the expression of IL-1β in the rat model of SCC and subsequently interferes in the phosphatidylinositol-3-kinase (PI3K)/AKT signaling pathway. Therefore, this study explored whether IL-1β affects the recovery of motor function in spinal cord injury by interfering with the PI3K/AKT signaling pathway.

**Method:** SCC rats were established by the Allen method. The Basso Beattie Bresnahan (BBB) scoring was used to assess motor function in the spinal cord of injured rats. Quantitative polymerase chain reaction and Western blot were used to determine the expression of genes and proteins of IL-1β, PI3K, and AKT1. Immunohistochemistry and immunofluorescence were used to locate and detect IL-1β and AKT1 proteins in spinal cord tissue. To further explore the underlying mechanism of IL-1β, lentivirus was constructed by RNA interfering (RNAi) technique to inhibit the expression of IL-1β, and bioinformatics was applied to show the relationship between IL-1β and AKT1.

**Results:** BBB scores decreased after SCC, and IL-1β and AKT1 was located in the cytoplasm of spinal cord anterior horn neurons. In the early stage of SCC, the expression level of IL-1β gene and protein in the experimental group was higher than that in the sham operated group. At the same time, expression of the AKT1 gene decreased. After expression of IL-1β mediated by lentivirus was inhibited, BBB scores increased significantly, and spinal cord motor function improved. Bioinformatic analysis revealed a relationship between IL-1β and AKT1. In addition, AKT1 gene expression was upregulated and PI3K expression was unchanged in the PI3K/AKT signaling pathway.

**Conclusion** IL-1β not only exacerbates the inflammatory response after SCC, but also interferes with motor function. Inhibition of IL-1β may promote recovery of spinal cord injury by upregulating AKT1 in the PI3K/AKT signaling pathway, which provides a new perspective for future clinical practice in treating spinal cord injury.

### Introduction

Spinal cord contusion (SCC) is a severe trauma of the central nervous system due to external forces. SCC can lead to sensorimotor dysfunction below the level of injury, with the possibility of disability and mortality[1]. Secondary complications of SCC due to primary spinal cord tissue damage include inflammation, edema, apoptosis among other conditions[1]. The complicated pathophysiologic process of SCC involves numerous factors such as inflammatory, growth, and signaling pathway factors. A
number of studies [2, 3] have explored whether or not inflammatory factors interfere with cell survival, regeneration, and apoptosis. However, results have not definitively demonstrated if in fact inflammatory factors are involved in the pathophysiologic process of SCC.

Interleukin-1 β (IL-1β) is one of the most extensively studied pro-inflammatory cytokines. IL-1β is produced by microglia, astrocytes, and neurons and has many functions after spinal cord injury (SCI) [4]. IL-1β is able to induce a number of cellular reactions such as changes in intracellular calcium concentration [5]. It has also been demonstrated to have strong effects on astrocytes by promoting their activation, as well as proliferation and production of neurotoxic mediators [2]. Interestingly, healthy neurons have been found to be injured when exposed to IL-1β, and IL-1β can also aggravate neurodegeneration that leads to cell death in experimental models of central nervous system (CNS) trauma [6, 7]. Moreover, in the compression model of SCI, IL-1β has been shown to exacerbate the lesion and activate microglia, which expands the lesion areas; this effect can be attenuated with exposure to the IL-1 receptor antagonist (IL-1ra) [8]. However, it was found that IL-1 gene-deficient mice had improved axon plasticity [9, 10]. It is known that IL-1β is important for tissue repair following SCI, but the mechanism of how IL-1β impacts the restoration of locomotor function in SCC has not yet been definitively reported.

The serine-threonine protein kinase encoded by the AKT1 gene, as the downstream target of phosphatidylinositol 3-kinase (PI3K), is believed to be at the core in PI3K/AKT signaling pathway [11]. AKT1 is activated by platelet-derived growth factor and is a critical mediator of growth factor induced neuronal survival in the developing nervous system. By activating AKT1, survival factors can inhibit apoptosis in a transcription-independent manner [12]. Moreover, AKT1 is able to suppress neuron apoptosis and thus effect restoration of motor function [13]. However, whether AKT1 is affected by IL-1β to restrain the restoration of motor function in SCI has not been reported, nor has the relationship between IL-1β and AKT1 in SCC been elucidated. Therefore, we assume that IL-1β hinders the restoration of motor function after SCC by interfering with the PI3K/AKT signaling pathway.

In this study, we applied the rat model of spinal cord contusion to explore the inhibition of IL-1β mRNA mediated by lentivirus to interfere with the expression of IL-1β in order to investigate whether IL-1β affected the recovery of spinal cord motor function, and to explore whether IL-1β in fact interferes with the PI3K/AKT signaling pathway. Our results indicate that IL-1β does appear to interrupt restoration of motor function following SCC in rats by disrupting the expression of AKT1 in the PI3K/AKT signaling pathway.

Materials And Methods

Animals and groupings

All experimental procedures were performed in accord with the National Institutes of Health Guide for the Care and Use of Laboratory Animals (2011) and were approved by the Institute of Translational
Neuroscience Center of Sichuan University, Chengdu, China and by the institutional ethics and licensing committee of Chengdu Medical College, Chengdu, China.

A total of 144 female Sprague-Dawley (SD) rats (each weighing 200±20 g) were purchased from the Animal Experimental Center, Medical Sciences Department of Sichuan University. The animals were individually housed, in quiet room, under 12/12 hours light/dark condition with free access to water and food. Animals were randomly divided into four groups: sham; spinal cord contusion injury (SCC); vector; and lentivirus mediated siRNA interference (IL-1\(\beta\)-RNAi-LV). Spinal cord contusion injury group was further divided into the following post-injury subgroups with six animals each: 6 hours (6h), 12 hours (12h), 1 day (1d), 3 days (3d), and 28 days (28d). This one and lentivirus-mediated siRNA interference groups consisted of the following subgroups of six animals each: 3 days (3d), 7 days (7d), and 28 days (28d).

**Preparation of lentiviral vector**

Information on siRNA fragment with the highest interference efficiency was provided by Gene-Copoeia, Guangzhou, China. We then constructed the IL-1\(\beta\) lentiviral expression vector, which expresses a gene encoding with red fluorescent protein (RFP). Thereafter, IL-1\(\beta\)-lentiviral expression vectors (5 \(\mu\)g) and viral packaging vectors (1 \(\mu\)L) were co-transfected into packaging cell line (293T) to produce lentiviral particles (IL-1\(\beta\)-RNAi-LV). The viral supernatant was harvested at 48 hours post-transfection and filtered through a 0.45 \(\mu\)m cellulose acetate filter. Then 5 mL of cell supernatant containing lentivirus was centrifuged (3500 \(\times\) g, 25 minutes). The precipitate was re-dissolved in 500 \(\mu\)L phosphate-buffered saline. Finally, the lentivirus was frozen at 80\(^\circ\)C. The negative plasmid was also packaged and used as a negative control, designated as NC-LV, which theoretically had no effect on any gene.

**Spinal cord contusion animal model**

Rats were anesthetized using intraperitoneal (IP) injection of 3.6% chloral hydrate (1 mL/100 g). Assess the depth of anesthesia by judging the toes of rats. Under aseptic conditions, an incision was made along the dorsal midline to expose the soft tissues and muscles, the yellow ligaments overlying the spinal end plate at T11, and finally the spinal cord. A micro-syringe with 10 \(\mu\)L virus mixture was injected 4 mm deep, 5\(\mu\)l each caudally and cranially to the spinal cord. At 48 hours post injection, bilateral laminectomy was performed at T11. To induce SCC, a 10 g weight was dropped from a height of 30 mm onto the exposed cord at T11. Urinary bladders of rats were manually expressed twice a day for one week until normal urination was restored. Rats in the sham group received laminectomy without cord injury. The animals were placed in a warm environment (33-35\(^\circ\)C) 24 hours following the procedures. During this period, they were observed for infection (blood in urine, foul odor, whitish color).

**Locomotor function assessment**

Restoration of hind limb function was assessed by the Basso Beattie Bresnahan (BBB) [14] locomotor scale on days 1, 3, 5, 7, 14, and 28 post-injury. The 28-day group animals were placed in a 2 meter square
cardboard box and observed by three people who were blinded to treatment of the rats. The animals were observed according to the standard BBB grading standards and the recovery of motor function in the hind limbs was recorded. All observations were performed simultaneously. Scoring criteria were as follows: 0-7, joint activity; 8-13, gait and coordination function; 14-21, claw movement. Maximum score was 21 and hind limb paralysis was scored as 0.

Bioinformatics analysis

GeneMANIA was used (www.genemania.org; University of Toronto, Toronto, Canada) to predict the relationships and functions of IL-1β and other genes of the rat species.

Quantitative polymerase chain reaction analysis (qPCR)

Rats were anesthetized by IP injection of 3.6% chloral hydrate (1 ml/100 g) and perfused with 4% paraformaldehyde solution for 30 minutes. Damaged spinal cord tissues were carefully dissected and pre-treated with the total RNA isolation reagent superfecTRI (Shanghai Pufei Biotechnology, Shanghai, China) and then centrifuged at 33500g for 10 minutes before homogenizing. Total mRNA was extracted per the manufacturer's protocol and reverse transcription to cDNA was performed with the Revert Aid First Strand cDNA Synthesis Kit (Thermo Scientic, Waltham, MA, USA). The kit was used for amplification, which included 160 μL TE, 40 μL cDNA, 12.5 μL 2X PCR Mix, 0.6 μL forward primer, 0.6 μL reverse primer, 0.6 μL TaqMan probe, and 7.7 μL water. The PCR mixture underwent 45 cycles of 95°C for 2 minutes, 95°C for 15 seconds, 52°C for 20 seconds, and 60°C for 40 seconds to gain PCR amplification products. During the process, the ΔCT value was recorded, the relative content based on 2-ΔΔCT value was calculated and inspected. Relative expressions were calculated with normalization to β-actin values. Design and synthesis of primers (Table 1) and fluorescent probes were made. Gene sequences were obtained from GenBank (www.ncbi.nlm.nih.gov/gene; National Center for Biotechnology Information, U.S. National Library of Medicine, Bethesda, MD, USA) and primers were designed using Primer Premier 5 (Premier Biosoft International, Palo Alto, CA, USA). TaqMan fluorescent probes were constructed based on Applied Biosystems 7300 Real Time Quantitative PCR System (Thermo Fisher Scientific) filter wavelength, FAM was selected as the fluorescent reporting group and TAMRA was chosen for the quenching group. The primers and probes were synthesized by Sangon Biotech, (Shanghai, China).

Western blotting

Western blotting was used to determine the changes in IL-1β protein in the spinal cord in response to different treatments. The spinal cords at the lesion sites were harvested at different time points post-injury. The rats were deeply anesthetized with IP injection of 3.6% chloral hydrate (1 mL/100 g), and the spinal cords were dissected immediately. The tissues were homogenized on ice in 400 μL of RIPA buffer (Thermo Fisher), containing a cocktail of phosphatase and protease, then centrifuged at 12000 × g for 30 minutes. Protein concentration of each sample was assayed with BCA reagent (Sigma, St. Louis, MO, USA). A 20 μL aliquot of the samples was loaded and electrophoresed on 8% and 12% SDS polyacrylamide gel for 1.5 hours at 120 volts. Proteins were transferred from the gel to a polyvinylidene
diﬀluoride membrane. Then the membrane was blocked with 5% nonfat dry milk for 120 minutes. Primary antibody of β-actin (1:1000; Santa Cruz Biotechnology, Dallas, TX, USA) and anti-IL-1β (1:100, rabbit; Abcam, Cambridge, MA, USA) were separately incubated with two target bands overnight at 4°C after the membranes were rinsed thrice in phosphate-buffered saline containing 0.05% Tween-20. The membrane was incubated for 2 hours with HRP-conjugated goat anti-rabbit IgG (1:2,000; Santa Cruz) or goat anti-rabbit IgG (1:5,000; ZSGB-BIO, Beijing, China) for 2 hours at room temperature. Finally, the membranes were rinsed thrice in buffer and the immune complexes were quantified using the Alpha Imager 2000 (Alpha Innotech, San Leandro, CA, USA) with Western Lightning Plus enhanced chemiluminescence substrate (Perkin Elmer, Waltham, MA, USA). Protein densitometry analysis was performed by Quantity One 1-D Analysis Software (Bio-Rad, Hercules, CA, USA).

**Immunohistochemistry**

Rabbit IL-1β polyclonal antibody (1:100; Abcam) was used as the primary antibody, while goat anti-rabbit IgG (1:100; Jackson Immunoresearch, Westgrove, PA, USA) was used as the second antibody, and DAB chromogenic liquid was applied for detection of positive expression. The epicenters of injured cord tissues were embedded in methyl methacrylate plastic after serial dehydration with a graded ethanol series to xylene. The immune-positive cells were identiﬁed using an optical microscope. Five immunohistochemical slices were taken from each tissue. The ventral and dorsal horns of the spinal cord could be separated based on the vertical straight line drawn between central tube and dorsal medial sulcus of the spinal cord. Immune-positive cells with nuclei in the ventral horn were counted under a 10×-40× stereo microscope (Motic, Carlsbad, CA, USA).

**Immunofluorescence**

Primary antibodies used in this study contained rabbit IL-1β polyclonal antibody (1:100; Abcam), rabbit AKT1 polyclonal antibody (1:150; Abcam), and NeuN (1:50, ZSGB-BIO). Damaged spinal cord tissues were dehydrated, ﬁxed, and embedded in parafﬁn, then cut into 5 µm thick slices. The epicenter of injured spinal cord tissues were incubated in blocking buffer (5% goat serum and 0.3% Triton X-100 in phosphate buffer saline) for 30 minutes at 37°C before incubating overnight with the primary antibodies at 4°C. Secondary antibodies were incubated at 37°C for 1 hour before washing with the phosphate buffer saline. The nucleus was stained using DAPI (0.8 µg/mL, Beyotime, Shanghai, China). The tissues were then mounted onto slides and viewed under a Leica ﬂuorescence inverted microscope (Leica Microsystems, Wetzlar, Germany).

**Statistical analysis**

Data were presented as mean ± standard deviation. Statistical analysis of physiologic parameters was performed with independent sample T test and one-way analysis of variance (ANOVA). The Dunnett family error rates were chosen for unequal sample sizes, otherwise the LSD test was selected. Statistical analysis was performed using SPSS17.0 software (IBM, Armonk, NY, USA). Statistical signiﬁcance was deﬁned as a P-value of < .05.
Results

Motor function evaluation and IL-1β expression in SCC rats

Throughout the 28-day study, BBB scores were 21 in the sham group (Fig. 1A). In comparison, motor function of rats following SCC was significantly suppressed on day 1, but BBB scores gradually increased on days 5, 7, 14, and 28 ($P < .05$) (Fig. 1A). qPCR results showed that the expression of IL-1β mRNA drastically increased ($P < .05$) at 6 hours after SCC, and returned to normal when compared with the sham group (Fig. 1B). IL-1β protein was detected by Western blot and showed that IL-1β was significantly increased after SCC at 12 hours, and 1 and 3 days (Figs. 1C). IL-1β immunoreactivity was found in the spinal cord anterior horn neurons. Compared with the sham group, IL-1β positive cells in the SCC rats were significantly upregulated at 6 hours ($P < .05$). Furthermore, through immunofluorescence IL-1β was observed in the cytoplasm of neurons (Figs. 1D,E).

IL-1β in the recovery of motor function after SCC

The recombinant IL-1β-RNAi-LV was then prepared for use (Fig. 2A). IL-1β lentiviral expression vector was prepared as follows. Three targets of IL-1β siRNA sequences (Figs. 2B F1, F2, F3) were transfected into 293T cells to screen out the most effective siRNA vector by real-time PCR. Results showed that IL-1β expression ratios of F1, F2, and F3 compared with the controls were decreased, thus F3 was considered the suitable target virus (Fig. 2B) and was then transfected into a primary culture of 293T cells. Immunofluorescence was used to determine expression of the vector. Results showed that IL-1β expression (red) could be found in 293T cells after transfection (Fig. 2C). To construct the IL-1β recombinant vector, a segment of F3 was inserted into pcDNA3 plasmid and enveloped by lentivirus with routing methods.

The IL-1β lentiviral vector was injected into the spinal cord of rats to verify the role of IL-1β in the spinal cord. qPCR was used to detect the expression of IL-1β in the spinal cord. Results found that the level of IL-1β mRNA decreased significantly when compared to the vector group ($P < .05$), indicating that the IL-1β lentiviral vector was successfully transfected on the spinal cord (Fig. 3A). Meanwhile, immunohistochemistry results showed that IL-1β immunoreactivity was located in spinal cord anterior horn neurons (Fig. 3B) and the numbers of IL-1β positive neurons were significantly less than the vector group on days 7 and 28 ($P < .05$) (Fig. 3C). Finally, movement of rat hind limbs was assessed using the BBB scale. Scores in the IL-1β-RNAi-LV group were markedly higher than the vector group on days 3, 5, 14, and 28 following spinal cord injury ($P < .05$) (Fig. 3D)

IL-1β in the AKT1 in PI3K/AKT signaling pathway

Bioinformatic analysis was used to predict the relationship of IL-1β and other possibly related genes. By constructing a gene network analysis, we found that IL-1β was related to AKT1 through the arrestin beta 2 (Arrb2), tribbles homolog 3 (Trib3), and tyrosine-protein phosphatase non-receptor type (PTPN2) genes.
Moreover, IL-1β was co-expressed with Arrb2, Trib3, and PTPN2. These three genes were co-expressed with AKT1 as well (Fig. 4A).

qPCR was used to detected the relationship between IL-1β, AKT1, and PI3K. Results showed that the expression of AKT1 mRNA in the IL-1β lentiviral group was significantly higher than in the vector group (P < .05) (Fig. 4B), while expression of PI3K mRNA in the IL-1β lentiviral group was not statistically significant compared to the vector group (Fig. 4C). These results suggest that IL-1β might be associated with AKT1 instead of PI3K. Furthermore, immunofluorescence showed that AKT1 was located in the cytoplasm of neurons, which co-expressed with IL-1β in the spinal cord (Fig. 4D).

Discussion

In this study, we demonstrated that in the rat model of SCC, increased expression of IL-1β in spinal cord tissue hinders the recovery of motor dysfunction after SCC. However, inhibition of IL-1β expression by lentivirus-mediated RNAi upregulates AKT1 expression in the PI3K/AKT signaling pathway, which is advantageous to tissue regeneration and recovery of spinal cord function following SCI. Therefore, our results appear to show that IL-1β has a dual role in spinal cord injury in that it not only affects the key site of AKT1 in the PI3K/AKT signaling pathway, but also affects the recovery of motor function after SCI.

The first effect of IL-1β that we explored was its increased expression in the spinal cord following SCC[8, 15, 16]. Results indicated that the BBB score was significantly lower in the spinal cord injury group compared with the sham-operated group. Furthermore, expression of IL-1β in motor neurons of the anterior horn of the spinal cord increased markedly compared with the control group at 6 hours and 1 and 3 days after SCC, and continued until day 28. These results suggest that increased expression of IL-1β led to reduced spinal cord motor function. To explore the second and opposite effect of IL-1β on the recovery of motor function following SCC, we constructed a lentiviral vector to inhibit expression of IL-1β. BBB score increased significantly in the IL-1β-RNAi inhibitory group than in the control group. In addition, expression of IL-1β decreased in the SCC groups. Results suggest that inhibiting expression of IL-1β promotes recovery of motor function after SCI.

IL-1β is one of the most extensively studied pro-inflammatory cytokines. However, its exact role in the central nervous system is incompletely understood. On the one hand, IL-1β may have deleterious effects on damaged nerve tissue and exacerbate secondary damage caused by central nervous system damage. IL-1β and its receptors are upregulated in astrocytes and microglia after spinal cord injury, with IL-1β mRNA peak expression occurring 12 hours after injury [17]. In addition, in mice, under systemic morphine administration, expression level of IL-1β is significantly increased, resulting in a marked decline in spinal motor function while intrathecal injection of IL-1β receptor antagonists can block the adverse effects of morphine on the recovery of spinal cord motor function[8]. On the other hand, there is evidence that IL-1β has beneficial effects on myelin formation and peripheral nerve regeneration after sciatic nerve injury[9]. Injection of IL-1β can also stimulate activation of macrophages and removal of myelin fragments in the
white matter of the spinal cord. However, no studies have investigated the effect of IL-1β on spinal motor function in SCC, thus it is unclear how IL-1β functions in SCC.

Our study, which investigated the role of IL-1β in SCC, had two aims: whether inhibition of IL-1β expression affected recovery of spinal motor function in SCC; and to understand the relationship between IL-1β and the PI3K/AKT signaling pathway. Bioinformatics analysis was used to first predict the relationship between IL-1β and the PI3K/AKT signaling pathway. The analysis showed that IL-1β was co-expressed with AKT1 and the intracellular proteins Arrb2, PTPN2, and Trib3 in the same cell. IL-1β is interconnected with AKT1 through the intracellular proteins Arrb2, PTPN2, and Trib3. Arrb2 regulates the activity of G protein-coupled receptors during the mitotic cycle and affects the survival of cells [18]. PTPN2 is a signaling molecule that regulates cell growth and differentiation [4]. Trib3 interferes with AKT1 phosphorylation and causes inhibits cell death [19]. Therefore, Arrb2, PTPN2, and Trib3 are important for regulating cell signal transduction, and IL-1β depends on these proteins to establish an association with AKT1. Subsequently, we observed the changes in the expression of AKT1 in SCC and the effect of IL-1β via AKT1 on the recovery of motor function in spinal cord injured rats. In our study, like IL-1β, AKT1 is expressed in motor neurons in the anterior horn of the spinal cord. In early spinal cord injury, expression of AKT1 decreased as the BBB score decreased. Hence, our results suggest that AKT1 affects the recovery of motor function in rats with spinal cord injury. Results by Felix et al [20], also showed that IL-1β disrupts NT-3 and BDNF-mediated neurotransmitter release by interfering with the AKT pathway, and interferes with the effects of NT-3 and BDNF on neuron survival.

The second aim in investigating the role of IL-1β in SCC was to elucidate the relationship between IL-1β and the PI3K/AKT signaling pathway in the repair of spinal cord injury. Following IL-1-β-RNAi inhibition, the mRNA expression of AKT1 was upregulated compared with the control group, while the mRNA expression of PI3K was unchanged. Thus, our results indicate that IL-1β acts on the AKT1 site, thereby upregulating the expression of AKT1 in the PI3K/AKT signaling pathway, and IL-1β participates in the functional recovery after spinal cord injury through AKT1.

PI3K/AKT is an important signaling pathway in cells. PI3K is located on the cell membrane and transmits growth factor and inflammatory factor signals upstream of the cell signaling pathway to the cell. PI3K then phosphorylates phosphatidylinositol 4,5 bisphosphate (PIP2) to PIP3. PIP3 recruits AKT to the plasma membrane and causes activation of the AKT protein [21, 22]. AKT is a serine threonine kinase that controls cell survival and metabolic processes by regulating nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB), cAMP-response element binding protein (CREB), and tumor protein p53 (p53), resulting in transcription of anti-apoptotic and proliferative genes [23]. PI3K/AKT are involved in different aspects of cell proliferation, cell activation, and inflammatory response in SCI. A series of experiments by Fu et al. showed that IL-1β may improve the outcome of spinal cord injury and inflammation by altering the PI3K/AKT signaling pathway. The xanthonoid gambogic acid was found to reduce levels of inflammatory cytokines such as tumor necrosis factor-α, IL-1β, and oxidative stress factors, and inhibit receptor activators of nuclear factor κB ligands, thus attenuating spinal cord injury and inflammation via inhibition of the AKT signaling pathway [24]. Therefore, our experimental
observations, combined with the results of other authors, show that IL-1β acts on the AKT1 site, thereby upregulating the expression of AKT1 in the PI3K/AKT signaling pathway. AKT1 plays an important role in the survival of spinal cord neurons, the mechanism of which may be through inhibiting the expression of IL-1β and indirectly activating the PI3K/AKT signaling pathway. As a result, the growth factor signaling pathway is improved, which enhances recovery of motor function following SCC. The PI3K/AKT/NF-κB axis is recognized as a potential treatment target for SCI.

**Conclusion**

In this study, we demonstrated that inhibition of IL-1β mediated by lentivirus contributed to the recovery of motor function in SCC rats. The novel perspective that IL-1β could impact recovery following SCC by upregulating AKT1 in the PI3K/AKT signaling pathway provides a possible alternative to the treatment of SCC in future clinical practice.

**Abbreviations List**

SCC: Spinal cord contusion

IL-1β: Interleukin-1 beta

AKT: protein kinase B

PI3K: phosphatidylinositol-3-kinase

BBB: Basso Beattie Bresnahan

RNAi: RNA interfering

SCI: spinal cord injury

CNS: central nervous system

IL-1ra: IL-1 receptor antagonist

SD: Sprague-Dawley

IP: intraperitoneal

**Declarations**

**Ethics Approval and consent to participate**

Not applicable

**Consent for Publication**
Not applicable

**Competing interests**

The authors declare that they have no competing interests.

**Funding**

Unit Aliated Hospital of Chengdu Medical College

Award Number: CYFY-GQ27I

Recipient: Yi-li Wang

**Acknowledgment**

This work was supported by National Foundation for Undergraduate Student Innovation (201713705010, 201713705012, 201613705028). The authors thank Nissi S. Wang, MSc, for substantive editing of the manuscript.

**Authors’ contributions**

This study was designed by Xiao Zhang and Ting-Hua Wang. Data were obtained by Xi Hu and Xiao-Ying Lin. Analysis was performed by Xiu-Ya Zhou. BBB scores were assessed by Nan Liang. The manuscript was written by Xi Hu and reviewed by Ke Xiao and Xi Zeng. All authors read and approved the final version of the manuscript.

**Availability of data and materials**

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

**Contributor Information**

Yi-li Wang, Email: 2807383079@qq.com

Xi Hu, Email: 2479256790@qq.com

Qin-xuan Li, Email: 1787038960@qq.com

Li-xin Zhang, Email: 534259627@qq.com

Qing-jie Xia, Email: 648278976@qq.com

Nan Liang, Email: 33722677@qq.com

Wei-hua Liu, Email: 872485363@qq.com
Xiao Zhang, Email: 954073462@qq.com

Author details

1 Chengdu Medical College, Chengdu 610500, Sichuan, China.

2 Affiliated Hospital of Chengdu Medical College, Chengdu 610500, Sichuan, China.

3 Institute of Neurological Disease, Translational Neuroscience Center, West China Hospital, Sichuan University, Chengdu, Sichuan 610041, China.

4 Yunnan Academy of Forestry, Kunming 650204, China

References

1. Arsalan A, Matthew DS, Soheila K-A: Traumatic Spinal Cord Injury: An Overview of Pathophysiology, Models and Acute Injury Mechanisms. Frontiers in neurology 2019, 10.

2. R JG, C LS, Xianyuan S, Mark R, F BC: IL-1-regulated responses in astrocytes: relevance to injury and recovery. Glia 2005, 49(2).

3. Wei J, Xing M, Xiaoshan H, Tiansheng Z, Baoyu Y, Jing W, Hongbin N, Jian J, Handong W, Weibang L: Protective effects of erythropoietin in traumatic spinal cord injury by inducing the Nrf2 signaling pathway activation. The journal of trauma and acute care surgery 2014, 76(5).

4. A S, N T, P W, J RN: Interleukin-1 and inflammatory neurodegeneration. Biochemical Society transactions 2007, 35(Pt 5).

5. E DS, Victor FA: Interleukin 1beta modulates rat subfomical organ neurons as a result of activation of a non-selective cationic conductance. The Journal of physiology 2003, 550(Pt 1).

6. Wang CX, Olschowka JA, Wrathall JR: Increase of interleukin-1β mRNA and protein in the spinal cord following experimental traumatic injury in the rat. Brain Research 1997, 759(2).

7. Wang X-j, Kong K-m, Qi W-I, Ye W-I, Song P-s: Interleukin-1 beta induction of neuron apoptosis depends on p38 mitogen-activated protein kinase activity after spinal cord injury. Acta Pharmacologica Sinica 2005, 26(8).

8. A HM, N WS, Georgina M, A WS, Denise P, H LK, W GJ: An IL-1 receptor antagonist blocks a morphine-induced attenuation of locomotor recovery after spinal cord injury. Brain, behavior, and immunity 2011, 25(2).

9. Boato F, Rosenberger K, Nelissen S, Geboes L, Peters EM, Nitsch R, Hendrix S: Absence of IL-1β positively affects neurological outcome, lesion development and axonal plasticity after spinal cord injury. BioMed Central 2013, 10(1).

10. Sato A, Ohtaki H, Tsumuraya T, Song D, Ohara K, Asano M, Iwakura Y, Atsumi T, Shioda S: Interleukin-1 participates in the classical and alternative activation of microglia/macrophages after spinal cord injury. BioMed Central 2012, 9(1).
11. Fei F, Xiaofei L, Li X, Deyang L, Zhipei Z, Xu G, Hushan Y, Zhinan C, Jinliang X: **CD147-CD98hc complex contributes to poor prognosis of non-small cell lung cancer patients through promoting cell proliferation via the PI3K/Akt signaling pathway.** *Annals of surgical oncology* 2014, **21**(13).

12. Anahita R, Danial K, Peyman K, Alireza S-H, Amir D-A: **Corrigendum to "neurogenesis and increase in differentiated neural cell survival via phosphorylation of Akt1 after fluoxetine treatment of stem cells".** *BioMed research international* 2015, 2015.

13. Xiuqing W, Hanmo Z, M AA, J YA, Liji X, Zhixun X: **Role of phosphatidylinositol 3-kinase (PI3K) and Akt1 kinase in porcine reproductive and respiratory syndrome virus (PRRSV) replication.** *Archives of virology* 2014, **159**(8).

14. Zeng H, Liu N, Yang YY, Xing HY, Liu XX, La GY, Huang MJ, Zhou MW: **Lentivirus-mediated downregulation of α-synuclein reduces neuroinflammation and promotes functional recovery in rats with spinal cord injury.** *Journal of neuroinflammation* 2019, **16**(1):283.

15. Mortezaee K, Khanlarkhani N, Beyer C, Zendedel A: **Inflammasome: Its role in traumatic brain and spinal cord injury.** *Journal of cellular physiology* 2018, **233**(7):5160-5169.

16. Huang SJ, Yan JQ, Luo H, Zhou LY, Luo JG: **IL-33/ST2 signaling contributes to radicular pain by modulating MAPK and NF-κB activation and inflammatory mediator expression in the spinal cord in rat models of noncompressive lumber disk herniation.** *Journal of neuroinflammation* 2018, **15**(1):12.

17. Paniagua-Torija B, Arevalo-Martin A, Molina-Holgado E, Molina-Holgado F, Garcia-Ovejero D: **Spinal cord injury induces a long-lasting upregulation of interleukin-1β in astrocytes around the central canal.** *Neuroscience* 2015, **284**.

18. N GE, J SW, C BT, E TH: **Protein tyrosine phosphatases: molecular switches in metabolism and diabetes.** *Trends in endocrinology and metabolism: TEM* 2015, **26**(1).

19. M S, M L, E G-T, E PG, D D, P Z-G, J MF, A R, Z H, D M-A et al: **Loss of Tribbles pseudokinase-3 promotes Akt-driven tumorigenesis via FOXO inactivation.** *Cell death and differentiation* 2015, **22**(1).

20. S FM, S B, F D, F M, A K, P G, V M: **Activation of Akt/FKHR in the medulla oblongata contributes to spontaneous respiratory recovery after incomplete spinal cord injury in adult rats.** *Neurobiology of disease* 2014, **69**.

21. Chiara DSM, Federico G, Cosimo CC, Miriam M, Emilio H: **Targeting PI3K signaling in cancer: Challenges and advances.** *Biochimica et biophysica acta Reviews on cancer* 2019, **1871**(2).

22. Huifang G, Peter G, Shanshan B, Sean B, Wei G, Xiangjie Q, Hongxiang L, Jiyong L, Eric J, B MG et al: **The PI3K/AKT Pathway and Renal Cell Carcinoma.** *Journal of genetics and genomics = Yi chuan xue bao* 2015, **42**(7).

23. Ethan C, Jianjiong G, Ugur D, E GB, Onur SS, Arman AB, Anders J, J BC, L HM, Erik L et al: **The cBio cancer genomics portal: an open platform for exploring multidimensional cancer genomics data.** *Cancer discovery* 2012, **2**(5).

24. Gambogic acid inhibits spinal cord injury and inflammation through suppressing the p38 and Akt signaling pathways. *Molecular Medicine Reports* 2018, **17**(1).
Table 1

Table 1 Selection of primers for qPCR experiments

| Gene name | Primer sequence (sense) | Primer sequence (antisense) |
|-----------|-------------------------|-----------------------------|
| β-actin   | GAAGATCAAGATCATTTGCTCCT | TACTCCTGCTTGCTGATCCA        |
| Il-1β     | GAGCTGAAAGCTCTCCACTTCTTCTTGGT | TTCCATCTTTCTTCTTTGGT        |
| AKT1      | GAGAACCTCATGCTGGAACAG   | GTCGTTGCTCCTCCAGCACTT       |
| PI3K      | TATGCTGCTCTGCTGTAGTGGT  | TAGTGACATTTAGGGAGT          |

Figures
Figure 1

Expression of IL-1β at the spinal cord. (A) BBB scores in the spinal cords of contused rats on days 1, 3, 5, 7, 14, and 28. Scores were significantly lower and increased gradually in the contusion group compared with the sham group. (B) Expression of IL-1β mRNA was detected by qPCR. Data showed that IL-1β mRNA drastically increased and peaked at 6 hours, but there was no obvious change on days 1 and 3 when compared with the sham group. (C, D) IL-1β was detected by Western blotting, with β-actin as standard value. Expression of IL-1β increased after spinal cord contusion when compared with the sham group. (E) Immunohistochemistry showed that IL-1β was located in the neurons of the anterior horn of the spinal cord. Bar = 40×10µm. (F) IL-1β positive cells were found in the sham group and significantly upregulated at 6 hours and on days 1 and 3. (G) Immunofluorescence double staining for IL-1β/NeuN in neurons of the anterior horn of the spinal cord. DAPI stained blue and IL-1β stained green, NeuN stained red. Co-expression of NeuN with IL-1β within the same cell showed orange (merged). Bar = 40×10µm. *P < .05 when compared with the sham group.
Figure 2

Preparation of IL-1β-RNAi-LV recombinants. (A) Schematic representation of IL-1β-RNAi-LV, in which, enhanced red fluorescent protein (mCherry FP) as the reporter gene was inserted into the plasmid. The framework also contains the antibiotic ampicillin and the plasma cloning vector pUC Ori, which were used as promoters for vector expression. (B) Screening of effective interference fragment for IL-1β inhibition showed that F3 exhibited the most effective interference of the three tested fragments. (C) Fluorescent image of IL-1β-RNAi-LV transfected into 293T cells.
Figure 3

IL-1β in rats following spinal cord contusion. (A) Expression of IL-1β mRNA was successfully decreased IL-1β-lentiviral group. Mean values in the RNAi group were lower than in the vector group. (B) IL-1β-positive staining was located in the cytoplasm of neurons in the anterior horn of the spinal cord. (C) Number of IL-1β-positive cells in the IL-1β-lentiviral group were decreased on days 7 and 28. *P < .05 when compared with the vector group. (D) BBB scores in the IL-1β-lentiviral group were significantly higher than the vector group on days 3, 5, 14, and 28.
IL-1β in rats with spinal cord contusion. (A) Bioinformatic analysis showed that IL-1β is related to AKT1 through arrestin beta 2 (Arrb2), tribbles homolog 3 (Trib3), tyrosine-protein phosphatase non-receptor type (PTPN2) genes. IL-1β are co-expressed with Arrb2, Trib3, and PTPN2; Arrb2, Trib3, and PTPN2 are co-expressed with AKT1. (B) Expression of AKT1 mRNA was detected by qPCR, which showed that expression of AKT1 mRNA was significantly increased on day 7 in the IL-1β-lentiviral group. (C) Expression of PI3K mRNA was detected by qPCR, which showed that expression of PI3K mRNA was not significant between the IL-1β-lentiviral group and vector group. (D) Immunofluorescence double staining of AKT/NeuN was showed DAPI staining blue (a) and NeuN staining green (b), AKT1 staining red (c). AKT1 was located in neurons of the anterior horn in the spinal cord (merged) and co-expressed with IL-1β. *P < .05 when compared to the vector group.