Scientific Research Publishing

Journal of Biosciences and Medicines, 2025, 13(3), 27-41
https://www.scirp.org/journal/jbm
ISSN Online: 2327-509X
ISSN Print: 2327-5081

SIRT1 Regulates Autophagy and Activation of Microglia through Smad3/TRIB3 Signaling Axis to Improve Spinal Cord Injury

Li Qian*, Fucui Tang*, Dekun Tang, Yuanmou Li, Wenyi Wang, Mei Qin, Na Yin, Ping Zhan, Xiaodan Liu#, Hongbo Chen#

Department of Rehabilitation Medicine, Qujing No. 1 Hospital, Qujing, China
Email: #18887428898@163.com, #18987435993@163.com

How to cite this paper: Qian, L., Tang, F.C., Tang, D.K., Li, Y.M., Wang, W.Y., Qin, M., Yin, N., Zhan, P., Liu, X.D. and Chen, H.B. (2025) SIRT1 Regulates Autophagy and Activation of Microglia through Smad3/TRIB3 Signaling Axis to Improve Spinal Cord Injury. *Journal of Biosciences and Medicines*, **13**, 27-41.
https://doi.org/10.4236/jbm.2025.133003

Received: January 16, 2025
Accepted: March 1, 2025
Published: March 4, 2025

Copyright © 2025 by author(s) and Scientific Research Publishing Inc.
This work is licensed under the Creative Commons Attribution International License (CC BY 4.0).
http://creativecommons.org/licenses/by/4.0/

Open Access

Abstract

Background and Purpose: Spinal cord injury (SCI) manifests as a central nervous system disorder causing sensory and motor impairments. Preventing an overabundance of microglial activation is crucial in managing SCI. SIRT1 as a NAD-dependent deacetylase has the effect of ameliorating SCI, but its mechanism of action in microglia activation is still poorly understood. The objective of this research was to explore the role and process of SIRT1 in activating microglia during SCI. **Methods:** Laminectomy was conducted on the thoracic T10 region of rats, followed by striking the revealed spinal cord using an IH-0400 spinal cord impactor to create a model for SCI animals (n = 30). To create a model of BV2 inflammation, BV2 cells underwent a 24 h treatment with 1 μg/mL LPS. The expression of related proteins was detected by Western blot. Immunofluorescence staining, HE staining and Nissl staining were used to evaluate BV2 cell activation and spinal cord tissue injury in rats. **Results:** The research indicated minimal expression of SIRT1 in SCI cases, with its heightened expression ameliorating pathological damage and boosting neuron survival rates in SCI rats. Conversely, treatment with LPS markedly reduced autophagy in BV2 cells. Elevated levels of SIRT1 enhanced the production of autophagy-associated proteins LC3-II/I and Beclin1, reduced p62 expression, and curtailed the expression of the activation indicator IBA-1 in BV2 cells. Mechanistically, overexpression of SIRT1 inhibits the expression of TRIB3 by inhibiting Smad3 nuclear metastasis, thereby activating autophagy, inhibiting microglial overactivation and alleviating the development of SCI. **Conclusion:** Enhancing SIRT1 expression could reduce SCI progression by curbing the overactivation of microglia.

*These authors contributed equally to this study.
#Corresponding author.

Keywords

Spinal Cord Injury, Microglial Activation, SIRT1, Smad3/TRIB3, Autophagy

1. Introduction

An injury to the spinal cord injury (SCI) may lead to significant damage to the central nervous system (CNS), potentially resulting in serious neurological impairments such as paralysis, incontinence, chronic pain, among others, and is marked by a high rate of morbidity and mortality [1]. A multitude of research indicates that SCI frequently coincides with intricate pathological and physiological alterations, primarily comprising two phases: the initial injury and the subsequent one, where the latter is crucial in restoring neurological abilities post-SCI [2] [3]. Multiple factors contribute to secondary injuries, primarily including blood-brain barrier malfunctions, neuroinflammatory reactions, activation of microglia, and neuronal demise [4] [5]. Microglia, residing in the CNS, play a role in sustaining CNS equilibrium through their interactions with both neurons and nonneuronal cells [6] [7]. Nonetheless, post-SCI, external triggers activate microglia, leading to the release of substantial quantities of inflammatory agents and mediators, thereby exacerbating the impairment of neurons' biological roles [8]. Consequently, exploring the processes that control the activation of microglia is crucial in treating SCI.

Lately, a growing body of research has been dedicated to examining autophagy's function in SCI [9] [10]. Autophagy is an important defense and protection mechanism of the body, preserving metabolism and equilibrium through the elimination of protein clusters and impaired organelles, and is involved in the emergence and progression of CNS disorders [11]. Earlier research has indicated that curcumin therapy can diminish neuronal cell death, enhance the health of the spinal cord, and curb inflammation through the initiation of autophagy, thus aiding in the restoration of spinal cord functionality in SCI model rats [12]. The enzyme SIRT1, belonging to the sirtuin family, is a well-preserved type of nicotinamide adenine dinucleotide-dependent class III protein deacetylase. Research indicates that SIRT1 is crucial in a range of metabolic and pathological functions, including controlling inflammation, autophagy, cell death, stress, differentiation, and the aging process [13] [14]. During SCI, MSC-sEVs triggered by microelectric fields can initiate autophagy via the MALAT1/miR-22-3p/SIRT1/AMPK pathway, thus reducing the rate of neuronal cell death [1]. Yet, research has not indicated any impact of SIRT1-regulated autophagy on microglia activation.

Sirt1 has been shown to mediate the deacetylation of Smad3 and inhibit TGF-β1-induced fibrosis [15]. In addition, recent studies have identified a potential role for Smad3 in autophagy [16]. As an illustration, Smad3 suppresses the TFEB-driven creation of lysosomes, leading to disrupted autophagy in DKD patients [17]. Furthermore, by blocking neuronal pyroptosis, Smad3 inhibitors are capable

of mitigating the onset of SCI in mice [18]. Conversely, TRIB3, a pseudokinase and part of the mammalian Tribbles analogs, is also crucial in controlling autophagy [19]. Earlier research indicates that TRIB3 enhances the growth and movement of glioblastoma cells by hindering autophagy [20]. Crucially, Smad3 plays a role in controlling the expression of TRIB3 [21]. Therefore, this study will explore the role of Smad3/TRIB3 signaling pathway in microglial autophagy and activation.

To sum up, the objective of this research was to thoroughly explore SIRT1's role and interplay in controlling microglial activation in SCI, and to offer innovative concepts and techniques for SCI therapy.

2. Materials and Methods

2.1. Construction of the Animal Model

For this research, thirty male SD rats, aged between 7 and 8 weeks and weighing 200 to 220 g, were acquired from Kunming Medical University's Animal Experiment Center. After 1 week of adaptive feeding, the experimental animals were randomly divided into 3 groups: Sham group, SCI group and SCI + OE-SIRT1 group, with 10 mice in each group. According to the previous research methods [22], rats' ventral areas were secured in a U-shaped stabilizer, anesthetized using 4% isoflurane, their back hair at T9 - T10 points was shaved using an electric shaver, followed by a T10 laminectomy. The modified Allen method was employed to induce SCI. A contusion was inserted into the T10 spinous process of the spinal cord using a specially designed IH-0400 spinal impactor (PSI, Lexington, KY, USA). In the sham group, rats underwent solely laminectomy without any contusions. Post-surgery, each rat was positioned on a heating pad until their complete recovery from anesthesia. To explore the effect of SIRT1 on rats with SCI, 3 days after SCI modeling, the lentivirus (2 μL) carrying OE-SIRT1 was diluted in PBS and then intrathecally injected using a glass micropipette. The injection was performed once a day for 3 consecutive days. The lentivirus carrying OE-SIRT1 was packaged by co-transfecting the plasmid encoding OE-SIRT1 and packaging plasmids (pMD2.G and psPAX2) into 293T cells. Subsequently, the cell supernatant containing the lentivirus was collected, and the lentivirus was concentrated and purified by ultracentrifugation. The titer of the purified lentivirus was determined by qPCR, and the concentration of the lentivirus used for intrathecal injection was 1×10^8 TU/mL. Finally, the successful transfection of the lentivirus in the spinal cord was verified by detecting the expression level of SIRT1 in the spinal cord tissue. The rats were sacrificed 28 days after surgery, and then a 10 mm long spinal cord (or the same spinal cord segment of normal rats) including the injury center was removed to detect the relevant indicators.

2.2. HE Staining

Tissues from the spinal cord, preserved in 4% paraformaldehyde, were encased in paraffin and sliced into sections 5 μm in thickness. Subsequently, the tissue samples underwent dewaxing in xylene for five minutes and were rehydrated using

gradient alcohols. Subsequently, the samples underwent a 15-minute staining process using hematoxylin (Solarbio, China), followed by two washes with tap water and a 2-minute eosin staining. After being dehydrated, transparent, and sealed with neutral resin (Sigma-Aldrich, USA), the stained spinal cord tissue was observed with a microscope (Nikon, Tokyo, Japan).

2.3. Nissl Staining

Spinal cord paraffin sections were deparaffinized by xylene, gradient alcohol hydration, washed with distilled water, stained with Nissl staining solution (Beyotime, China) for 5 min, then washed twice with distilled water, and the stained spinal cord tissue was observed with a microscope (Nikon, Tokyo, Japan).

2.4. BV2 Cell Culture and Treatment

BV2 mouse microglia, acquired from Shenzhen OTWO Biotech, were grown in cells enriched with 10% fetal calf serum (Gibco, Billings, MT, USA) and 100 U/mL of penicillin/streptomycin (Sigma, Saint Louis, MO, USA), using DMEM (Gibco, USA) in a standard incubator at 37˚C and 5% CO_2. Following earlier techniques [23], for creating the BV2 inflammation model, 1 μg/mL LPS (Solarbio, China) was introduced into the BV2 cell culture medium for a day. For evaluating autophagy in BV2 cells pre-LPS treatment, 5 mM autophagy inhibitor 3-MA (MedChem Express, USA) was introduced into the medium for two hours.

BV2 cells, once cultured, were grown in 24-well plates for an entire night. Upon achieving a cell density near 60% - 70%, there was an overexpression of SIRT1 (OE-SIRT1) and TRIB3 (OE-SIRT1), as per the guidelines provided by the Lipofectamine 3000 reagent (Invitrogen, USA). TRIB3) along with its negative control (OE-NC) (GenePharma, China) underwent transfection into BV2 cells, followed by cultivation at 37˚C in an incubator with 5% CO_2 for 48 hours, post which the efficiency of the transfection was assessed.

2.5. Western Blot Analysis

The complete protein was extracted from both rat spinal cord tissues and BV2 cells. Following the processes of lysis and centrifugation, proteins were collected. Protein concentrations were measured using a BCA protein concentration assay kit (Beyotime, China). Post mixing with a 5× concentration buffer (Beyotime, China), the samples underwent boiling at 100˚C for 10 minutes. Samples of protein (100 μg) underwent separation via SDS-PAGE and were then moved onto polyvinylidene fluoride membranes (Millipore, Billerica, MA). Following a 1-hour block using 5% milk at ambient temperature, the cells underwent an overnight incubation with the primary antibody at 4˚C. Subsequently, the membrane underwent incubation with a secondary antibody (1:4000, ab97051, Abcam, UK) at ambient temperature for an hour. The images were captured using a Fluor Chem E system (Protein Simple, San Jose, CA) and examined

through ImageJ software. Utilized primary antibodies included: SIRT1 (1:1000, ab110304, Abcam, UK), LC3B (1:2000, ab192890, Abcam, UK), Beclin1 (1:1000, ab210498, Abcam, UK), p62 (1:1000, ab109012, Abcam, UK), TRIB3 (1:2000, PA5-15480, Thermo Fisher Scientific, USA), and β-actin (1:1000, ab8226, Abcam, UK).

2.6. Immunofluorescence

BV2 cells underwent fixation in 4% paraformaldehyde, were made permeable using 0.5% Triton X-100 for a duration of 10 minutes, and then treated with bovine serum albumin for an hour. Following this, they were subjected to an overnight incubation at 4˚C with primary antibodies targeting IBA-1 (1:200, ab178846, Abcam, UK) or Smad3 (1:200, ab84177, Abcam, UK). Following a 1-hour incubation with the respective secondary antibody, the sections were marked with DAPI (Thermo Fisher Scientific, USA) for five minutes and examined using a fluorescence microscope (NiKon, Japan).

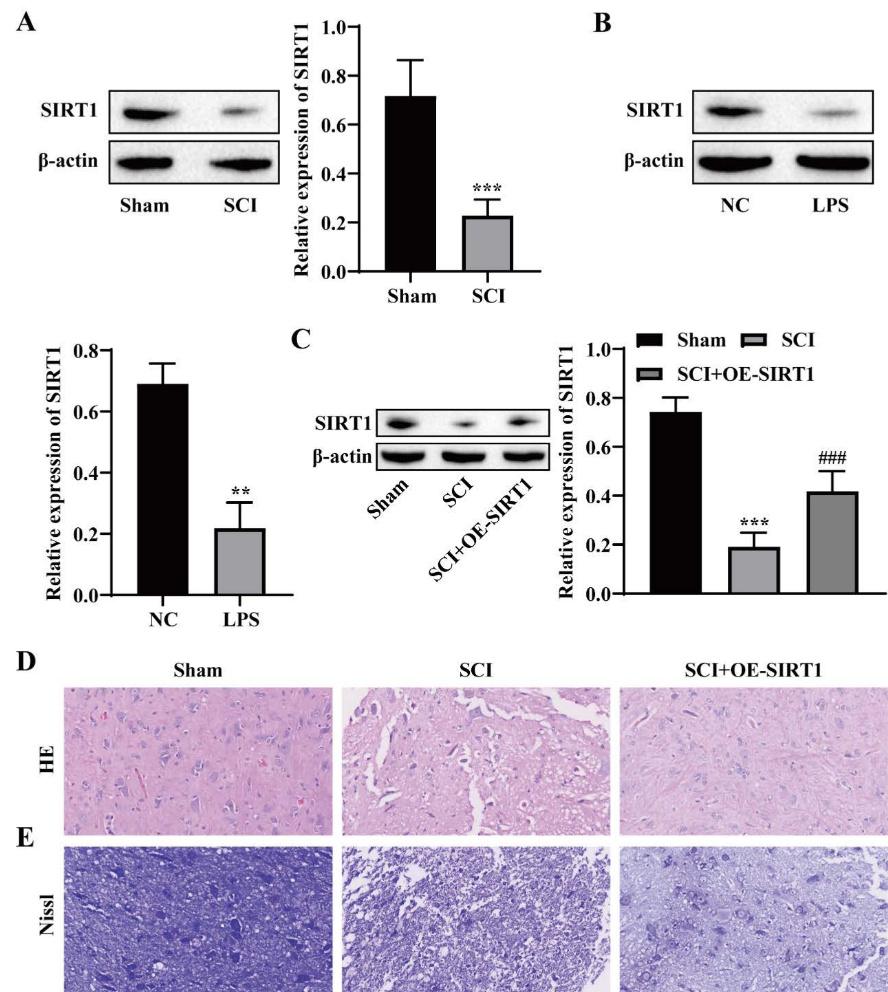
2.7. Statistical Analysis

The analysis and graphical representation of data were conducted using the GraphPad Prism version 8 software (GraphPad, USA). Data are expressed as mean ± standard deviation (SD), with each experiment being conducted a minimum of three times. Statistical analysis used *t*-test to evaluate the difference between the two groups of data. One-way ANOVA or two-way ANOVA were used to assess differences between multiple sets of data, and multiple comparisons were performed using Tukey's post-hoc test. A *P*-value below 0.05 was deemed to hold statistical significance.

3. Results

3.1. Overexpression of SIRT1 Improves Neural Recovery after SCI

We first examined the expression of SIRT1 in animal and cell models of SCI. The Western blot test showed a decrease in SIRT1 expression in SCI rats and LPS-stimulated BV2 cells relative to the control group (**Figure 1(A)**, **Figure 1(B)**). To detect the effect of SIRT1 on SCI rats, we overexpressed SIRT1 in rats. The results showed that the expression of SIRT1 in the SCI + OE-SIRT1 group was significantly up-regulated compared with the SCI group (**Figure 1(C)**). HE staining outcomes showed that the SCI group, in contrast to the sham group, exhibited spinal cord hemorrhage and structural harm, with SIRT1 overexpression lessening the extent of hemorrhage and damage in the affected spinal cord tissue (**Figure 1(D)**). Nissl staining outcomes showed a notable reduction in neuron count in the SCI group compared to the control group, with SIRT1 overexpression boosting neuron survival rates (**Figure 1(E)**). The above results showed that SIRT1 was under-expressed in SCI, and overexpression of SIRT1 could improve the neurological recovery after SCI in rats.



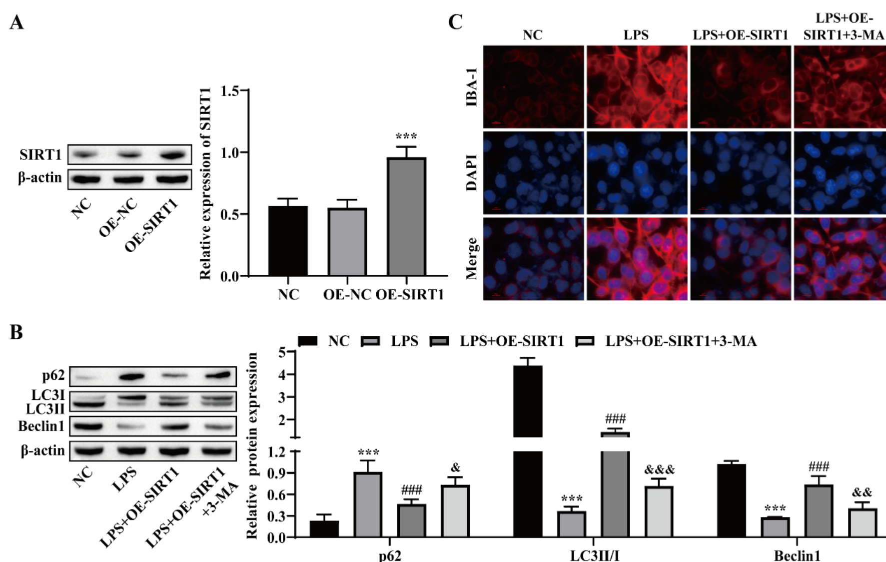
(A): Western blot was used to detect the expression of SIRT1 in rat spinal cord tissues; (B): Western blot was used to detect the expression of SIRT1 in BV2 cells; (C): Western blot was used to detect the expression of SIRT1 in rat spinal cord tissues; (D): HE staining was used to detect the histopathology of rat spinal cord; (E): Nissl staining was used to detect the survival of neurons in the spinal cord of rats. $^{**}P < 0.01$, $^{***}P < 0.001$ vs. Sham or NC; $^{###}P < 0.001$ vs. SCI.

Figure 1. Overexpression of SIRT1 improves neural recovery after SCI.

3.2. Overexpression of SIRT1 Inhibits Excessive Activation of BV2 Cells by Promoting Autophagy

As previously stated, triggering autophagy may facilitate the restoration of spinal cord activity in SCI model rats [12]. Investigating if SIRT1 influences BV2 cell activation through autophagy regulation, we increased SIRT1 levels in BV2 cells and added the autophagy inhibitor 3-MA. First, the transfection efficiency of SIRT1 was measured, and the results showed that the expression of SIRT1 in the OE-SIRT1 group was significantly increased (Figure 2(A)). Subsequently, the detection of autophagy markers was made. In contrast to the NC group, treatment with LPS notably enhanced p62 expression while suppressing LC3-II/I and Beclin1 expression. Overexpression of SIRT1 markedly reduced p62 expression

while enhancing LC3-II/I and Beclin1 levels. Further addition of 3-MA partially weakened the effect of overexpression of SIRT1 (**Figure 2(B)**). Finally, the expression of IBA-1, a marker of BV2 cell activation, was detected by immunofluorescence, and the results showed that compared with the NC group, LPS treatment significantly promoted the expression of IBA-1, overexpressed SIRT1 inhibited the expression of IBA-1, and further addition of 3-MA partially weakened the overexpression of SIRT1 (**Figure 2(C)**). These results suggest that overexpression of SIRT1 inhibits BV2 cell overactivation by promoting autophagy.

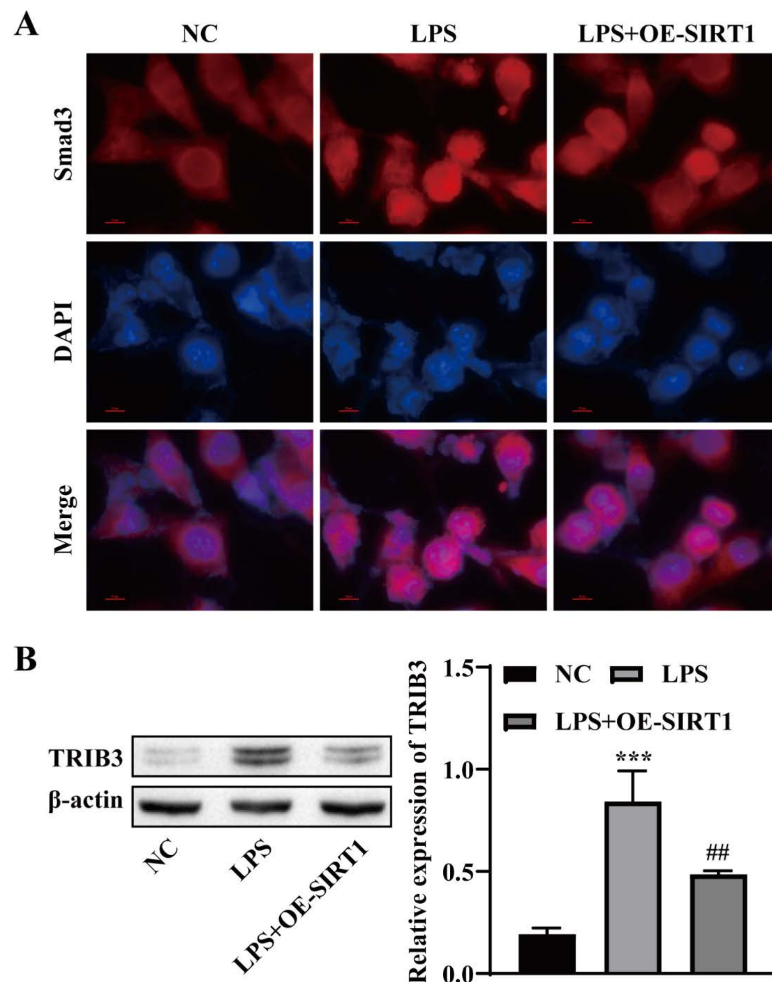


(A): The transfection efficiency of SIRT1 in BV2 cells was detected by Western blot; (B): Identification of autophagy-associated proteins p62, LC3-II/I, and Beclin1 in BV2 cells through Western blot; (C): Identification of the activation indicator IBA-1 in BV2 cells via immunofluorescence. $^{***}P < 0.001$ vs. NC; $^{###}P < 0.001$ vs. LPS; $^{\&}P < 0.05$, $^{\&\&}P < 0.01$, $^{\&\&\&}P < 0.001$ vs. LPS + OE-SIRT1.

Figure 2. Overexpression of SIRT1 inhibits excessive activation of BV2 cells by promoting autophagy.

3.3. SIRT1 Represses TRIB3 Expression by Inhibiting Smad3 Nuclear Translocation

Subsequently, we delve into the subsequent control process of SIRT1 and reveal that Smad3 and TRIB3 both play roles in autophagy regulation [17] [20]. Furthermore, SIRT1 is capable of controlling Smad3 [24]. Consequently, the regulatory influence of SIRT1 on the Smad3/TRIB3 signaling pathway was observed. The results of immunofluorescence showed that compared with the NC group, LPS treatment could promote the nuclear metastasis of Smad3, and further overexpression of SIRT1 inhibited the nuclear metastasis of Smad3 (**Figure 3(A)**). The results of Western blot showed that compared with the NC group, LPS treatment significantly promoted the expression of TRIB3, and further overexpression of SIRT1 inhibited the expression of TRIB3 (**Figure 3(B)**). The findings revealed that SIRT1 suppressed TRIB3 expression by hindering the nuclear transfer of Smad3.

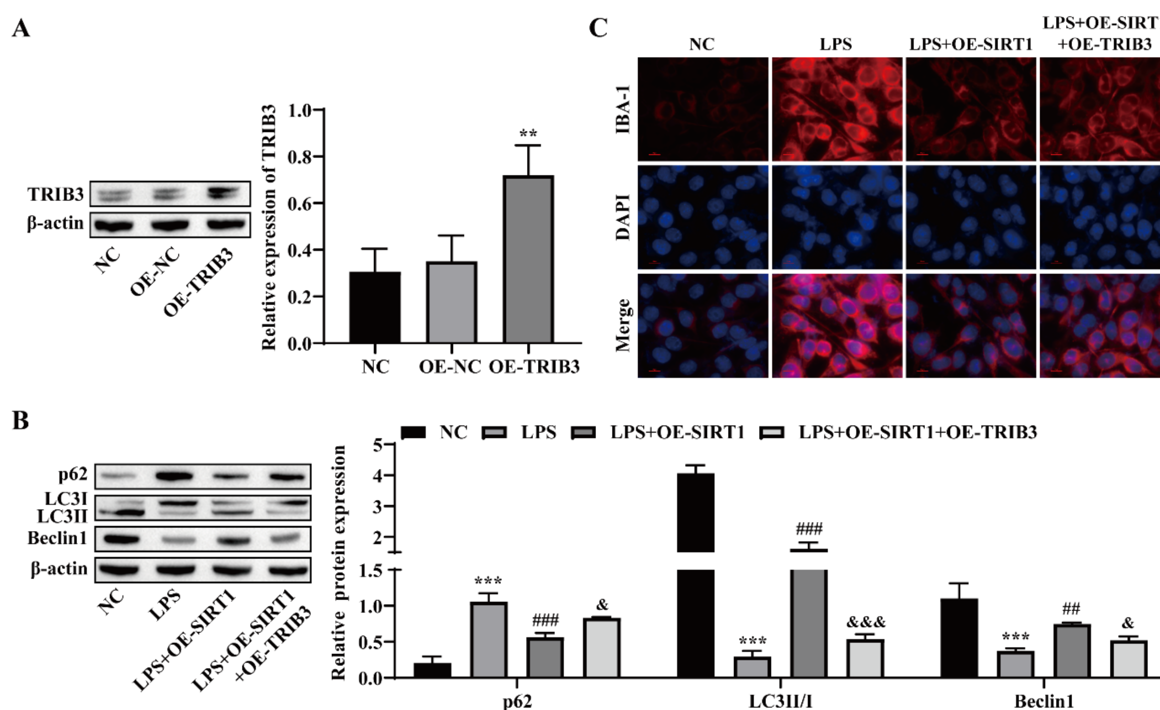


(A): Identification of Smad3 nuclear transfer in BV2 cells through immunofluorescence; (B): Identification of TRIB3 expression in BV2 cells via Western blot. $^{***}P < 0.001$ vs. NC; $^{\#\#}P < 0.01$ vs. LPS.

Figure 3. SIRT1 represses TRIB3 expression through the inhibition of Smad3 nuclear translocation.

3.4. SIRT1 Inhibits Excessive Activation of BV2 Cells through the TRIB3 Autophagy Pathway

To verify whether SIRT1 inhibits BV2 cell activation via the TRIB3 autophagy pathway, we transfected OE-TRIB3 into BV2 cells. The results showed that the expression of TRIB3 in the OE-TRIB3 group was significantly up-regulated (Figure 4(A)). Autophagy marker detection results showed that overexpression of TRIB3 could partially weaken the effect of overexpression of SIRT1, promote the expression of p62, and inhibit the expression of LC3-II/I and Beclin1 (Figure 4(B)). The immunofluorescence analysis of IBA-1, an activation marker, in BV2 cells showed that TRIB3 overexpression notably enhanced IBA-1 expression, in contrast to the LPS + OE-SIRT1 group (Figure 4(C)). The findings suggest that SIRT1 enhances autophagy by suppressing TRIB3 expression, thus preventing the overactivation of BV2 cells.



(A): Efficiency of TRIB3 transfection in BV2 cells via Western blot; (B): Identification of autophagy-associated proteins p62, LC3-II/I, and Beclin1 in BV2 cells through Western blot; (C): Identification of the activation indicator IBA-1 in BV2 cells via immunofluorescence. $^{**}P < 0.01$, $^{***}P < 0.001$ vs. NC; $^{\#\#}P < 0.01$, $^{\#\#\#}P < 0.001$ vs. LPS; $^{\&}P < 0.05$, $^{\&\&\&}P < 0.001$ vs. LPS + OE-SIRT1.

Figure 4. SIRT1 inhibits excessive activation of BV2 cells through the TRIB3 autophagy pathway.

4. Discussion

SCI, a central nervous system injury condition characterized by minimal reversibility, significant morbidity, and a high rate of disability, imposes a substantial strain on patients, families, society, and a high consumption of medical resources [25]. SCI results in oxidative stress, inflammation, ischemia, and neuronal death, significantly influencing patient prognoses. Consequently, it's critically important to discover an innovative and efficient strategy to decelerate the advancement of SCI. Latest research indicates a strong connection between SIRT1 and various neurodegenerative disorders, such as Alzheimer's, ischemic stroke, and CNS traumatic brain injury [26] [27]. Chen [28] *et al.* have been documented that the suppression of miR-138-5p curtails cellular apoptosis and diminishes inflammation through the elevation of SIRT1 expression, thus enhancing the progression of SCI. In addition, Chen [29] *et al.* documented that hyperbaric oxygen treatment curtailed the inflammatory sequence and cellular death by stimulating SIRT1, thus aiding in the recuperation of motor impairments in SCI rats. In this study, we also found that SIRT1 expression was down-regulated in SCI rats and LPS-induced BV2 cells, and overexpression of SIRT1 could improve neural recovery after SCI in rats. Our study further confirms the important role of SIRT1 in SCI recovery.

In contrast to primary irreversible SCI, secondary injury can be reversed as a pathological condition [30]. The primary factors exacerbating nerve damage and

leading to unfavorable neurological outcomes post-SCI are secondary injuries such as neuroinflammation, oxidative stress, lipid peroxidation, and electrolyte imbalance [31] [32]. Consequently, the crucial aspect in managing SCI lies in the methods of intervening and alleviating subsequent injuries. Microglia, as protectors of the nervous system, are rapidly stimulated following a spinal cord injury. Stimulated microglia emit substantial quantities of inflammatory agents and ROS, exacerbating the neuroinflammatory reaction and resulting in ROS buildup, which in turn leads to mitochondrial harm and oxidative stress [33] [34]. Furthermore, numerous deceased cells emit harmful substances, intensifying the neuroinflammatory reaction [35] [36]. The relentless sequence of neuroinflammation, oxidative stress, and mitochondrial harm significantly complicates the management of SCI. Therefore, intervening the activation of microglia is a key method to improve SCI. Research indicates that SIRT1 could suppress the activation of microglia by reducing Wnt/β-catenin signals post-SCI, thus serving a neuroprotective function [37]. Similarly, inhibition of HDAC3 can inhibit microglial activation by activating the SIRT1/Nrf2 signaling pathway, thereby improving SCI progression [38]. In this study, we also found that overexpression of SIRT1 inhibited LPS-induced BV2 cell activation. This study focused on the effect of SIRT1 overexpression on SCI rats, but the importance of its potential side effects on clinical transformation cannot be ignored. In this study, although the basic physiological indicators of the rats were monitored, there may still be some subtle changes that were not detected. In order to achieve clinical translation, more comprehensive safety assessments are needed in the future.

Furthermore, autophagy, crucial for the body's defense and defense, significantly contributes to the activation of microglia. As an illustration, blocking the PI3K/AKT/mTOR pathway may enhance autophagy, consequently preventing the activation of microglia [39]. In this study, we also found that overexpression of SIRT1 could inhibit the expression of autophagy marker p62 and promote the expression of LC3-II/I and Beclin1, while the addition of 3-MA partially weakened the overexpression of SIRT1 and promoted the activation of BV2 cells. In summary, we found that overexpression of SIRT1 inhibits BV2 cell overactivation by promoting autophagy. The results of this study further enrich the understanding of the role of SIRT1 in SCI. The new mechanism of action we discovered provides a new perspective for further understanding the pathophysiological processes and therapeutic targets of SCI.

Studies correlating SIRT1 reveal that SIRT1 has the ability to attach to Smad3, and SSR may amplify CKD's antifibrotic impact through the enhancement of the SIRT1/Smad3 deacetylation pathway [24]. Smad3, playing a crucial role in the TGF-β signaling pathway, has a close connection to biological mechanisms like inflammation, oxidative stress, and cellular apoptosis [40]. Li [41] *et al.* documented that stromal cells originating from adipose tissue can diminish the area of injury and aid in functional recuperation post-SCI through the activation of the TGF-β1/Smad3/PLOD2 pathway in spinal cord neurons. Liu [22] *et al.* docu-

mented that miR-10b-5p has the potential to alleviate SCI through the activation of the TGF-β1/Smad3 pathway. Furthermore, Smad3 influences the development of adipocytes linked to cancer through the activation of TRIB3 [21]. Furthermore, TRIB3 is significantly involved in controlling autophagy. As an illustration, cepharantine hampers autophagy by focusing on the TRIB3-FOXO3-FOXM1 pathway and makes gastric cancer cells more susceptible to chemotherapy [42]. The interaction between TRIB3 and SQSTM1 fosters tumor growth and advancement by hindering autophagy and proteasomal breakdown [43]. Therefore, we hypothesized that the Smad3/TRIB3 signaling pathway may be involved in SIRT1-regulated BV2 cell activation. We found that LPS treatment promoted Smad3 nuclear transfer and TRIB3 expression, while overexpression of SIRT1 inhibited TRIB3 expression by inhibiting Smad3 nuclear metastasis. In addition, overexpression of TRIB3 can promote the overactivation of BV2 cells by inhibiting autophagy, and partially weaken the effect of overexpression of SIRT1. Our study confirms the facilitating role of the Smad3/TRIB3 signaling pathway in BV2 cell activation.

In conclusion, our study demonstrated that overexpression of SIRT1 inhibits the expression of TRIB3 by inhibiting Smad3 nuclear metastasis, which in turn activates autophagy, inhibits microglial overactivation, and alleviates the development of SCI. Our study provides new ideas and targets for the treatment of SCI. However, there are some limitations to this study. First, the study was only conducted in rat models, and there are certain differences between animal models and human SCI, and the applicability of the results in humans needs to be further validated. Second, while the potential side effects of SIRT1 overexpression have been initially explored, the evaluation has not been comprehensive. In future studies, we are committed to validating it in larger-scale animal experiments to provide a more reliable basis for clinical translation. It is also necessary to further improve the safety evaluation system of SIRT1 overexpression to fully understand its potential side effects.

Funding

The study was supported by the Yunnan Provincial Department of Education Science Research [2023J0314].

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

References

[1] Li, K., Liu, Z., Wu, P., Chen, S., Wang, M., Liu, W., *et al.* (2023) Micro Electrical Fields Induced MSC-sEVs Attenuate Neuronal Cell Apoptosis by Activating Autophagy via lncRNA MALAT1/miR-22-3p/SIRT1/AMPK Axis in Spinal Cord Injury. *Journal of Nanobiotechnology*, **21**, Article No. 451. https://doi.org/10.1186/s12951-023-02217-2

[2] Quadri, S.A., Farooqui, M., Ikram, A., Zafar, A., Khan, M.A., Suriya, S.S., *et al.* (2018) Recent Update on Basic Mechanisms of Spinal Cord Injury. *Neurosurgical Review*,

43, 425-441. https://doi.org/10.1007/s10143-018-1008-3

[3] Kirshblum, S., Snider, B., Eren, F. and Guest, J. (2021) Characterizing Natural Recovery after Traumatic Spinal Cord Injury. *Journal of Neurotrauma*, **38**, 1267-1284. https://doi.org/10.1089/neu.2020.7473

[4] Li, Y., Gu, R., Zhu, Q. and Liu, J. (2018) Changes of Spinal Edema and Expression of Aquaporin 4 in Methylprednisolone-Treated Rats with Spinal Cord Injury. *Annals of Clinical & Laboratory Science*, **48**, 453-459.

[5] Caponegro, M.D., Torres, L.F., Rastegar, C., Rath, N., Anderson, M.E., Robinson, J.K., *et al.* (2018) Pifithrin-μ Modulates Microglial Activation and Promotes Histological Recovery Following Spinal Cord Injury. *CNS Neuroscience & Therapeutics*, **25**, 200-214. https://doi.org/10.1111/cns.13000

[6] Kettenmann, H., Hanisch, U., Noda, M. and Verkhratsky, A. (2011) Physiology of Microglia. *Physiological Reviews*, **91**, 461-553. https://doi.org/10.1152/physrev.00011.2010

[7] Nimmerjahn, A., Kirchhoff, F. and Helmchen, F. (2005) Resting Microglial Cells Are Highly Dynamic Surveillants of Brain Parenchyma *in vivo*. *Science*, **308**, 1314-1318. https://doi.org/10.1126/science.1110647

[8] Zhang, Y., Deng, Q., Hong, H., Qian, Z., Wan, B. and Xia, M. (2024) Caffeic Acid Phenethyl Ester Inhibits Neuro-Inflammation and Oxidative Stress Following Spinal Cord Injury by Mitigating Mitochondrial Dysfunction via the SIRT1/PGC1*α*/DRP1 Signaling Pathway. *Journal of Translational Medicine*, **22**, Article No. 304. https://doi.org/10.1186/s12967-024-05089-8

[9] Li, Y., Lei, Z., Ritzel, R.M., He, J., Li, H., Choi, H.M.C., *et al.* (2022) Impairment of Autophagy after Spinal Cord Injury Potentiates Neuroinflammation and Motor Function Deficit in Mice. *Theranostics*, **12**, 5364-5388. https://doi.org/10.7150/thno.72713

[10] Liao, H., Wang, Z., Ran, R., Zhou, K., Ma, C. and Zhang, H. (2021) Biological Functions and Therapeutic Potential of Autophagy in Spinal Cord Injury. *Frontiers in Cell and Developmental Biology*, **9**, Article 761273. https://doi.org/10.3389/fcell.2021.761273

[11] Kim, K.H. and Lee, M. (2014) Autophagy—A Key Player in Cellular and Body Metabolism. *Nature Reviews Endocrinology*, **10**, 322-337. https://doi.org/10.1038/nrendo.2014.35

[12] Li, W., Yao, S., Li, H., Meng, Z. and Sun, X. (2019) Curcumin Promotes Functional Recovery and Inhibits Neuronal Apoptosis after Spinal Cord Injury through the Modulation of Autophagy. *The Journal of Spinal Cord Medicine*, **44**, 37-45. https://doi.org/10.1080/10790268.2019.1616147

[13] Chen, H., Ji, H., Zhang, M., Liu, Z., Lao, L., Deng, C., *et al.* (2017) An Agonist of the Protective Factor Sirt1 Improves Functional Recovery and Promotes Neuronal Survival by Attenuating Inflammation after Spinal Cord Injury. *The Journal of Neuroscience*, **37**, 2916-2930. https://doi.org/10.1523/jneurosci.3046-16.2017

[14] Li, B., Li, W., Zheng, M., Wang, Y., Diao, Y., Mou, X., *et al.* (2023) Corilagin Alleviates Intestinal Ischemia/Reperfusion Injury by Relieving Oxidative Stress and Apoptosis via AMPK/Sirt1-Autophagy Pathway. *Experimental Biology and Medicine*, **248**, 317-326. https://doi.org/10.1177/15353702221147560

[15] Chen, Q., Zeng, Y., Yang, X., Wu, Y., Zhang, S., Huang, S., *et al.* (2022) Resveratrol Ameliorates Myocardial Fibrosis by Regulating Sirt1/Smad3 Deacetylation Pathway in Rat Model with Dilated Cardiomyopathy. *BMC Cardiovascular Disorders*, **22**, Article No. 17. https://doi.org/10.1186/s12872-021-02401-y

[16] Chien, L., Deng, J., Jiang, W., Chou, Y., Lin, J. and Huang, G. (2023) Evaluation of Lung Protection of Sanghuangporus Sanghuang through TLR4/NF-κB/MAPK, Keap1/Nrf2/HO-1, CaMKK/AMPK/Sirt1, and TGF-β/SMAD3 Signaling Pathways Mediating Apoptosis and Autophagy. *Biomedicine & Pharmacotherapy*, **165**, Article 115080. https://doi.org/10.1016/j.biopha.2023.115080

[17] Yang, C., Chen, X., Li, Z., Wu, H., Jing, K., Huang, X., *et al*. (2020) SMAD3 Promotes Autophagy Dysregulation by Triggering Lysosome Depletion in Tubular Epithelial Cells in Diabetic Nephropathy. *Autophagy*, **17**, 2325-2344. https://doi.org/10.1080/15548627.2020.1824694

[18] Zhu, J., Fu, Y. and Tu, G. (2020) Role of Smad3 Inhibitor and the Pyroptosis Pathway in Spinal Cord Injury. *Experimental and Therapeutic Medicine*, **20**, 1675-1681. https://doi.org/10.3892/etm.2020.8832

[19] Zhu, P., Xue, J., Zhang, Z., Jia, Y., Tong, Y., Han, D., *et al*. (2017) *Helicobacter pylori* VacA Induces Autophagic Cell Death in Gastric Epithelial Cells via the Endoplasmic Reticulum Stress Pathway. *Cell Death & Disease*, **8**, Article No. 3207. https://doi.org/10.1038/s41419-017-0011-x

[20] Tang, Z., Chen, H., Zhong, D., Wei, W., Liu, L., Duan, Q., *et al*. (2020) TRIB3 Facilitates Glioblastoma Progression via Restraining Autophagy. *Aging*, **12**, 25020-25034. https://doi.org/10.18632/aging.103969

[21] Gao, T., Li, J., Cheng, T., Wang, X., Wang, M., Xu, Z., *et al*. (2024) Ovarian Cancer-Derived TGF-β1 Induces Cancer-Associated Adipocytes Formation by Activating SMAD3/TRIB3 Pathway to Establish Pre-Metastatic Niche. *Cell Death & Disease*, **15**, Article No. 930. https://doi.org/10.1038/s41419-024-07311-3

[22] Liu, H., Liang, C., Liu, H., Liang, P. and Cheng, H. (2024) MiR-10b-5p Attenuates Spinal Cord Injury and Alleviates LPS-Induced PC12 Cells Injury by Inhibiting TGF-β1 Decay and Activating TGF-β1/Smad3 Pathway through PTBP1. *European Journal of Medical Research*, **29**, Article No. 554. https://doi.org/10.1186/s40001-024-02133-7

[23] Wang, C., Wang, Q., Lou, Y., Xu, J., Feng, Z., Chen, Y., *et al*. (2017) Salidroside Attenuates Neuroinflammation and Improves Functional Recovery after Spinal Cord Injury through Microglia Polarization Regulation. *Journal of Cellular and Molecular Medicine*, **22**, 1148-1166. https://doi.org/10.1111/jcmm.13368

[24] Wang, M., Yang, L., Yang, J. and Wang, C. (2019) Shen Shuai IIRecipe Attenuates Renal Injury and Fibrosis in Chronic Kidney Disease by Regulating NLRP3 Inflammasome and Sirt1/smad3 Deacetylation Pathway. *BMC Complementary and Alternative Medicine*, **19**, Article No. 107. https://doi.org/10.1186/s12906-019-2524-6

[25] Russo, G.S., Mangan, J.J., Galetta, M.S., Boody, B., Bronson, W., Segar, A., *et al*. (2020) Update on Spinal Cord Injury Management. *Clinical Spine Surgery*: *A Spine Publication*, **33**, 258-264. https://doi.org/10.1097/bsd.0000000000000956

[26] Cattelan, A., Ceolotto, G., Bova, S., Albiero, M., Kuppusamy, M., De Martin, S., *et al*. (2015) NAD^+-Dependent SIRT1 Deactivation Has a Key Role on Ischemia-Reperfusion-Induced Apoptosis. *Vascular Pharmacology*, **70**, 35-44. https://doi.org/10.1016/j.vph.2015.02.004

[27] Mei, Z., Huang, Y., Feng, Z., Luo, Y., Yang, S., Du, L., *et al*. (2020) Electroacupuncture Ameliorates Cerebral Ischemia/Reperfusion Injury by Suppressing Autophagy via the SIRT1-FOXO1 Signaling Pathway. *Aging*, **12**, 13187-13205. https://doi.org/10.18632/aging.103420

[28] Chen, J. and Qin, R. (2020) MicroRNA-138-5p Regulates the Development of Spinal Cord Injury by Targeting SIRT1. *Molecular Medicine Reports*, **22**, 328-336.

https://doi.org/10.3892/mmr.2020.11071

[29] Chen, H., Xing, R., Yin, X. and Huang, H. (2023) Activation of SIRT1 by Hyperbaric Oxygenation Promotes Recovery of Motor Dysfunction in Spinal Cord Injury Rats. *International Journal of Neuroscience*, **135**, 52-62. https://doi.org/10.1080/00207454.2023.2285707

[30] Ma, H., Wang, C., Han, L., Kong, F., Liu, Z., Zhang, B., *et al*. (2023) Tofacitinib Promotes Functional Recovery after Spinal Cord Injury by Regulating Microglial Polarization via JAK/STAT Signaling Pathway. *International Journal of Biological Sciences*, **19**, 4865-4882. https://doi.org/10.7150/ijbs.84564

[31] Hellenbrand, D.J., Quinn, C.M., Piper, Z.J., Elder, R.T., Mishra, R.R., Marti, T.L., *et al*. (2023) The Secondary Injury Cascade after Spinal Cord Injury: An Analysis of Local Cytokine/Chemokine Regulation. *Neural Regeneration Research*, **19**, 1308-1317. https://doi.org/10.4103/1673-5374.385849

[32] Maki, S., Furuya, T., Inoue, T., Yunde, A., Miura, M., Shiratani, Y., *et al*. (2024) Machine Learning Web Application for Predicting Functional Outcomes in Patients with Traumatic Spinal Cord Injury Following Inpatient Rehabilitation. *Journal of Neurotrauma*, **41**, 1089-1100. https://doi.org/10.1089/neu.2022.0383

[33] Dai, C., Qu, B., Peng, B., Liu, B., Li, Y., Niu, C., *et al*. (2023) Phosphoglycerate Mutase 5 Facilitates Mitochondrial Dysfunction and Neuroinflammation in Spinal Tissues after Spinal Cord Injury. *International Immunopharmacology*, **116**, Article 109773. https://doi.org/10.1016/j.intimp.2023.109773

[34] Patil, V., Bohara, R., Winter, C., Kilcoyne, M., McMahon, S. and Pandit, A. (2022) An Insight into New Glycotherapeutics in Glial Inflammation: Understanding the Role of Glycosylation in Mitochondrial Function and Acute to the Chronic Phases of Inflammation. *CNS Neuroscience & Therapeutics*, **29**, 429-444. https://doi.org/10.1111/cns.14016

[35] Wu, C., Wang, L., Chen, S., Shi, L., Liu, M., Tu, P., *et al*. (2023) Iron Induces B Cell Pyroptosis through Tom20-Bax-Caspase-Gasdermin E Signaling to Promote Inflammation Post-Spinal Cord Injury. *Journal of Neuroinflammation*, **20**, Article No. 171. https://doi.org/10.1186/s12974-023-02848-0

[36] Jiang, J., Xu, L., Yang, L., Liu, S. and Wang, Z. (2023) Mitochondrial-Derived Peptide Mots-C Ameliorates Spared Nerve Injury-Induced Neuropathic Pain in Mice by Inhibiting Microglia Activation and Neuronal Oxidative Damage in the Spinal Cord *via* the AMPK Pathway. *ACS Chemical Neuroscience*, **14**, 2362-2374. https://doi.org/10.1021/acschemneuro.3c00140

[37] Lu, P., Han, D., Zhu, K., Jin, M., Mei, X. and Lu, H. (2019) Effects of Sirtuin 1 on Microglia in Spinal Cord Injury: Involvement of Wnt/β-Catenin Signaling Pathway. *NeuroReport*, **30**, 867-874. https://doi.org/10.1097/wnr.0000000000001293

[38] Chen, S., Ye, J., Wu, G., Shi, J., Li, X., Chen, X., *et al*. (2023) Histone Deacetylase 3 Inhibition Ameliorates Microglia-Mediated Neuro-Inflammation via the SIRT1/Nrf2 Pathway after Traumatic Spinal Cord Injury. *Neurorehabilitation and Neural Repair*, **37**, 503-518. https://doi.org/10.1177/15459683231183716

[39] Li, J., Cheng, X., Fu, D., Liang, Y., Chen, C., Deng, W., *et al*. (2022) Autophagy of Spinal Microglia Affects the Activation of Microglia through the PI3K/AKT/mTOR Signaling Pathway. *Neuroscience*, **482**, 77-86. https://doi.org/10.1016/j.neuroscience.2021.12.005

[40] Chen, K., Chen, W., Liu, S.L., Wu, T.S., Yu, K.F., Qi, J., *et al*. (2018) Epigallocatechingallate Attenuates Myocardial Injury in a Mouse Model of Heart Failure through TGF-β1/Smad3 Signaling Pathway. *Molecular Medicine Reports*, **17**, 7652-

7660. https://doi.org/10.3892/mmr.2018.8825

[41] Li, F., Liu, H., Zhang, K., Xiao, D., Wang, C. and Wang, Y. (2021) Adipose-Derived Stromal Cells Improve Functional Recovery after Spinal Cord Injury through TGF-β1/Smad3/PLOD2 Pathway Activation. *Aging*, **13**, 4370-4387. https://doi.org/10.18632/aging.202399

[42] Lu, Y., Fang, Y., Wang, S., Guo, J., Song, J., Zhu, L., *et al*. (2024) Cepharanthine Sensitizes Gastric Cancer Cells to Chemotherapy by Targeting TRIB3-FOXO3-FOXM1 Axis to Inhibit Autophagy. *Phytomedicine*, **135**, Article 156161. https://doi.org/10.1016/j.phymed.2024.156161

[43] Hua, F., Li, K., Yu, J. and Hu, Z. (2015) The TRIB3-SQSTM1 Interaction Mediates Metabolic Stress-Promoted Tumorigenesis and Progression via Suppressing Autophagic and Proteasomal Degradation. *Autophagy*, **11**, 1929-1931. https://doi.org/10.1080/15548627.2015.1084458