Int J Clin Exp Med 2018;11(10):10487-10495
www.ijcem.com /ISSN:1940-5901/IJCEM0073702

Original Article

Protective effect of Lithium on Schwann cell transplantation via Wnt/β-catenin signaling pathway after spinal cord injury in vitro and vivo

Lei Yang[1], Fan Yang[2], Jianrong Liu[1], Shuhu Sun[1], Linlin Wang[1], Xingxing Zhang[2], Feng Bi[3], Junhong Wang[4], Xiaoliang Wang[5], Haikang Zhao[1]

[1]Neurosurgery, The Second Affiliated Hospital of Xi'an Medical College, Xi'an 710038, Shaanxi Province, China; [2]Department of Obstetrics and Gynecology, The Second Affiliated Hospital of Xi'an Medical College, Xi'an 710038, Shaanxi Province, China; [3]Neurosurgery, People's Hospital of Dali County, Dali 715100, Shaanxi Province, China; [4]Neurosurgery, People's Hospital of Hu County, Xi'an 710300, Shaanxi Province, China; [5]Neurosurgery, Boai Hospital of Yanan City, Yanan 716000, Shaanxi Province, China

Received March 31, 2017; Accepted May 15, 2018; Epub October 15, 2018; Published October 30, 2018

Abstract: Schwann cell is one of the most widely studied cell types for repair of the spinal cord. Following the spinal cord injury (SCI). For transplantation, large numbers of Schwann cells are necessary to fill injury-induced cystic cavities. Lithium chloride could exhibit neuroprotection after SCI, however, the molecular mechanism of which remains unknown. This study demonstrated that lithium chloride exhibited neuroprotective effects on survival and proliferation of cultured SCs in vitro and in vivo of a SCI model via activating the Wnt/β-catenin signaling pathway. We constructed the Dominant-activeβ-cantenin-GFP tet-off lentivirus and obtained the Wnt signaling activated SCs in vitro. Then transplanted them to explore the effect on long-term survival, proliferation and regeneration of axons in the injured spinal cord of mice. Results showed that primary cultured SCs treated with lithium chloride significantly up-regulated the expression of β-catenin and decreased the β-catenin phosphorylation (p-β-catenin), improved the survival and proliferation of cultured SCs, and increased the axonal growth of dorsal root ganglion (DRG) in vitro; moreover, the same protective effects were also obtained with SCs transplantation after SCI in vivo.

Keywords: Schwann cells, spinal cord injury, Lithium chloride, Wnt/β-catenin signaling pathway

Introduction

Schwann cells (SCs) are dynamic participants in peripheral nerve function and repair, and have been widely studied as a therapy for central nervous system (CNS) injury. In the spinal cord injury (SCI) research, SCs have been studied in various contexts ranging from peripheral neuronal transplantation [1, 2], SC-seeded channel engraftment [3, 4] to cell suspension injection into or adjacent to the site of injury. Hormonal therapy combined with SC transplantation has also been reported [5, 6]. The potential benefits of SCs in the injured spinal cord are many [7]; nonetheless, key limitations to SC transplantation as a single therapy for SCI persist. Approximately 80% of transplanted cells die by necrosis and apoptosis by the end of the first week after transplantation [8]. SCs can support considerable growth of propriospinal neurons and axons of other origin [9], however, these fibers fail to survive and go through the lesion site area after transplantation into the host tissue. Adding a neuroprotective treatment prior to SC transplantation may enhance its survival and repair for spinal cord injury.

Lithium is a well-recognized direct inhibitor of the kinase activity of glycogen synthase kinase (GSK)-3 β, of which the main function is to make β-catenin phosphorylated and was degraded after entering the ubiquitin proteasome system. β-catenin is the key effector molecules of classical Wnt signaling pathway, and decides the activation of Wnt signaling pathway. Wnts are a family of glycoproteins that participate in neural development, axonal guidance, cell proliferation, and neural cell survival [10, 11]. Several studies suggested that the Wnt/β-catenin signaling pathway is activated after SCI

and promotes functional recovery [12-14]. In addition, the Wnt/β-catenin signaling pathway reportedly regulates apoptotic activity in several diseases, including acute respiratory distress syndrome and colorectal cancer [15, 16]. Work performed in zebrafish embryos and in SC lines implicated Wnt/β-catenin signals in SC proliferation and apoptosis and as positive regulators of myelination [17-19]. The role of Wnt/β-catenin signal in the late stage of SC development is unclear.

In this study, we hypothesized that lithium chloride exerts neuroprotective effects on SCI by activating the Wnt/β-catenin signaling pathway. We aimed to define the role of lithium chloride in SCs proliferation and survival in vitro, and its neuroprotective mechanism in SCI treatment.

The findings of this study may provide a novel mechanism by which lithium chloride exerts its protective effects on SCs survival and may be of potential therapeutic value in future treatments of SCI.

Materials and methods

Cell lines

Schwann cells (SCs) were purified and expanded as previously described [20]. In brief, SCs were harvested from sciatic nerves ligated KM mice provided by the animal experimental research center of The Fourth Military Medical University (Chongqing, China), followed by purification and expansion in culture for 7 days. The nerves were washed with PBS (0.01 M, ZSGB-BIO, China) for 3 times, stripping the epineurium tissue and cutting into pieces, digesting with 0.25% separation trypsin (Gibco, USA) at 37°C. After centrifugation, cells were supernatant with Dulbecco's modified Eagle Medium (DMEM) containing 10% FBS (Gibco, USA) and then plated onto 25 cm^2 culture-flasks which were handled by 10 g/ml Laminin at 37°C for 2 hours. Experiments were routinely performed using SCs collected at passages 3-5. Cultures were > 98% pure SCs upon collection for transplantation [21].

Dominant-active β-cantenin-GFP tet-off construction of lentiviral vector

SCs were infected with construction of Dominant-active β-cantenin-GFP tet-off lentivirus. The GFP infection group and the control group were digested by 0.125% trypsin to make cellular suspension for cell sorting by flow cytometry. GFP positive and negative cells were collected and cultured in vitro, respectively. Purified cells were collected for transplantation into the spinal lesion cavity.

Detection of survival and proliferation of SCs

After the suspension of the sample was stained with placental blue, the survival cells and dead cells were counted in 3 minutes. Each group of purified SCs was added 10 mol/L BrdU and incubated for 24 hours. BrdU incorporation was determined by fluorescence microscopy in fixed cells. For this, the cells were fixed with 4% paraformaldehyde and processed for BrdU detection according to the manufacturer's protocol. Additionally, SCs were labeled with S100 and nuclei were stained with Hoechst to reveal the total number of cells. Cultures were assayed in triplicate samples in each experimental condition.

DRG/SCs co-culture conditions in vitro

Cultures of purified embryonic dissociated dorsal root ganglion (DRG) neurons were established as described previously. The DRG bodies were dissected from new born mice and then dissociated with 0.25% trypsin at 37°C for 30 min. The cell suspensions were plated onto the GFP positive or negative SCs single cell layer and the Laminin coated plastic culture plate respectively as the cell number 5×10^5/ml. The culture medium was removed and replaced for the serum free medium after 24 hours. After co-cultured for 10 days, the cultures were fixed by 4% paraformaldehyde for 30 minutes using immunofluorescence staining to assay axonal growth.

SCI model

An SCI model was established using the modified weight-drop method as previously described (Yacoub et al. 2014). In brief, mice were intraperitoneal anesthesia by 10% chloral hydrate (5 ml/kg weight) and in a prone position. Under the operating microscope, the T8 spinous process was central, and the dorsal side of the spinal cord was opened to cut the dura mater, making the transection of spinal cord. Sham animals underwent surgery but did not receive injury.

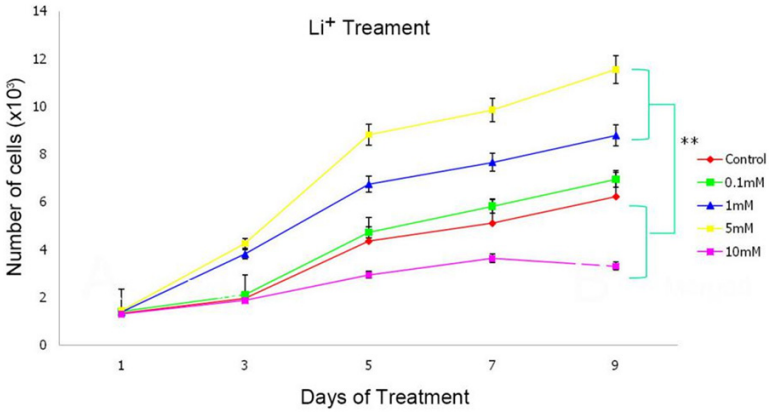

Figure 1. Effects of different dose of lithium chloride (0.1 mM, 1 mM, 5 mM and 10 mM) on SCs viability. Cells were cultured with or without lithium chloride for 9 days. The number of SCs in each group was counted every day. Data are expressed as the mean ± standard error of the mean of three independent experiments. Data are of at least three independent experiments. **, $P < 0.01$.

GFP negative SCs injection and sham operation group were taken as control groups; each group has least 12 mice. All animals received subcutaneous injection of 5 mL 0.9% saline for hydration and were monitored for 24 h in housing with controlled temperature. Animals were returned to the campus animal housing facility under veterinarian-guided observation and care upon recovery. A laboratory animal technician assisted in animal care. All procedures and surgeries were approved under the Guide for the Care and Use of Laboratory Animals.

GFP-SC transplantation

Transplantation of GFP-SCs was slightly modified from previously published methods [5]. Four weeks after injury, GFP-SCs were harvested from culture flasks using 0.125% Trypsin/EDTA, washed and suspended in DMEM with 10% FBS for cell transplantation. In preparation for transplantation, injured and sham rats were anesthetized and the surgical site was reopened. Two groups of animals, GFP positive SCs and GFP negative SCs were designated for SC transplantation into the lesion cavity epicenter. The spine was stabilized as described, and cell suspension (1×10^6 GFP-SCs in 5 μl DMEM medium) was stereotaxically injected into the lesion epicenter at a depth of 5 mm through a glass micropipette with an outer diameter of 50-70 μm and beveled sharpened tip at a rate of ~1 μl/min. After injection, the pipette was left in place for 2 min to prevent cell leakage. Animals not receiving SC injection were surgically opened and the sham laminectomy or injury site exposed. Following surgery, animals were allowed to recover under conditions described for the initial surgical procedures.

Western blot analysis

The western blot analysis was performed as described previously [22]. Briefly, the proteins were extracted from the harvested cells with radio immunoprecipitation assay (RIPA). A total of 20 μg proteins were supplement and separated with 10% SDS-PAGE gel, then transferred onto a PVDF membrane (Millipore). The membrane was incubated overnight at 4°C with primary antibody Rabbit β-catenin or p-β-catenin (1:1,000; Beyotime Institute of Biotechnology). Whenever appropriate, the unchanged expression of β-actin is shown as a control of equal cellular protein loading. Signals were detected by ECL substrate (Bio Rad, USA) and quantified using image analysis system Image J software.

Statistical analysis

Analysis between two groups was assessed by Student's *t* test and statistical significance between multiple groups was determined using a one-way ANOVA with post hoc analysis using the GraphPad Prism5 software (GraphPad, Inc., La Jolla, CA). All the experiments were repeated for at least three times. Data are presented as the mean and standard error of the mean (mean ± SD). Differences were termed statistically significant at $P < 0.05$. Differences were termed statistically significant at $P < 0.05$.

Results

Effects of lithium chloride on the growth activity of SCs

Compared with the control group, there is no significant difference in different concentrations of lithium chloride treatment after 24 h for the survival number of SCs. After 5 days, the

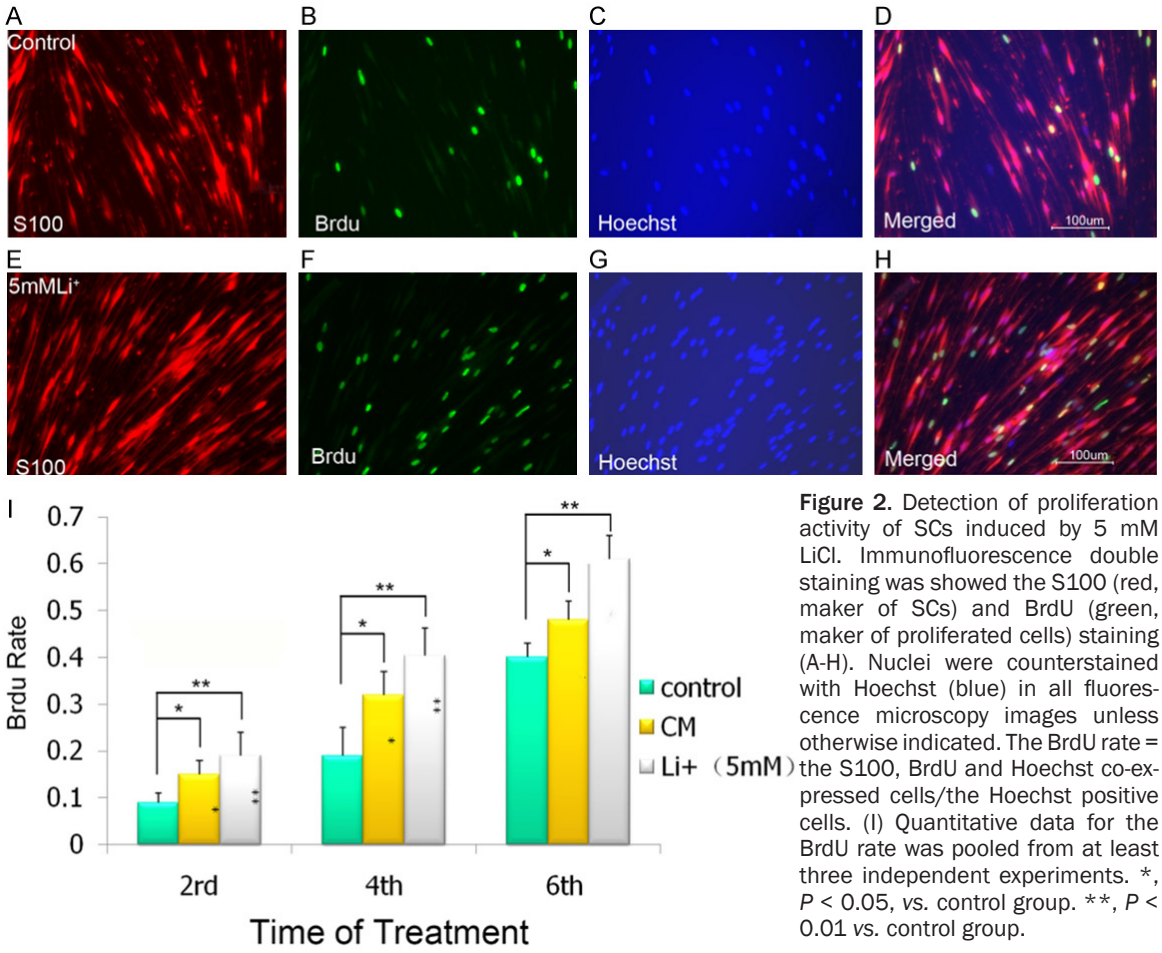

Figure 2. Detection of proliferation activity of SCs induced by 5 mM LiCl. Immunofluorescence double staining was showed the S100 (red, maker of SCs) and BrdU (green, maker of proliferated cells) staining (A-H). Nuclei were counterstained with Hoechst (blue) in all fluorescence microscopy images unless otherwise indicated. The BrdU rate = the S100, BrdU and Hoechst co-expressed cells/the Hoechst positive cells. (I) Quantitative data for the BrdU rate was pooled from at least three independent experiments. *, $P < 0.05$, *vs.* control group. **, $P < 0.01$ *vs.* control group.

survival number of SCs in 1 mM and 5 mM concentrations of lithium chloride was significantly increased compared to the control group ($P < 0.01$). Moreover, 5 mM concentration of lithium chloride was tested for having the best effect, and the low concentration (0.1 mM) has no obvious effect, however, the high concentration of lithium chloride (10 mM) plays a significant inhibitory effect on the survival of SCs, all the differences were statistically significant (**Figure 1**, $P < 0.01$).

5 mM lithium chloride induced proliferation activity of SCs

As having the best effects on the growth, we applied 5 mM lithium chloride to assay the effects of the proliferation activity of SCs via BrdU staining. The results showed that the number of S100 and BrdU positive SCs was significantly increased in lithium chloride treatment group compared to the control group (**Figure 2**, $P < 0.05$).

Effects of SCs pretreatment with lithium chloride on the growth of DRG axons

Immunofluorescence staining was used to detect the growth of DRG axons via the expression of neurofilament 200 (NF200). Results showed that the growth of neurons was slow when directly inoculated on Laminin, and only a few small protrusions were observed after 2 days. The average of the total neurite length and the longest projections was significantly increased in single neuron (**Figure 3**, $P < 0.05$).

Effects of lithium chloride on Wnt/β-catenin signaling activation

Western blot was used to test the expression of β-catenin and p-β-catenin protein after the lithium chloride treatment in SCs. The result showed that lithium chloride could significantly increase the expression of β-catenin and decrease the expression of p-β-catenin, sug-

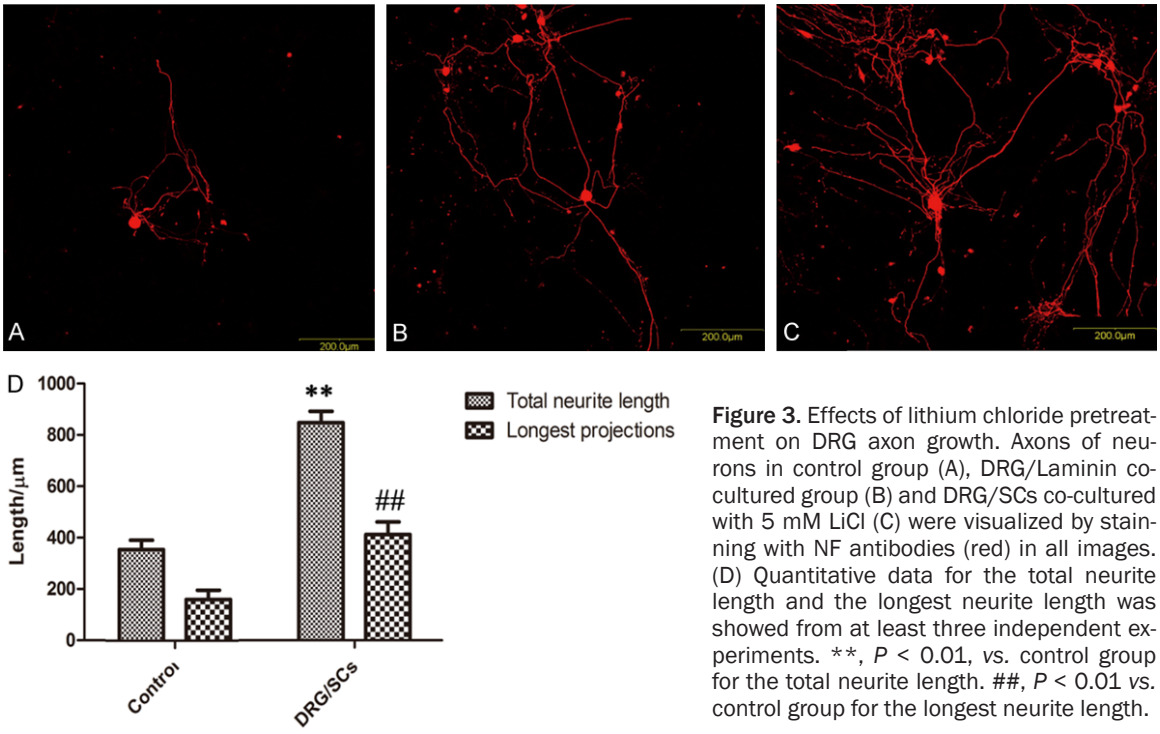

Figure 3. Effects of lithium chloride pretreatment on DRG axon growth. Axons of neurons in control group (A), DRG/Laminin co-cultured group (B) and DRG/SCs co-cultured with 5 mM LiCl (C) were visualized by staining with NF antibodies (red) in all images. (D) Quantitative data for the total neurite length and the longest neurite length was showed from at least three independent experiments. **, $P < 0.01$, *vs.* control group for the total neurite length. ##, $P < 0.01$ *vs.* control group for the longest neurite length.

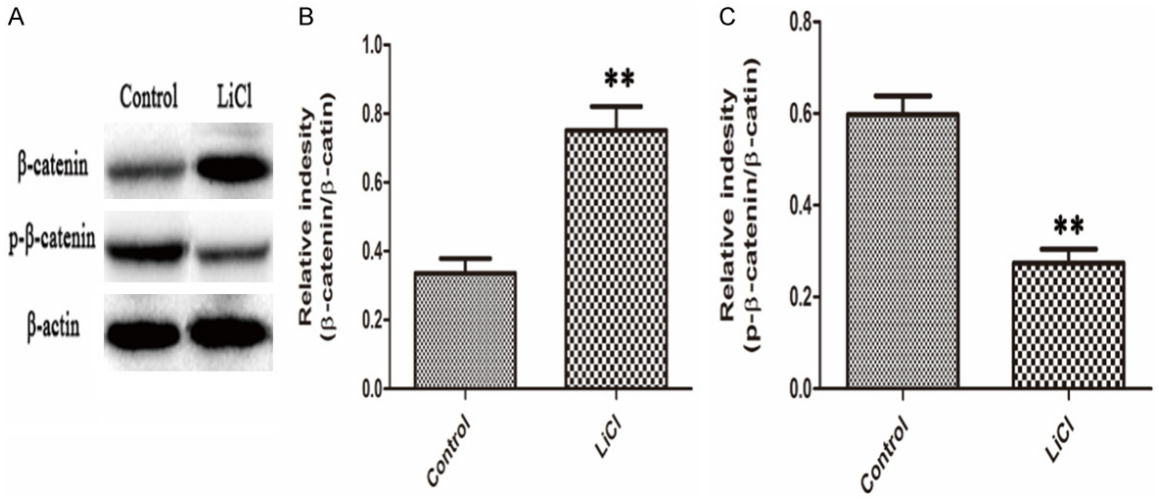

Figure 4. Western blot analysis was showed that β-catenin expression was increased and β-catenin phosphorylation expression was decreased in LiCl-treated SCs compared with the control (A-C). Cells were treated with LiCl and collected for western blot analysis at 24 h post-stimulation. Increased β-catenin levels are likely due to reduced degradation of the β-catenin protein in response to LiCl treatment. **, $P < 0.01$, *vs.* control group.

gesting that Wnt/β-catenin signaling activation may play an important role in SCs survival and proliferation (**Figure 4**).

Survival and migration of GFP-SCs in spinal cord injury area under continuous stimulation of lithium chloride

To assay the effect of SCs with lithium treatment induced Wnt/β-catenin signaling activation in spinal cord injury, the GFP labeled-β-cantenin-tet-off lentiviral vector was constructed by using the system of lentivirus to infect the primary culture of SCs and then the cell suspension was transplanted into spinal cord injured mice. The result was showed that there were a large number of transplanted SCs went through the lesion site and migrated long distances after 5 weeks transplantation (**Figure 6**).

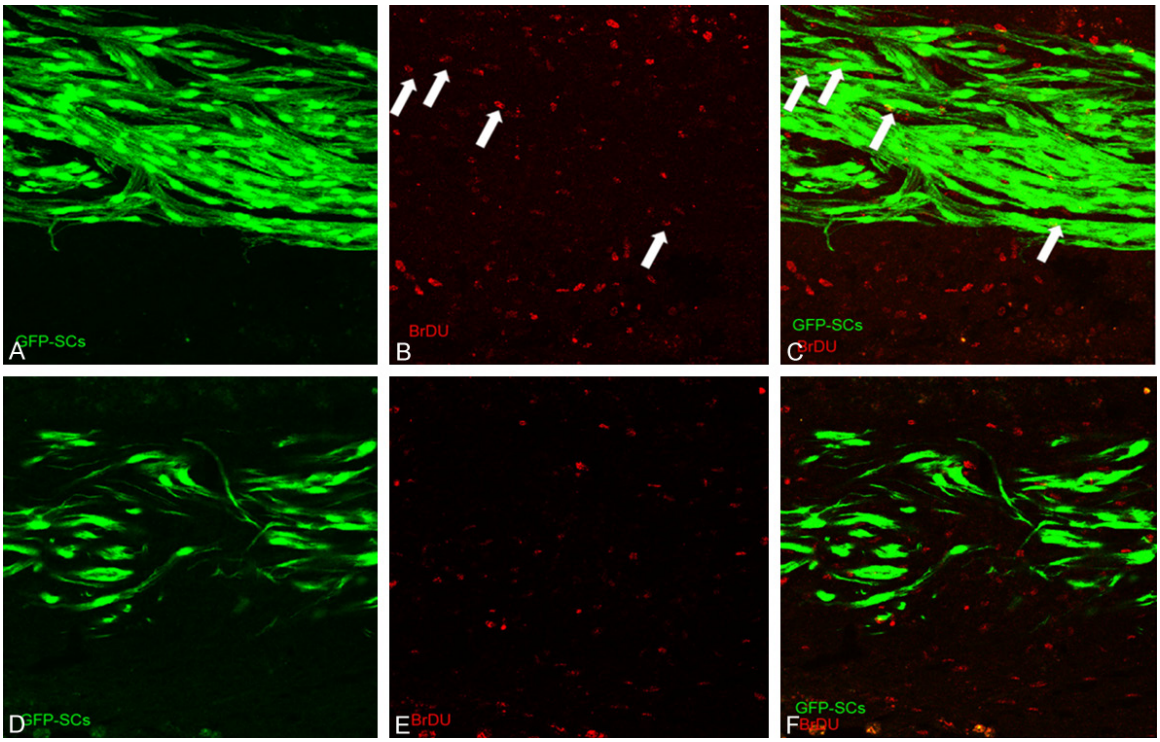

Figure 5. Proliferation of GFP-SCs after transplantation in vivo. Immunofluorescence double staining was showed the BrdU (red) and GFP-SCs (green) staining in the LiCl-treated group (A-C) and the control group (D-F).

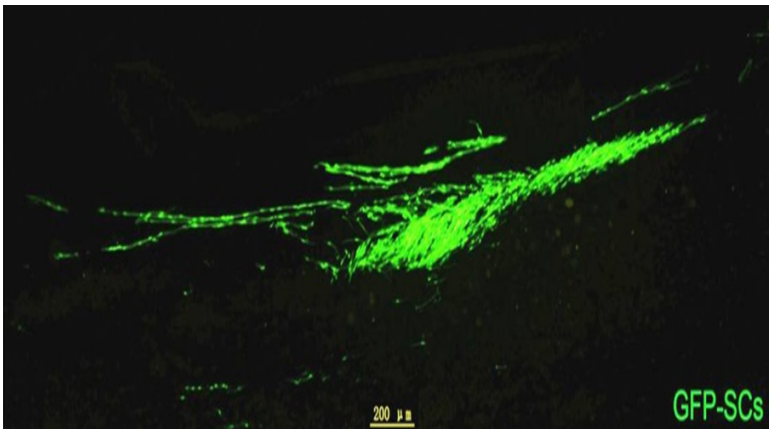

Figure 6. SCs transplantation promoted axonal growth within the lesion. Considerable axonal growth immunolabeled by GFP (green) into the lesion site was found after transplantation for 5 weeks.

Effects of lithium chloride on proliferation of GFP-SCs after transplantation

Immunofluorescence staining found that the transplanted group without lithium chloride treatment (**Figure 5D-F**) was not detected cell proliferation, however, there was still a small amount of proliferation in the lithium chloride treatment group (**Figure 5A-C**) after 5 weeks transplantation.

Effects of lithium chloride on remyelination after SCs transplantation

The transplanted SCs with lithium chloride treatment could support and guide the NF200 positive fibers and then to develop the re myelination at the junction of the lesion site area and the normal tissue (**Figure 7**).

Discussion

After spinal cord injury, axonal regeneration is crucial for significant functional recovery; however, neurons of the mature central nervous system (CNS) are known to have low regenerative ability [23]. The elegant studies demonstrated that the peripheral nerve milieu, mainly composed of SCs, was favorable for regeneration of injured CNS axons [24]. Since then, many therapeutic strategies containing SCs, such as SCs transplantation or SCs isolated have been established [25, 26], which strongly suggest the important role of SCs for repair after SCI. The potential

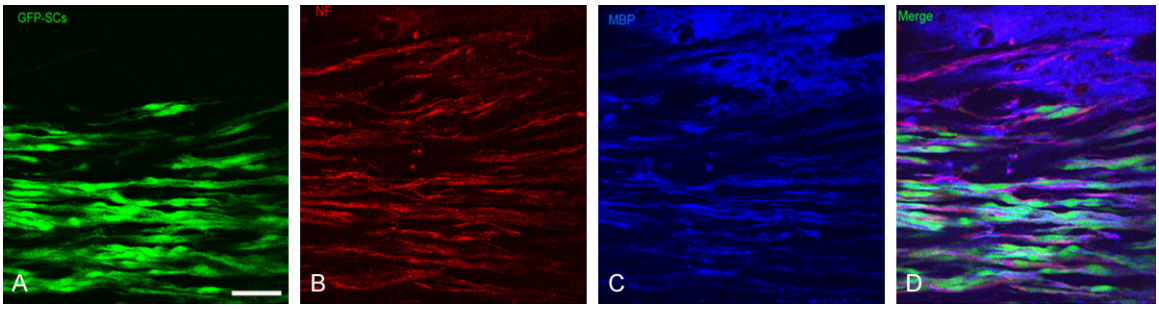

Figure 7. Effects of transplanted SCs on remyelination in vivo. Immunofluorescence triple staining was showed the GFP-SCs (A, green), NF (B, red) and MBP (C, myelin basic protein, blue) staining in the LiCl-treated group. The Merger of GFP-SCs, NF and MBP was showed as (D).

benefits of SCs in the injured spinal cord are existent, for example, key limitations to SCs transplantation as a single therapy for SCI. About 80% of transplanted SCs die by necrosis and apoptosis by the end of the first week after transplantation [8, 27]. Although SCs can support considerable growth of propriospinal [9], reticulospinal [28], raphespinal [29] and axons of other origin, these fibers fail to exit the graft into caudal host tissue due to the scars that encapsulates the contusive lesion.

Lithium modulates several of cellular processes by means of interaction with diverse intracellular signaling systems in a manner similar to ligands that activate tyrosine kinase and G protein-coupled receptors. The interaction of lithium with these signal pathways has provided a molecular basis for its widespread role in stem cell lineage specification [30], proliferation [31], and differentiation in a variety of cell types [32, 33].

In the present study, we first applied different concentrations of lithium chloride to detect the effects on the growth activity of SCs in vitro and found that 5 mM concentration of lithium chloride having the best effect; then immunofluorescence staining results showed that lithium chloride treatment SCs could make a benefit on the growth of DRG axons. Moreover, western blot showed that the expression of β-catenin protein in SCs treatment with lithium chloride group was increased but p-β-catenin expression was decreased significantly compared to the control group, suggesting that Wnt/β-catenin signaling was activated by lithium chloride, which could play a key role for the SCs survival.

Wnts are a family of glycoproteins that participate in neural development, axonal guidance, cell proliferation, and neural cell survival [10, 34]. Several studies suggested that the Wnt/β-catenin signaling pathway is activated after SCI and promotes functional recovery [13, 14]. In this study, to determine whether the Wnt/β-catenin signaling activation by lithium chloride was necessary for SCs survival and neuroprotection, the GFP labeled -β-catenin-tet-off lentiviral vector was constructed by using the system of lentivirus to infect the primary culture of SCs and then the cell suspension was transplanted into spinal cord injured mice. The results showed that the transplanted group without lithium chloride treatment was not detected cell proliferation; however, there was still a small amount of proliferation in the lithium chloride treatment group after 5 weeks transplantation. In addition, transplanted SCs with lithium chloride treatment group could support and guide the NF positive fibers and then to develop the re myelination at the junction of the lesion site area and the normal tissue, indicating that Wnt/β-catenin signaling activation induced by lithium chloride may have a critic role for SCs survival and neuroprotection after SCI.

In conclusion, our findings indicate that lithium chloride could make a benefit on the survival of SCs and the growth of DRG axons; Wnt/β-catenin signaling was activated by lithium chloride, which thus contributing to the survival of SCs and neuroprotection after SCI. Our study provides a theoretical basis and new insights into the treatment of SCI.

Acknowledgements

We thank the other researchers for the valuable technical assistance in this work. The authors have declared that they have no conflict of interests for this work and project.

Disclosure of conflict of interest

None.

Address correspondence to: Haikang Zhao, Neurosurgery, The Second Affiliated Hospital of Xi'an Medical College, No. 167, Fangdong Street, Baqiao District, Xi'an 710038, Shaanxi Province, China. E-mail: zhaohaik2@163.com

References

[1] Houle JD and Cote MP. Axon regeneration and exercise-dependent plasticity after spinal cord injury. Ann N Y Acad Sci 2013; 1279: 154-163.

[2] Tom VJ, Sandrow-Feinberg HR, Miller K, Domitrovich C, Bouyer J, Zhukareva V, Klaw MC, Lemay MA and Houle JD. Exogenous BDNF enhances the integration of chronically injured axons that regenerate through a peripheral nerve grafted into a chondroitinase-treated spinal cord injury site. Exp Neurol 2013; 239: 91-100.

[3] Deng LX, Deng P, Ruan Y, Xu ZC, Liu NK, Wen X, Smith GM and Xu XM. A novel growth-promoting pathway formed by GDNF-overexpressing Schwann cells promotes propriospinal axonal regeneration, synapse formation, and partial recovery of function after spinal cord injury. J Neurosci 2013; 33: 5655-5667.

[4] Deng LX, Hu J, Liu N, Wang X, Smith GM, Wen X and Xu XM. GDNF modifies reactive astrogliosis allowing robust axonal regeneration through Schwann cell-seeded guidance channels after spinal cord injury. Exp Neurol 2011; 229: 238-250.

[5] Hu JG, Wang XF, Deng LX, Liu NK, Gao X, Chen JH, Zhou FC and Xu XM. Cotransplantation of glial restricted precursor cells and Schwann cells promotes functional recovery after spinal cord injury. Cell Transplant 2013; 22: 2219-2236.

[6] Siriphorn A, Chompoopong S and Floyd CL. 17beta-estradiol protects Schwann cells against H2O2-induced cytotoxicity and increases transplanted Schwann cell survival in a cervical hemicontusion spinal cord injury model. J Neurochem 2010; 115: 864-872.

[7] Oudega M and Xu XM. Schwann cell transplantation for repair of the adult spinal cord. J Neurotrauma 2006; 23: 453-467.

[8] Hill CE, Hurtado A, Blits B, Bahr BA, Wood PM, Bartlett Bunge M and Oudega M. Early necrosis and apoptosis of Schwann cells transplanted into the injured rat spinal cord. Eur J Neurosci 2007; 26: 1433-1445.

[9] Iannotti C, Ping Zhang Y, Shields CB, Han Y, Burke DA and Xu XM. A neuroprotective role of glial cell line-derived neurotrophic factor following moderate spinal cord contusion injury. Exp Neurol 2004; 189: 317-332.

[10] Gonzalez-Fernandez C, Fernandez-Martos CM, Shields SD, Arenas E and Javier Rodriguez F. Wnts are expressed in the spinal cord of adult mice and are differentially induced after injury. J Neurotrauma 2014; 31: 565-581.

[11] Zhang S, Li J, Lea R, Vleminckx K and Amaya E. Fezf2 promotes neuronal differentiation through localised activation of Wnt/beta-catenin signalling during forebrain development. Development 2014; 141: 4794-4805.

[12] Fernandez-Martos CM, Gonzalez-Fernandez C, Gonzalez P, Maqueda A, Arenas E and Rodriguez FJ. Differential expression of Wnts after spinal cord contusion injury in adult rats. PLoS One 2011; 6: e27000.

[13] Suh HI, Min J, Choi KH, Kim SW, Kim KS and Jeon SR. Axonal regeneration effects of Wnt3a-secreting fibroblast transplantation in spinal cord-injured rats. Acta Neurochir (Wien) 2011; 153: 1003-1010.

[14] Sun L, Pan J, Peng Y, Wu Y, Li J, Liu X, Qin Y, Bauman WA, Cardozo C, Zaidi M and Qin W. Anabolic steroids reduce spinal cord injury-related bone loss in rats associated with increased Wnt signaling. J Spinal Cord Med 2013; 36: 616-622.

[15] Hsu HC, Liu YS, Tseng KC, Tan BC, Chen SJ and Chen HC. LGR5 regulates survival through mitochondria-mediated apoptosis and by targeting the Wnt/beta-catenin signaling pathway in colorectal cancer cells. Cell Signal 2014; 26: 2333-2342.

[16] Li B, Zeng M, He W, Huang X, Luo L, Zhang H and Deng DY. Ghrelin protects alveolar macrophages against lipopolysaccharide-induced apoptosis through growth hormone secretagogue receptor 1a-dependent c-Jun N-terminal kinase and Wnt/beta-catenin signaling and suppresses lung inflammation. Endocrinology 2015; 156: 203-217.

[17] Jacob C, Christen CN, Pereira JA, Somandin C, Baggiolini A, Lotscher P, Ozcelik M, Tricaud N, Meijer D, Yamaguchi T, Matthias P and Suter U. HDAC1 and HDAC2 control the transcriptional program of myelination and the survival of Schwann cells. Nat Neurosci 2011; 14: 429-436.

[18] Makoukji J, Shackleford G, Meffre D, Grenier J, Liere P, Lobaccaro JM, Schumacher M and Massaad C. Interplay between LXR and Wnt/beta-catenin signaling in the negative regulation of peripheral myelin genes by oxysterols. J Neurosci 2011; 31: 9620-9629.

[19] Tawk M, Makoukji J, Belle M, Fonte C, Trousson A, Hawkins T, Li H, Ghandour S, Schumacher M and Massaad C. Wnt/beta-catenin signaling is

an essential and direct driver of myelin gene expression and myelinogenesis. J Neurosci 2011; 31: 3729-3742.
[20] Morrissey TK, Kleitman N and Bunge RP. Isolation and functional characterization of Schwann cells derived from adult peripheral nerve. J Neurosci 1991; 11: 2433-2442.
[21] Xu XM, Chen A, Guenard V, Kleitman N and Bunge MB. Bridging Schwann cell transplants promote axonal regeneration from both the rostral and caudal stumps of transected adult rat spinal cord. J Neurocytol 1997; 26: 1-16.
[22] Wang WG, Chen SJ, He JS, Li JS and Zang XF. The tumor suppressive role of RASSF1A in osteosarcoma through the Wnt signaling pathway. Tumour Biol 2016; 37: 8869-8877.
[23] Steward O, Zheng B, Tessier-Lavigne M, Hofstadter M, Sharp K and Yee KM. Regenerative growth of corticospinal tract axons via the ventral column after spinal cord injury in mice. J Neurosci 2008; 28: 6836-6847.
[24] Bray GM, Villegas-Perez MP, Vidal-Sanz M and Aguayo AJ. The use of peripheral nerve grafts to enhance neuronal survival, promote growth and permit terminal reconnections in the central nervous system of adult rats. J Exp Biol 1987; 132: 5-19.
[25] Houle JD, Tom VJ, Mayes D, Wagoner G, Phillips N and Silver J. Combining an autologous peripheral nervous system "bridge" and matrix modification by chondroitinase allows robust, functional regeneration beyond a hemisection lesion of the adult rat spinal cord. J Neurosci 2006; 26: 7405-7415.
[26] Wiliams RR and Bunge MB. Schwann cell transplantation: a repair strategy for spinal cord injury? Prog Brain Res 2012; 201: 295-312.
[27] Hill CE, Moon LD, Wood PM and Bunge MB. Labeled Schwann cell transplantation: cell loss, host Schwann cell replacement, and strategies to enhance survival. Glia 2006; 53: 338-343.
[28] Schaal SM, Kitay BM, Cho KS, Lo TP Jr, Barakat DJ, Marcillo AE, Sanchez AR, Andrade CM and Pearse DD. Schwann cell transplantation improves reticulospinal axon growth and forelimb strength after severe cervical spinal cord contusion. Cell Transplant 2007; 16: 207-228.
[29] Novikova LN, Pettersson J, Brohlin M, Wiberg M and Novikov LN. Biodegradable poly-beta-hydroxybutyrate scaffold seeded with Schwann cells to promote spinal cord repair. Biomaterials 2008; 29: 1198-1206.
[30] Kim JS, Chang MY, Yu IT, Kim JH, Lee SH, Lee YS and Son H. Lithium selectively increases neuronal differentiation of hippocampal neural progenitor cells both in vitro and in vivo. J Neurochem 2004; 89: 324-336.
[31] Zhu Z, Yin J, Guan J, Hu B, Niu X, Jin D, Wang Y and Zhang C. Lithium stimulates human bone marrow derived mesenchymal stem cell proliferation through GSK-3beta-dependent beta-catenin/Wnt pathway activation. FEBS J 2014; 281: 5371-5389.
[32] Arioka M, Takahashi-Yanaga F, Sasaki M, Yoshihara T, Morimoto S, Hirata M, Mori Y and Sasaguri T. Acceleration of bone regeneration by local application of lithium: Wnt signal-mediated osteoblastogenesis and Wnt signal-independent suppression of osteoclastogenesis. Biochem Pharmacol 2014; 90: 397-405.
[33] Su H, Chu TH and Wu W. Lithium enhances proliferation and neuronal differentiation of neural progenitor cells in vitro and after transplantation into the adult rat spinal cord. Exp Neurol 2007; 206: 296-307.
[34] Zhang YP, Iannotti C, Shields LB, Han Y, Burke DA, Xu XM and Shields CB. Dural closure, cord approximation, and clot removal: enhancement of tissue sparing in a novel laceration spinal cord injury model. J Neurosurg 2004; 100: 343-352.