doi: 10.4149/gpb_2024024

Down-regulated miR-10a protects against spinal cord injury by up-regulating SIRT1

Chao Song[1] and Yan Zhang[1]

[1] *Department of Orthopaedics, Gongli Hospital of Pudong New Area, Shanghai, China*

Abstract. MicroRNAs (miRNAs) are essential modulators of gene expression and are associated with various pathological processes, including spinal cord injury (SCI). This investigation aimed to elucidate miR-10a activity in SCI and its potential interaction with sirtuin 1 (SIRT1). The SCI rat model was established to assess hind limb movement, measure levels of miR-10a, SIRT1, neuronal survival, and inflammatory factors. An *in-vitro* SCI cell model was also developed to evaluate cell viability and inflammatory factor levels. The interaction between miR10a and SIRT1 was verified. Upregulated miR-10a and downregulated SIRT1 expression were found in the tissues of SCI rats. miR-10a knockdown in SCI rats enhanced the recovery of motor function, increased neuronal survival, and reduced the levels of inflammatory cytokines. Luciferase reporter assays confirmed that miR-10a targeted SIRT1 directly. In PC12 cells, downregulation of miR-10a increased SIRT1 expression, enhanced cell viability, and reduced inflammatory factor levels after LPS stimulation. Conversely, SIRT1 knockdown inhibited the protective effects of downregulated miR-10a on cell viability and inflammatory responses. The results suggest that miR-10a downregulation protects against SCI by upregulating SIRT1 expression, improving functional recovery, and reducing inflammation. Targeting the miR-10a/SIRT1 axis is a promising strategy for SCI treatment.

Key words: Spinal cord injury (SCI) — miR-10a — SIRT1 — Functional recovery — Inflammation

Abbreviations: 3'-UTRs, 3'-untranslated regions; AT, ambient temperature; BBB score, Basso, Beattie, and Bresnahan score; BDNF, brain-derived neurotrophic factor; BSCB, blood-spinal cord barrier; CFH, complement factor-H; IL-1β, interleukin 1β; IL-6, interleukin 6; LPS, lipopolysaccharide; miRNAs, microRNAs; MSCs, mesenchymal stem cells; MT, mutant; NF-κB, nuclear factor kappa B; SCI, spinal cord injury; SIRT1, Sirtuin 1; WT, wild-type.

Introduction

Spinal cord injury (SCI) is neurological damage that causes severe sensorimotor deficits and other complications (Ortega et al. 2023). The most frequent and severe disabilities caused by SCI include tetraplegia and paraplegia, resulting in marked impairment in affected individuals (González-Viejo et al. 2023). SCI pathophysiology is associated with complex mechanisms, including oxidative stress, inflammation, and apoptosis, contributing to secondary injury and worsening functional outcomes (Feng et al. 2023; Zuo et al. 2023). Although the underlying SCI pathophysiology has been comprehensively elucidated, effective treatment does not exist because of abnormal cellular and tissue response to the injury.

MicroRNAs (miRNAs) are non-coding RNAs that modulate gene expression by translational splicing and

Electronic Supplementary material. The online version of this article (doi: 10.4149/gpb_2024024) contains Supplementary material.

Correspondence to: Yan Zhang, Department of Orthopaedics, Gongli Hospital of Pudong New Area, No. 219 Miaopu Road, Shanghai, China, 200135
E-mail: rmyyzh@126.com

post-transcriptional inhibition (Busseau et al. 2023). They interact directly with the 3'-untranslated regions (3'-UTRs) of mRNAs, resulting in the inhibition of translation and suppression of the mRNA (DiVincenzo et al. 2023). Studies have shown the involvement of miRNAs in a variety of biological processes, including cell growth, differentiation, and apoptosis (Hu et al. 2023; Jeong and Hwang 2023; Ma et al. 2023). miR-10a has been reported to be involved in inflammation and apoptosis (Lu et al. 2023), and its expression is upregulated in epileptic hippocampal neurons, renal tissues affected by renal ischemia-reperfusion, and osteoarthritic tissues (Ma et al. 2019; Xu et al. 2020). Additionally, studies using miR-10a mimics have shown that it can enhance the expression of TNF-α, interleukin 1β (IL-1β), and interleukin 6 (IL-6) (Lu et al. 2023), while IL-1β-induced apoptosis was prevented by transfection with a miR-10a-5p inhibitor (Ma et al. 2019). Xu et al. (2020) found that the overexpression of miR-10a worsened renal damage and promoted cell apoptosis *in vivo*.

Moreover, miR-10a has been associated with several neurological disorders, such as Alzheimer's disease (Gui et al. 2015; Jia et al. 2021), Parkinson's disease (Jia et al. 2021), and ischemic stroke (Ryu et al. 2020), indicating its possible role in neurodegenerative mechanisms. Studies have demonstrated that suppression of miRNA-10a-5p enhanced the specialization of mesenchymal stem cells (MSCs) by specifically targeting brain-derived neurotrophic factor (BDNF). This process ultimately facilitated the healing of damaged tissues following SCI (Zhang T et al. 2020). Altered expression of several miRNAs has been observed in adult rats after traumatic SCI (Han T et al. 2023; Han Z et al. 2023; Yue et al. 2023). These results suggest that miRNAs may play an important role in SCI. However, the association of miR-10a in SCI requires further elucidation.

Sirtuin 1 (SIRT1) is a NAD+-dependent protein deacetylase that has been extensively studied for its roles in cellular homeostasis and stress responses (Barangi et al. 2023). SIRT1 has neuroprotective activity against various neurological disorders, such as SCI (Rao et al. 2023). It regulates various cellular processes, including inflammation, apoptosis, and oxidative stress, through its deacetylase activity and interaction with various transcription factors and co-regulators (Sun et al. 2022; Tang et al. 2022). Jiang et al.'s research demonstrated that an absence of SIRT1 in endothelial cells resulted in significant damage to the blood-spinal cord barrier (BSCB). This damage led to inflammation throughout the body, neural cell death, and poor functional recovery after SCI (Jiang et al. 2023). Furthermore, numerous studies have demonstrated that SIRT1 expression is reduced in tissues and cells affected by SCI. Conversely, upregulation of SIRT1 was found to reduce apoptosis in the cells of SCI models (Yu et al. 2019; Chen and Qin 2020). Additionally, SIRT1 serum levels were observed to be strongly correlated with the extent of the injury and thus may play a crucial role in determining the restoration of neurological functioning in SCI (Zhong et al. 2021).

In this study, the potential interaction sites between SIRT1 and miR-10a were investigated using bioinformatics analysis. It was hypothesized that miR-10a modulates the levels of SIRT1 and, therefore, could have therapeutic implications for SCI. This hypothesis was tested on *in vivo* and *in vitro* SCI models; furthermore, the functional recovery, neuronal survival, and inflammatory responses following the modulation of miR-10a and SIRT1 expression were assessed.

Materials and Methods

Animals

Sprague-Dawley rats (weight = 200–300 g, male, $n = 60$, age = 6–8 weeks) provided by the Jiesijie Experimental Animal Company (Shanghai, China) were housed under standard conditions of 23 ± 2°C, 12 h light/dark cycle, 40–60% relative humidity, and ad libitum chow and water. The authors' affiliated institutions granted approval for this research through the Institutional Review Board (IRB).

Rat SCI model development

The SCI model was developed as previously described (Gruner 1992; Khan et al. 1999). Briefly, 40 rats were anesthetized by intraperitoneal injections of pentobarbital (30 mg/kg), their skin was shaved, sterilized with betadine, and incised (20 mm) from the midline to expose the thoracic region of the vertebral column (T8–T11). The paravertebral muscle was dissected, and T10 laminectomy was performed to access the dorsal cord surface without disruption of the dura. To induce SCI, a 10 g rod was dropped from 5.0 cm height onto the T10 spinal cord level, after which the incision was sutured, and the animals were transferred to warm boxes for recovery. In the sham group, SCI induction was omitted, and only the surgical area was exposed ($n = 20$).

To elucidate the activity of miR-10a, Genechem (Shanghai, China) provided anti-miR-10a lentiviral vectors (LV-anti-miR-10a) and negative control (LV-NC) vectors. These were intrathecally injected in the rats 3 days before SCI induction (Sachdeva et al. 2020). The rats were then divided into the LV-anti-miR-10a ($n = 10$) and LV-NC ($n = 10$) groups. Seven days after the surgical procedure, correlations between miR-10a levels and brain function were examined using the Basso, Beattie, and Bresnahan (BBB) score. Blood samples were collected from the femoral vein and centrifuged (3000×*g*, 10 min) to obtain the serum. Subsequently, after intraperitoneal anesthesia by pentobarbital (40 mg/

kg), the animals were sacrificed by cervical dislocation, and their spinal cord tissues were sampled. The sequences of anti-miR-10a and anti-NC were as follows: anti-miR-10a, 5'-CACAAAUUCGGA UCUACAGGGUA-3'; anti-NC, 5'-CAGUACUUUUGUGUAGUACAA-3'.

Behavioral assessment

To elucidate the motor activities of the hind limbs, a BBB motor-rating scale was applied 7 days post-surgery (Wang et al. 2018). The scale assesses motor ability using a 21-point open-field locomotor scale, where 0 = no locomotion and 21 = normal motor functions. The rats' hindlimb movements, stability, trunk position, coordination, stepping, toe clearance, paw placement, and tail position were evaluated by two independent researchers, and the mean of their scored values was used.

Quantitative real-time polymerase chain reaction (qRT-PCR)

Total RNA was extracted from SCI tissues and PC12 cells using TRIzol (Invitrogen, Waltham, MA, USA), according to the provided directions. The RNA was then reverse-transcribed to cDNA using a cDNA Synthesis Kit (Applied Biosystems, MA, USA). qPCR was performed using SYBR Green Master Mix (Applied Biosystems) and an ABI Prism 7000 Sequence Detection system (Applied Biosystems). The experimental conditions were as follows: the temperature was set to 95°C and maintained for 5 min, followed by 40 cycles of PCR with steps at 95°C (30 s), 60°C (30 s), and 72°C (30 s). The miR-10a (sense primer: 5'-CGCG-CAAATTCGTATCTAGG-3'; antisense primer: 5'-AGT-GCAGGGTCCGAGGTATT-3') and U6 primers (sense primer: 5'-CTCGCTTCGGCAGCACA-3'; antisense primer: 5'-AACGCTTCACGAATTTGCGT-3'), were obtained from Sangon Biotech (Shanghai, China), with the U6 primer was used as a reference. The relative miR-10a expression was measured using the $2^{-\Delta\Delta Ct}$ method.

Western blotting

Tissues from SCI lesion site and treated PC12 cells were homogenized in RIPA lysis buffer (Beyotime, Shanghai, China) and the protein concentrations were measured using a BCA kit (Beyotime). The proteins were then separated on 8% SDS-PAGE gels and transferred to PVDF membranes. The membranes were incubated first with primary antibodies against SIRT1 (1:500, ab110304, Abcam, UK) and GAPDH (1:1,0000, ab8245, Abcam) antibodies and then a secondary antibody (1:1000, ab6789, Abcam). Bands were visualized by chemiluminescence (ECL Prime Western Blotting System; GE Healthcare) and quantified by ImageJ software (NIH, USA). The GAPDH was used as the loading control.

Immunohistochemistry

Samples of SCI tissues were embedded in paraffin, sectioned (4-μm thick), deparaffinized in xylene (Sigma-Aldrich, St Louis, MO, USA), treated with 3% H_2O_2, and heated for 30 min in citrate buffer for antigen retrieval. The sections were then incubated overnight with the primary anti-SIRT1 antibody (1:200, ab110304, Abcam) at 4°C, then for 45 min with the secondary antibody (1:1000, ab6789, Abcam) at ambient temperature (AT), stained with 3,3'diaminobenzidine (DAB), rinsed, and counterstained with hematoxylin for 30 s. Lastly, the samples were evaluated and imaged under a microscope (Olympus, Japan) and assessed by ImageJ software.

Nissl body staining

The sections were incubated overnight with anhydrous ethanol:chloroform (1:1 ratio) at AT, then with alcohol (95%) and absolute ethanol (100%), rinsed three times with distilled water, and stained at 37°C for 10 min with pre-warmed tar purple (0.1%, pH 3). The sections were then washed three times with distilled water, differentiated using alcohol (95%) for 5 min, dehydrated for 5 min with xylene and 100% anhydrous ethanol, and mounted. The effects of treatments on motor function were assessed by randomly selecting motor neurons from the anterior horn of five stained sites.

Enzyme-linked immunosorbent assays (ELISA)

The levels of TNF-α (KRC3011) and IL-6 (BMS625) in sera and culture supernatants were measured using rat ELISA Kits (Invitrogen), following the guidelines of the kits. Absorbance was measured at 450 nm using a microplate reader (Bio-Rad, CA, USA).

Cell culture and transfection

PC12 cells (American Type Culture Collection) were grown in Dulbecco's Modified Eagle Medium (DMEM; Gibco, Waltham, MA, USA) supplemented with 10% fetal bovine serum (FBS, Gibco) and 1% penicillin/streptomycin at 37°C in a humidified incubator with 5% CO_2.

Genepharma (Shanghai, China) provided the SIRT1 small interfering RNA (si-SIRT1, 5'-GAACAAAGUU-GACGAUUUAGATT-3') and negative control (si-control, 5'- UUCUCCGAACGUGUCACGUTT-3'); the cells were transfected with LV-anti-miR-10a alone or with si-control or si-SIRT1 *via* Lipofectamine 3000 (Invitrogen), according to the provided directions. The transfection efficiency of the cells was measured at 48 h after transfection using Western blotting and qRT-PCR analysis.

PC12 cells were exposed to lipopolysaccharides (LPS, L2630, Sigma-Aldrich) at concentrations of 50, 100, and 200 ng/ml for 6 h. The MTT assay revealed about a 50% decrease in cell viability at a concentration of 100 ng/ml (Supplementary material, Figure S1), prompting its selection for further experiments aimed at establishing an *in vitro* model of SCI (Ding et al. 2023).

Dual-luciferase reporter gene analysis

TargetScan was used to predict the putative miR-10a and SIRT1 binding sites. The 3'-UTR of the SIRT1 sequence was amplified and cloned into a pGL3 luciferase reporter vector (Promega, Madison, WI, USA) to establish a wide-type plasmid (SIRT1-WT). For the mutant-type (SIRT1-MT) plasmid, a Directed Mutagenesis system (Invitrogen) was utilized. PC12 cells were co-transfected with SIRT1-WT or -MT plasmids and miR-10a mimic (sense, 5'-CAAAUUCGGAUCUACAGGGUAUU-3', and anti-sense, 5'-UACCCUGUAGAUCCGAAUUUGUG-3') or miR-NC (sense, 5'-UUCUCCGAACGUGUCACGUTT-3', and anti-sense, 5'-UACCCUGUAGAUCCGAAUUUGUG-3') using Lipofectamine 3000 (Invitrogen), according to the provided instructions. The luciferase activities in lysed cells were assessed using a Luciferase Assay Kit (GeneCopoeia, Rockville, MD, USA).

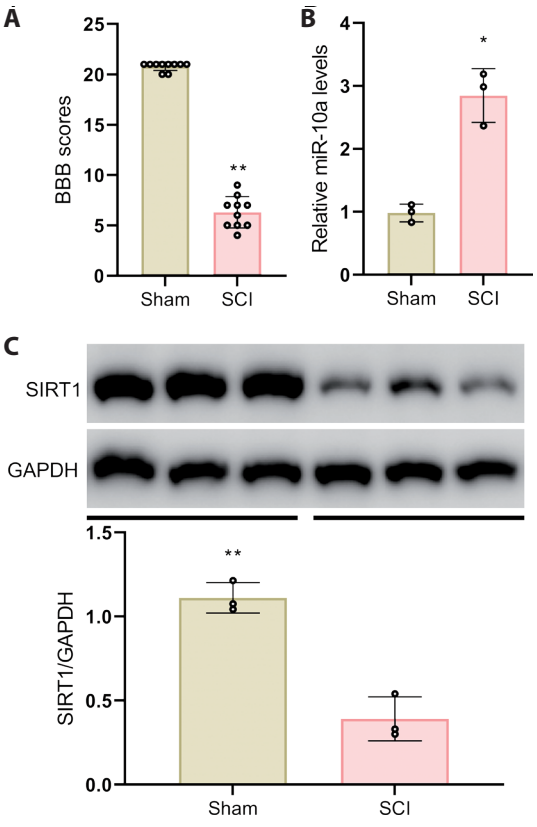

Figure 1. miR-10a and SIRT1 expression in spinal cord tissues. **A.** BBB score was used to assess hindlimb motor function 7 days after SCI induction (n = 10). **B.** miR-10a expression in the spinal cord tissues of sham and SCI rat models was quantified *via* qRT-PCR (n = 3). **C.** SIRT1 protein expression was assessed by Western blotting (n = 3). * $p < 0.05$, ** $p < 0.01$.

MTT assay

PC12 cells (1×10^5 *per* well) were seeded in 96-well plates and grown at 37°C for 24 h. After removal of the culture supernatants, the cells were incubated with LV-anti-miR-10a, LV-anti-miR-10a+si-control, or LV-anti-miR-10a+si-SIRT1 for 48 h at 37°C, after which the cells were treated with LPS (100 ng/ml) for 6 h. Subsequently, 10 µl of MTT reagent were then added to each well and incubated for 4 h at 37°C. The formazan crystals were then dissolved in DMSO (100 µl; Nanjing KeyGen Biotech Co., Ltd.), and absorbances at 490 nm were measured in a microplate reader.

Statistical measurements

Data were analyzed using SPSS 20.0 (IBM Corp., Armonk, NY, USA) and are presented as mean ± SD. Intergroup comparisons were performed with Student's t-tests, and multi-group comparisons, with one-way ANOVA. $p < 0.05$ was considered statistically significant.

Results

Altered expression of miR-10a and SIRT1 in SCI tissues

The BBB scores in the SCI group were markedly lower than those in the control group, indicating that rats' hindlimb motor functions were significantly affected (Fig. 1A). miR-10a expression in SCI tissues was significantly increased (Fig. 1B), while expression of SIRT1 was markedly decreased in SCI tissues (Fig. 1C) relative to the control group.

Knockdown of miR-10a promoted recovery of motor function and inhibited inflammation in SCI rats

The *in vivo* effect of miR-10a after SCI surgery was assessed by LV-anti-miR-10a treatment. miR-10a levels in the spinal cords of the SCI rats were reduced by miR-10a knockdown (Fig. 2A), while SIRT1 levels were increased (Fig. 2B,C). Furthermore, examination of the BBB scores indicated that miR-10a knockdown improved neurological function in SCI rats compared with the LV-NC treatment (Fig. 3A). To further elucidate the neuroprotective effects induced by miR-10a knockdown, Nissl bodies were stained and evaluated in the anterior horns of the spinal cords (Fig. 3B). The SCI group

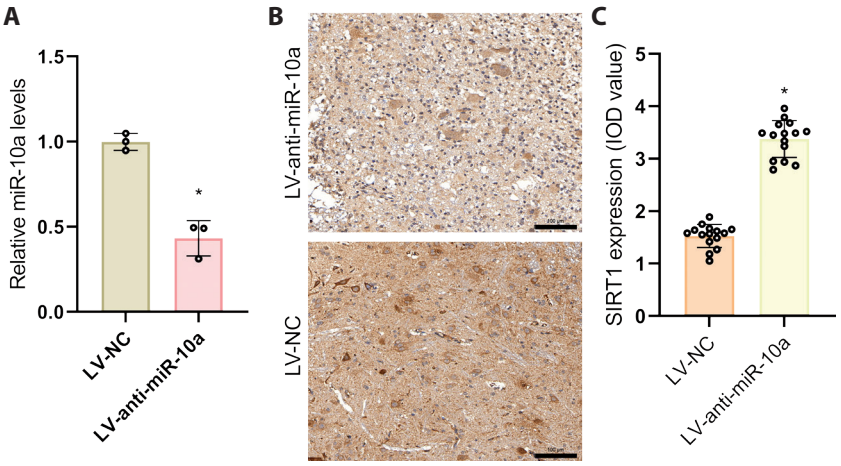

Figure 2. miR-10a knockdown increased SIRT1 expression in SCI rats. **A.** The miR-10a expression in spinal cord tissues of SCI rats injected with LV-NC or LV-anti-miR-10a was elucidated by qRT-PCR (n = 3). **B.,C.** Immunohistochemistry was used to measure the levels of SIRT1 in spinal cord tissues (n = 3, 2 cm length of the spinal cord centered at the injury site). * $p < 0.05$, scale bar = 100 μm.

showed fewer Nissl bodies compared to the sham group. Conversely, there was a significant increase in the number of Nissl bodies observed in the SCI+LV-anti-miR-10a group when compared to the SCI group (Fig. 3C). Unsurprisingly, LV-anti-miR-10a reduced the serum levels of TNF-α (Fig. 3D) and IL-6 (Fig. 3E) in SCI rats.

miR-10a is predicted to target SIRT1 specifically

A potential binding site for miR-10a was predicted in the SIRT1-3'-UTR sequence (Fig. 4A). Transfection with the SIRT1 wild-type (WT) and mutant (MT) luciferase vectors revealed that miR-10a reduced the luciferase activity of the

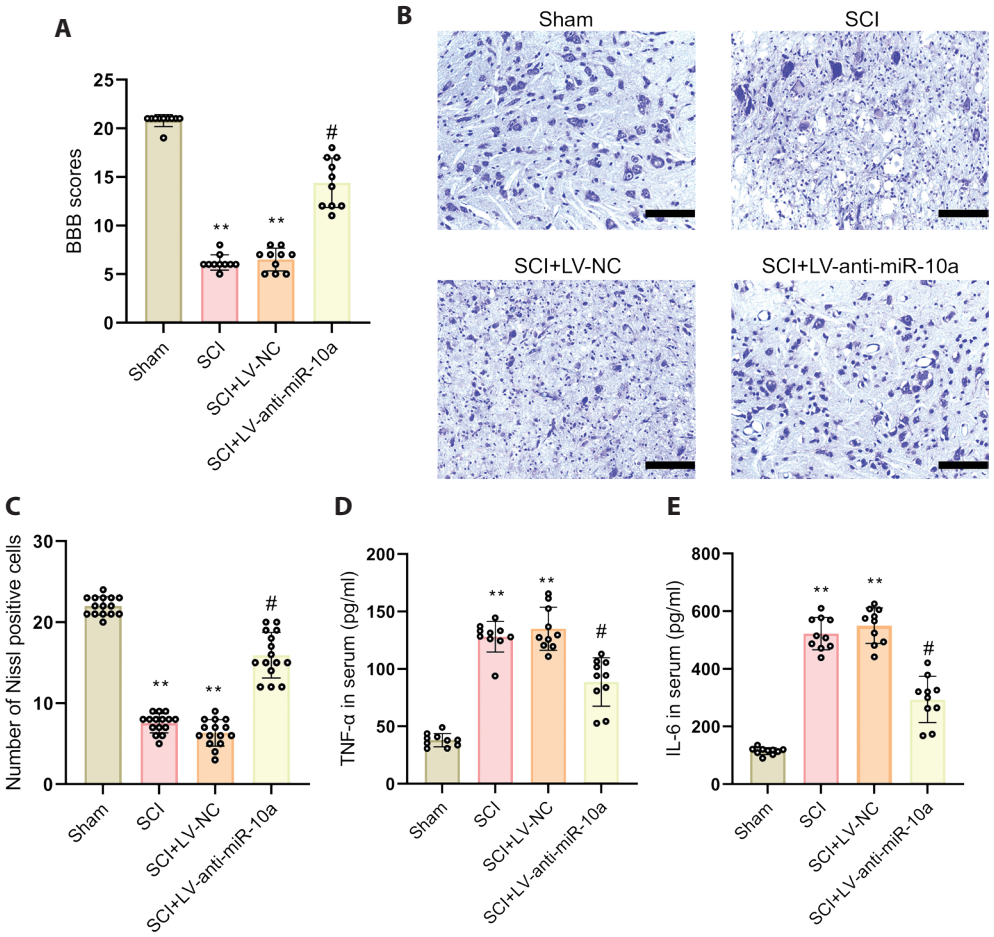

Figure 3. Knockdown of miR-10a promoted recovery of motor function and inhibited inflammation in SCI rats. **A.** BBB scores were used to assess functional recovery in LV-NC- or LV-anti-miR-10a-treated SCI rats (n = 10). **B.,C.** The Nissl bodies of the anterior horn of the spinal cord were stained to assess neuronal survival rates in SCI rats (n = 3). Scale bar = 100 μm. **D.,E.** The serum levels of inflammatory cytokines (TNF-α and IL-6) were assessed by ELISA (n = 10). ** $p < 0.01$ *vs.* Sham group; # $p < 0.05$, *vs.* SCI group.

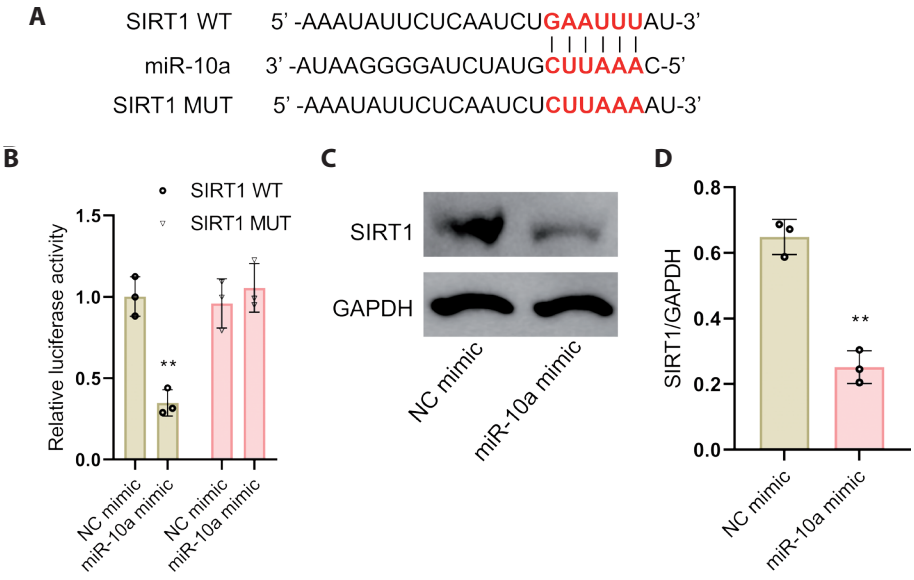

Figure 4. miR-10a targets SIRT1 directly. **A.** Predicted binding sites of miR-10a in the 3'-UTR sequences of SIRT1-WT and SIRT1-MUT. **B.** PC12 cells were co-transfected with SIRT1-MUT or SIRT1-WT 3'-UTR reporter plasmids together with NC mimic or miR-10a mimic, and after 48 h, their luciferase activity was assessed by dual-luciferase reporter assays. **C.,D.** Expression of SIRT1 protein in PC12 cells transfected with miR-10a mimic or NC mimic were analyzed by Western blotting. $^{**}p < 0.01$ *vs.* NC mimic group.

SIRT1-WT vectors but did not affect SIRT1-MUT vectors (Fig. 4B). Additionally, the in-vitro SIRT1 levels were also markedly reduced by miR-10a (Fig. 4C,D). Therefore, miR-10a is suggested to directly modulate SIRT1 levels.

miR-10a inhibition alleviates inflammation in the in-vitro SCI model.

To elucidate the function of miR-10a in an *in vitro* SCI model, LV-NC, LV-anti-miR-10a+si-control, LV-anti-miR-10a, or LV-anti-miR-10a+si-SIRT1 transfected cells were treated with 100 ng/ml LPS for 6 h. It was observed that LPS treatment markedly increased the levels of miR-10a relative to the control group, while levels were markedly reduced in LV-anti-miR-10a-transfected cells relative to LV-NC-transfected cells (Fig. 5A). Additionally, SIRT1 protein expression was substantially decreased in the LPS group compared with the control. SIRT1 levels were also markedly increased in the LV-anti-miR-10a group compared with the LPS group; this elevation was reversed by SIRT1 knockdown with SIRT1-siRNA (Fig. 5B,C). MTT assays for cell viability indicated significantly reduced viability in the LPS group compared with the control. However, miR-10a knockdown markedly increased cell viability compared with the LPS group, but this effect was reversed by si-SIRT1 (Fig. 5D). Moreover, the levels of inflammatory factors assessed by ELISA showed significant increases in both TNF-α and IL-6 in the LPS group relative to the control. miR-10a knockdown significantly reduced inflammatory factor levels compared with the group treated only with LPS and were markedly reduced after SIRT1 knockdown (Fig. 5E,F).

Discussion

SCI is a neurological disorder that causes severe motor, autonomic, and sensory dysfunction (Ortega et al. 2023). The pathophysiology of SCI derives from both primary and secondary damage to the spinal cord, with the latter including inflammation, oxidative stress, apoptosis, and glial scar formation (Feng et al. 2023; Zuo et al. 2023). miRNAs regulate these processes and, therefore, might be effective targets for treating SCI (Busseau et al. 2023; DiVincenzo et al. 2023). This investigation indicated that miR-10a was upregulated in SCI-affected tissues, while miR-10a knockdown improved the recovery of motor function, enhanced neuronal survival, and reduced the levels of inflammatory cytokines.

The results of this research are consistent with those of previous studies indicating that miRNAs are crucially associated with SCI pathogenesis. For example, it has been documented that miR-21 is increased in SCI and enhances neuronal cell death by specifically targeting phosphatase and tensin homolog (PTEN) (Kar et al. 2021). Moreover, miR-124 is decreased in SCI, this miRNA provides defense against neuronal death by specifically targeting Bax (Xu et al. 2019). Furthermore, numerous studies have demonstrated the involvement of miRNAs in the development of neurodegenerative disorders. Specifically, the disruptions in miRNA expression have been proposed to serve as a dependable and efficient method for diagnosing Parkinson's disease without the need for intrusive procedures (Kumar et al. 2024). The upregulation of miR-125b in the brain afflicted with Alzheimer's disease is induced by the transcription factor nuclear factor kappa B (NF-κB), potentially leading to neuroinflammation through the modulation of complement factor-H (CFH) mRNA (Lukiw and Alexandrov 2012). Studies have

demonstrated that miR-34a and miR-146a are positively associated with inflammatory mediators (Dos Santos et al. 2024; Liu et al. 2024). In summary, our results confirm the association of miRNAs with neuroinflammation. Knockdown of miR-10a could reduce the concentrations of inflammatory cytokines. These findings further contribute to our understanding by indicating that miR-10a is also disrupted in SCI, and is linked to inflammation, perhaps playing a role in the development of the condition.

Interestingly, it was observed that miR-10a directly targets SIRT1, a sirtuin family member of NAD+-dependent protein deacetylases, which modulate cellular processes, including apoptosis, inflammation, and senescence (Sun et al. 2022; Tang et al. 2022; Barangi et al. 2023). SCI rats showed reduced SIRT1 expression, while SIRT1 expression was increased by miR-10a downregulation, consistent with previous studies showing that miRNAs modulate SIRT1 expression. Moreover, miR-34a has been reported to target SIRT1 directly and to induce cellular senescence and inflammation in human endothelial cells (Raucci et al. 2021) as well as promoting neuronal survival in an ischemic stroke model (Wang et al. 2023). Here, it was revealed that miR-10a may exert its effects on SCI by modulating SIRT1 expression.

Cell experiments showed that miR-10a downregulation increased cell viability and reduced the levels of inflammatory factors after LPS treatment, suggesting that miR-10a may regulate the inflammatory response in SCI, a major contributor to secondary injury and a key target for therapeutic intervention. This is consistent with previous results showing that miR-10a plays a role in inflammatory reactions. Specifically, treatment with miR-10a mimics has been shown to enhance the secretion of TNF-α, IL-1β, and IL-6 (Lu et al. 2023), Conversely, inhibition of miR-10a-5p has been demonstrated to mitigate apoptosis triggered by IL-1β (Ma et al. 2019). It was observed that SIRT1 knockdown reduced the protective effects of miR-10a downregulation on cell viability and inflammatory responses, indicating that SIRT1 might mediate miR-10a effects on SCI. Previous research

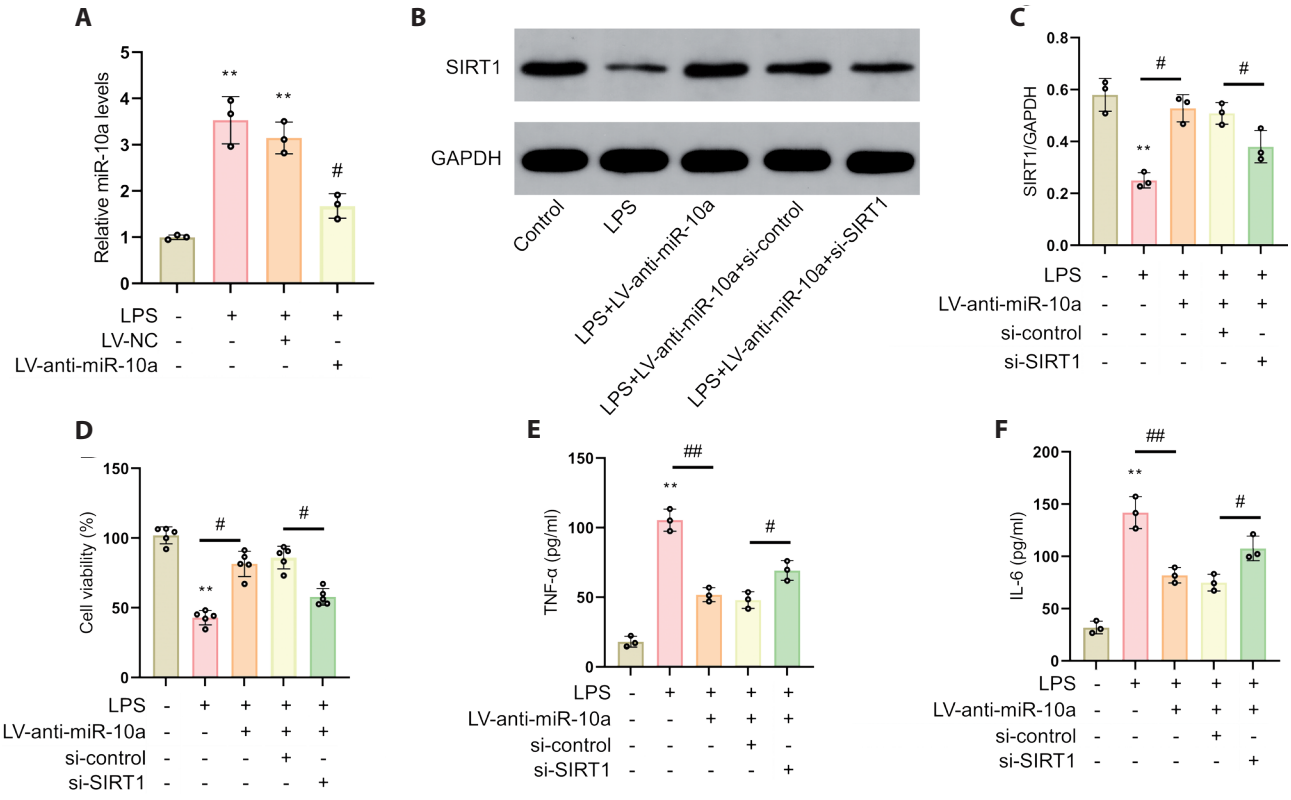

Figure 5. miR-10a downregulation or SIRT1 silencing affects cell viability and inflammatory factor levels. LV-NC, LV-anti-miR-10a+si-control, LV-anti-miR-10a, or LV-anti-miR-10a+si-SIRT1 were transfected for 48 h into PC12 cells. Cells were then treated for 6 h with LPS (100 ng/ml) **A.** qRT-PCR was used to measure miR-10a expression in PC12 cells after the indicated treatments. Values are depicted as the mean ± SD of 3 experimental replicates. ** $p < 0.01$ *vs.* control group; # $p < 0.05$ *vs.* LPS group. **B.,C.** SIRT1 protein expression in PC12 cells after indicated treatments. **D.** MTT assays measuring cell viability. ELISAs were used to measure the levels of the inflammatory factors TNF-α (**E**) and IL-6 (**F**). Values are shown as the mean ± SD of 3 experimental replicates. ** $p < 0.01$ *vs.* control group; # $p < 0.05$, ## $p < 0.01$ *vs.* as indicated.

has shown that SIRT1 can protect against neuronal injury and inflammation in various neurological disorders. For instance, it protected against neuronal death in an ischemic stroke model by deacetylating p53 (Zhang X et al. 2020) and suppressed inflammation in a model of multiple sclerosis by deacetylation of NF-κB (Schiaffino et al. 2018). The present results suggest that SIRT1 may be a key mediator of the effects of miR-10a on SCI.

Conclusion

In summary, the findings of this study provide new insights into the association of miR-10a and SIRT1 in SCI pathogenesis. Furthermore, it suggested that miR-10a may contribute to SCI by targeting SIRT1 and modulating the inflammatory response, which may represent a novel SCI therapeutic strategy. However, further research is required to verify these results and explore the potential therapeutic effects of miR-10a inhibitors or SIRT1 activators.

Conflict of interest. The authors declare that they have no conflict of interest.

Funding. The study was funded by the National Natural Science Foundation of China (Grant No. 81772383) and the key Discipline Construction project of Pudong Health Bureau of Shanghai (Grant No. PWZxk2022-09).

References

Barangi S, Hayes AW, Karimi G (2023): The role of lncRNAs/miRNAs/Sirt1 axis in myocardial and cerebral injury. Cell Cycle **22,** 1062-1073 https://doi.org/10.1080/15384101.2023.2172265

Busseau I, Mockly S, Houbron É, Somaï H, Seitz H (2023): Evaluation of microRNA variant maturation prior to genome edition. Biochimie **217,** 86-94 https://doi.org/10.1016/j.biochi.2023.06.007

Chen J, Qin R (2020): MicroRNA-138-5p regulates the development of spinal cord injury by targeting SIRT1. Mol. Med. Rep. **22,** 328-336 https://doi.org/10.3892/mmr.2020.11071

Ding W, Xu W, Lu D, Sheng H., Xu X, Xu B, Zheng A (2023): Inhibition of TERC inhibits neural apoptosis and inflammation in spinal cord injury through Akt activation and p-38 inhibition via the miR-34a-5p/XBP-1 axis. Open Med. (Wars) **18,** 20220619 https://doi.org/10.1515/med-2022-0619

DiVincenzo MJ, Angell CD, Suarez-Kelly LP, Ren C, Barricklow Z, Moufawad M, Fadda P, Yu L, Backes FJ, Ring K et al. (2023): Expression of microRNAs and their target genes in melanomas originating from gynecologic sites. PLoS One **18,** e0285804 https://doi.org/10.1371/journal.pone.0285804

Dos Santos BL, Dos Santos CC, da Silva KC, Nonaka CKV, Souza BSF, David JM, de Oliveira JVR, Costa MFD, Butt AM, da Silva VDA, Costa SL (2024): The phytochemical agathisflavone modulates miR146a and miR155 in activated microglia involving STAT3 signaling. Int. J. Mol. Sci. **25,** 2547 https://doi.org/10.3390/ijms25052547

Feng F, Song X, Tan Z, Tu Y, Xiao L, Xie P, Ma Y, Sun X, Ma J, Rong L, He L (2023): Cooperative assembly of a designer peptide and silk fibroin into hybrid nanofiber gels for neural regeneration after spinal cord injury. Sci. Adv. **9,** eadg0234 https://doi.org/10.1126/sciadv.adg0234

González-Viejo MA, Avellanet M, Boada-Pladellorens A, Montesinos-Magraner L, Jaúregui-Abrisqueta ML, Bárbara-Bataller E, Méndez-Ferrer B, Sánchez-Raya J, Cívicos N, Méndez-Suarez JL, et al. (2023): International Spinal Cord Injury Community Survey: Socioeconomic and healthcare satisfaction in Spain. Global Spine J. **16,** 21925682231183972 https://doi.org/10.1177/21925682231183972

Gruner (1992): A monitored contusion model of spinal cord injury in the rat. J. Neurotrauma **9,** 123-128 https://doi.org/10.1089/neu.1992.9.123

Gui Y, Liu H, Zhang L, Lv W, Hu X (2015): Altered microRNA profiles in cerebrospinal fluid exosome in Parkinson disease and Alzheimer disease. Oncotarget **6,** 37043-53 https://doi.org/10.18632/oncotarget.6158

Han T, Song P, Wu Z, Liu Y, Ying W, Shen C (2023): Inflammation modifies miR-21 expression within neuronal extracellular vesicles to regulate remyelination following spinal cord injury. Stem. Cell. Rev. Rep. **19,** 2024-2037 https://doi.org/10.1007/s12015-023-10560-y

Han Z, Mou Z, Jing Y, Jiang R, Sun T (2023): CircSmox knockdown alleviates PC12 cell apoptosis and inflammation in spinal cord injury by miR-340-5p/Smurf1 axis. Immun. Inflamm. Dis. **11,** e824 https://doi.org/10.1002/iid3.824

Hu C, Feng X, Ma Y, Wei D, Zhang L, Wang S, Ma Y (2023): CircADAMTS16 inhibits differentiation and promotes proliferation of bovine adipocytes by targeting miR-10167-3p. Cells **12,** 1175 https://doi.org/10.3390/cells12081175

Jeong HR, Hwang IT (2023): MicroRNAs as novel biomarkers for the diagnosis and treatment of pediatric diseases. Clin. Exp. Pediatr. **67,**119-125 https://doi.org/10.3345/cep.2023.00171

Jia L, Zhu M, Yang J, Pang Y, Wang Q, Li Y, Li T, Li F, Wang Q, Li Y, Wei Y (2021): Prediction of P-tau/Aβ42 in the cerebrospinal fluid with blood microRNAs in Alzheimer's disease. BMC Med. **19,** 264 https://doi.org/10.1186/s12916-021-02142-x

Jiang T, Qin T, Gao P, Tao Z, Wang X, Wu M, Gu J, Chu B, Zheng Z, Yi J, et al. (2023): SIRT1 attenuates blood-spinal cord barrier disruption after spinal cord injury by deacetylating p66Shc. Redox Biol. **60,** 102615 https://doi.org/10.1016/j.redox.2023.102615

Kar AN, Lee SJ, Sahoo PK, Thames E, Yoo S, Houle JD, Twiss JL (2021): MicroRNAs 21 and 199a-3p regulate axon growth potential through modulation of Pten and mTor mRNAs. eNeuro **8,** ENEURO.0155-21.2021 https://doi.org/10.1523/ENEURO.0155-21.2021

Khan T, Havey RM, Sayers ST, Patwardhan A, King WW (1999): Animal models of spinal cord contusion injuries. Lab. Anim. Sci. **49,** 161-172

Kumar A, Kouznetsova VL, Kesari S, Tsigelny IF (2024): Parkinson's disease diagnosis using miRNA biomarkers and deep learning. Front. Biosci. **29,** 4 https://doi.org/10.31083/j.fbl2901004

Liu Y, Meng XK, Shao WZ, Liu YQ, Tang C, Deng SS, Tang CF, Zheng L, Guo W (2024): miR-34a/TAN1/CREB axis engages in alleviating oligodendrocyte trophic factor-induced myelin repair function and astrocyte-dependent neuroinflammation in the early stages of Alzheimer's disease: The Anti-neurodegenerative effect of treadmill exercise. Neurochem. Res. **49,** 1105-1120 https://doi.org/10.1007/s11064-024-04108-w

Lu Y, Wang W, Ma Y, Fan Z, Xiong L, Zhao J, He Y, Li C, Wang A, Xiao N, et al. (2023): miR-10a induces inflammatory responses in epileptic hippocampal neurons of rats via PI3K/Akt/mTOR signaling pathway. Neuroreport **34,** 526-534 https://doi.org/10.1097/WNR.0000000000001920

Lukiw WJ, Alexandrov PN (2012): Regulation of complement factor H (CFH) by multiple miRNAs in Alzheimer's disease (AD) brain. Mol. Neurobiol. **46,** 11-9 https://doi.org/10.1007/s12035-012-8234-4

Ma J, Wen X, Xu Z, Xia P, Jin Y, Lin J, Qian J (2023): Abnormal regulation of miR-29b-ID1 signaling is involved in the process of decitabine resistance in leukemia cells. Cell Cycle **22,** 1215-1231 https://doi.org/10.1080/15384101.2023.2200312

Ma Y, Wu Y, Chen J, Huang K, Ji B, Chen Z, Wang Q, Ma J, Shen S, Zhang J (2019): miR-10a-5p promotes chondrocyte apoptosis in osteoarthritis by targeting HOXA1. Mol. Ther. Nucleic Acids **14,** 398-409 https://doi.org/10.1016/j.omtn.2018.12.012

Ortega MA, Fraile-Martinez O, García-Montero C, Haro S, Álvarez-Mon MÁ, De Leon-Oliva D, Gomez-Lahoz, AM, Monserrat J, Atienza-Pérez M, Díaz D, et al. (2023): A comprehensive look at the psychoneuroimmunoendocrinology of spinal cord injury and its progression: mechanisms and clinical opportunities. Mil. Med. Res. **10,** 26 https://doi.org/10.1186/s40779-023-00461-z

Rao Y, Li J, Qiao R, Luo J, Liu Y (2023): Tetramethylpyrazine and Astragaloside IV have synergistic effects against spinal cord injury-induced neuropathic pain via the OIP5-AS1/miR-34a/Sirt1/NF-κB axis. Int. Immunopharmacol. **115,** 109546 https://doi.org/10.1016/j.intimp.2022.109546

Raucci A, Macrì F, Castiglione S, Badi I, Vinci MC, Zuccolo E (2021): MicroRNA-34a: the bad guy in age-related vascular diseases. Cell Mol. Life Sci. **78,** 7355-7378 https://doi.org/10.1007/s00018-021-03979-4

Ryu CS, Oh SH, Lee KO, Park HS, An HJ, Lee JY, Ko EJ, Park HW, Kim OJ, Kim NK (2020): MiR-10a, 27a, 34b/c, and 300 polymorphisms are associated with ischemic stroke susceptibility and post-stroke mortality. Life (Basel) **10,** 309 https://doi.org/10.3390/life10120309

Sachdeva R, Krassioukov AV, Bucksot JE, Hays SA (2020): Acute cardiovascular responses to vagus nerve stimulation after experimental spinal cord injury. J. Neurotrauma **37,** 1149-1155 https://doi.org/10.1089/neu.2019.6828

Schiaffino L, Bonafede R, Scambi I, Parrella E, Pizzi M, Mariotti R (2018): Acetylation state of RelA modulated by epigenetic drugs prolongs survival and induces a neuroprotective effect on ALS murine model. Sci. Rep. **8,** 12875 https://doi.org/10.1038/s41598-018-30659-4

Sun K, Wu Y, Zeng Y, Xu J, Wu L, Li M, Shen B (2022): The role of the sirtuin family in cartilage and osteoarthritis: molecular mechanisms and therapeutic targets. Arthritis Res. Ther. **24,** 286 https://doi.org/10.1186/s13075-022-02983-8

Tang Y, Xie J, Chen X, Sun L, Xu L, Chen X (2022): A novel link between silent information regulator 1 and autophagy in cerebral ischemia-reperfusion. Front. Neurosci. **16,** 1040182 https://doi.org/10.3389/fnins.2022.1040182

Wang J, He P, Tian Q, Luo Y, He Y, Liu C, Gong P, Guo Y, Ye Q, Li M (2023): Genetic modification of miR-34a enhances efficacy of transplanted human dental pulp stem cells after ischemic stroke. Neural. Regen. Res. **18,** 2029-2036

Wang Y, Pang QJ, Liu JT, Wu HH, Tao DY (2018): Down-regulated miR-448 relieves spinal cord ischemia/reperfusion injury by up-regulating SIRT1. Braz. J. Med. Biol. Res. **51,** e7319 https://doi.org/10.1590/1414-431x20177319

Xu D, Li W, Zhang T, Wang G (2020): miR-10a overexpression aggravates renal ischemia-reperfusion injury associated with decreased PIK3CA expression. BMC Nephrol. **21,** 248 https://doi.org/10.1186/s12882-020-01898-3

Xu Z, Zhang K, Wang Q, Zheng Y (2019): MicroRNA-124 improves functional recovery and suppresses Bax-dependent apoptosis in rats following spinal cord injury. Mol. Med. Rep. **19,** 2551-2560 https://doi.org/10.3892/mmr.2019.9904

Yu X, Zhang S, Zhao D, Zhang X, Xia C, Wang T, Zhang M, Liu T, Huang W, Wu B (2019): SIRT1 inhibits apoptosis in in vivo and in vitro models of spinal cord injury via microRNA-494. Int. J. Mol. Med. **43,** 1758-1768 https://doi.org/10.3892/ijmm.2019.4106

Yue X, Gu M, Jia T (2023): Upregulated miR-125b mitigates inflammation, astrocyte activation, and dysfunction of spinal cord injury by inactivating the MAPK pathway. Histol. Histopathol. **39,** 225-237

Zhang T, Liu C, Chi L (2020): Suppression of miR-10a-5p in bone marrow mesenchymal stem cells enhances the therapeutic effect on spinal cord injury via BDNF. Neurosci. Lett. **714,** 134562 https://doi.org/10.1016/j.neulet.2019.134562

Zhang XS., Lu Y, Tao T, Wang H, Liu GJ, Liu XZ, Liu C, Xia DY, Hang CH, Li W (2020): Fucoxanthin mitigates subarachnoid hemorrhage-induced oxidative damage via sirtuin 1-dependent pathway. Mol. Neurobiol. **57,** 5286-5298 https://doi.org/10.1007/s12035-020-02095-x

Zhong G, Yang Y, Huang X, Chen J, Feng D, Wei K, Chen J, Chen H (2021): The serum SIRT1 protein is associated with the severity of injury and neurological recovery in mice with traumatic spinal cord injury. Neuroscience **469,** 103-109 https://doi.org/10.1016/j.neuroscience.2021.06.025

Zuo Y, Ye J, Cai W, Guo B, Chen X, Lin L, Jin S, Zheng H, Fang A, Qian X, et al. (2023): Controlled delivery of a neurotransmitter-agonist conjugate for functional recovery after severe spinal cord injury. Nat. Nanotechnol. **18,** 1230-1240 https://doi.org/10.1038/s41565-023-01416-0

Received: January 20, 2024
Final version accepted: June 4, 2024

doi: 10.4149/gpb_2024024

Supplementary Material

Down-regulated miR-10a protects against spinal cord injury by up-regulating SIRT1

Chao Song[1] and Yan Zhang[1]

[1] *Department of Orthopaedics, Gongli Hospital of Pudong New Area, Shanghai, China*

Supplementary Figure

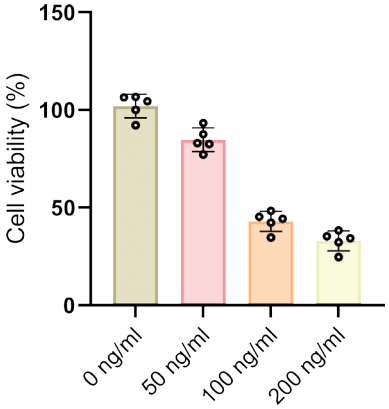

Figure S1. MTT assays measuring cell viability.