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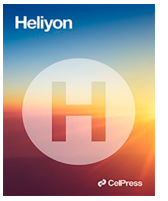

Combined transcriptomics and proteomics studies on the effect of electrical stimulation on spinal cord injury in rats

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ABSTRACT

Electrical stimulation (ES) of the spinal cord is a promising therapy for functional rehabilitation after spinal cord injury (SCI). However, the specific mechanism of action is poorly understood. We designed and applied an implanted ES device in the SCI area in rats and determined the effect of ES on the treatment of motor dysfunction after SCI using behavioral scores. Additionally, we examined the molecular characteristics of the samples using proteomic and transcriptomic sequencing. The differential molecules between groups were identified using statistical analyses. Molecular, network, and pathway-based analyses were used to identify group-specific biological features. ES (0.5 mA, 0.1 ms, 50 Hz) had a positive effect on motor dysfunction and neuronal regeneration in rats after SCI. Six samples (three independent replicates in each group) were used for transcriptome sequencing; we obtained 1026 differential genes, comprising 274 upregulated genes and 752 downregulated genes. A total of 10 samples were obtained: four samples in the ES group and six samples in the SCI group; for the proteome sequencing, 48 differential proteins were identified, including 45 up-regulated and three down-regulated proteins. Combined transcriptomic and proteomic studies have shown that the main enrichment pathway is the hedgehog signaling pathway. Western blot results showed that the expression levels of Sonic hedgehog (SHH) ($P < 0.001$), Smoothened (SMO) ($P = 0.0338$), and GLI-1 ($P < 0.01$) proteins in the ES treatment group were significantly higher than those in the SCI group. The immunofluorescence results showed significantly increased expression of SHH ($P = 0.0181$), SMO ($P = 0.021$), and GLI-1 ($P = 0.0126$) in the ES group compared with that in the SCI group. In conclusion, ES after SCI had a positive effect on motor dysfunction and anti-inflammatory effects in rats. Moreover, transcriptomic and proteomic sequencing also provided unique perspectives on the complex relationships between ES on SCI, where the SHH signaling pathway plays a critical role. Our study provides a significant theoretical foundation for the clinical implementation of ES therapy in patients with SCI.

Abbreviations: SCI, Spinal cord injury; ES, Electrical stimulation; BBB, Basso-Beattie-Bresnahan; GO, Gene ontology; Shh, Sonic hedgehog; KEGG, Kyoto Encyclopedia of Genes and Genomes; PPi, Protein–protein interaction; GEO, Gene expression omnibus; BP, Biological process; CC, Cellular component; MF, Molecular function.

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1. Introduction

Spinal cord injury (SCI) disturbs spinal structure and autonomic dysreflexia [1], causing motor and sensory dysfunction at the level of the injury and below [2,3]. According to a recent estimate, the annual incidence of SCI is approximately 54 cases per million people in the United States, or approximately 18,000 new SCI cases each year, not including those who die at the location of the incident that caused the SCI [4]. SCI is one of the most common causes of death and paralysis worldwide [5]. Early treatment of motor dysfunction caused by SCI is the most effective way to improve the patients' quality of life and can effectively prevent serious complications in long-term bedridden patients. Although the various strategies are employed for the treatment of SCI, until now there is no proper cure for the treatment of SCI.

Electrical stimulation (ES) is the current clinical standard used to restore function and provide therapy in a variety of clinical applications [6]. ES of the spinal cord is a promising therapy for functional rehabilitation after SCI [7]. The limb movements of patients with chronic quadriplegia caused by high cervical SCI can be restored by coordinating the ES of the peripheral muscles and nerves [8]. ES of the spinal cord can result in autonomous movement after SCI [9]. However, the specific mechanism of action is poorly understood.

Transcriptomics focuses on gene expression at the RNA level and provides genome-wide information on gene structure and function to uncover molecular mechanisms involved in specific biological processes [10]. Proteomics provides complementary information to genomics and transcriptomics, improving the molecular-level understanding of complex biochemical processes [11]. According to our previous report [12], SCI causes cell-composition alterations within the spinal cord, including astrocytes (Gfap+), microglia (Cd68+),

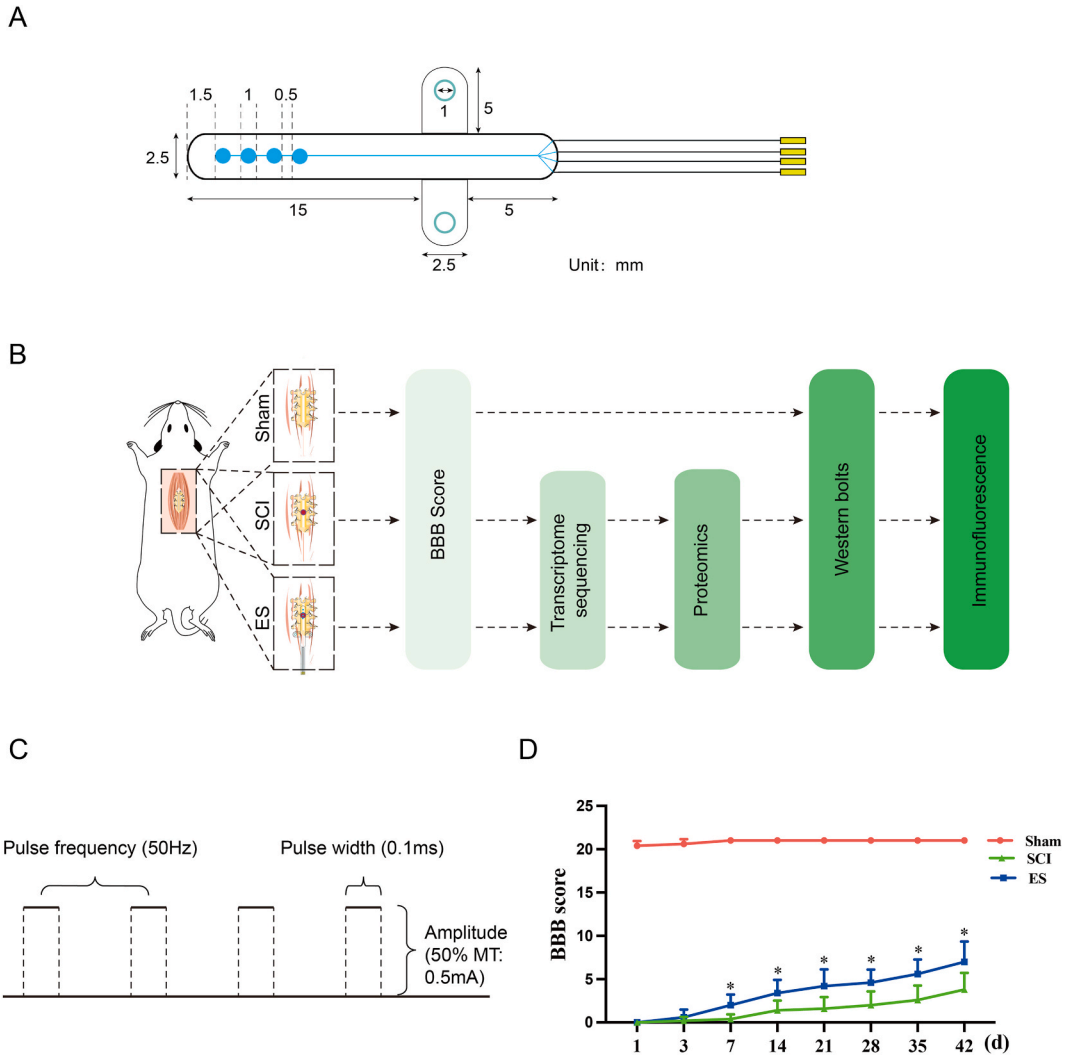

Fig. 1. ES electrode design and its effectiveness in SCI. A, ES electrode design; all measured values are in millimeters. B, Flow chart of the study. Red dots represent the injury area. C, ES parameters (frequency 50 Hz, pulse width 0.1 ms, amplitude 50 % motor threshold [MT], 0.5 mA). D, Basso-Beattie-Bresnahan scores of rats with different types of SCI (n = 4). *$P < 0.05$ vs. SCI group. ES: electrical stimulation, SCI: spinal cord injury.

and oligodendrocytes (Mbp+). Inspired by preclinical and early clinical studies, we designed a flexible electrode for ES treatment to treat hindlimb motor dysfunction caused by SCI in rats and explored the underlying molecular mechanisms using combined transcriptomics and proteomics analyses.

2. Method

2.1. Animals

Female Wistar rats (220–230 g, 8 weeks old, SPF Biotechnology Co., Ltd., Beijing, China, certificate no. SCXK [Jing] 2019-0010) were randomly divided into three groups: sham, SCI, and ES. Choosing female rats can better avoid severe urinary dysfunction [13]. Rats were housed in cages under a 12-h light/dark cycle at a constant temperature of 28 °C. Rats had free access to food and water. All experimental procedures were approved by the Animal and Ethics Committee of the Experimental Animal Center of Air Force Medical University and conducted in accordance with The Code of Practice for Care and Use of Animals for Experimental Purposes.

The timeline of the experimental design is briefly described as follows: treatment of hindlimb function in rats was analyzed using the Basso-Beattie-Bresnahan locomotor (BBB) rating scale at 1, 3, 7, 14, 21, 28, 35, and 42 days post-injury (dpi) after SCI. The rats' spinal cord tissues were collected and fixed for immunofluorescence staining. Simultaneously, spinal cord tissue was processed and subjected to transcriptomic and proteomic analyses. Western blot analysis was performed at 7 and 14 days after SCI.

2.2. Contusion SCI model and ES treatment

As described in a previous report [14], the rats were anesthetized with an intraperitoneal injection of 3 % sodium pentobarbital (40 mg/kg). The skin was incised, the muscle was isolated, and the spinous processes were fully exposed. The triangular direction formed by T9, T10, and T11 was used to accurately locate the spinous process, given that the T9 spinous process points caudally, the T10 dorsally, and the T11 rostrally [14]. The spinous processes and laminae of T9 were removed to fully expose the spinal cord. The sham control group received only laminectomy. A modified Allen impactor was used to fabricate a contusion SCI model. The actual contact area between the percussion device and the spinal cord was 4.91 mm^2 (2.5 mm diameter). A 10 g weight was dropped from a height of 50 mm to form the SCI model. Rats were observed to check for spasmodic tail wagging and bilateral hind limb twitching. The tissue was then sutured layer by layer, while ensuring body temperature was maintained during the operation. After the surgery, assisted urination (twice a day) was also conducted until spontaneous urination recovered. Buprenorphine (0.05 mg/kg) was injected subcutaneously daily for 3 days, and cefazolin (50 mg/kg) was administered subcutaneously once daily for 7 days. The rats' general condition was assessed at each day of observation, and wounds, infections, and other alterations were monitored.

Flexible electrodes were designed for ES treatment (manufactured by Kedou Brain-Computer Technology Co., Ltd, Suzhou, China); the whole body was made of flexible materials (silica gel, polyimide, and polydimethylsiloxane), and the contacts were made of pure platinum. The structure of the flexible electrode is illustrated (Fig. 1A), and the flow chart of the entire process was shown (Fig. 1B). The time of implantation of the flexible electrodes was set after the injury model was successful. Additional removal of the T12 lamina was required for better placement of the flexible electrodes to fit the spinal cord surface. ES signals were provided by a multichannel physiological signal recorder (version RM6240E; Chengdu Instrument Factory, Chengdu, China). One day after flexible electrode implantation, ES (0.5 mA, 0.1 ms, 50 Hz) was applied at 10:00 a.m. for 1 h each day for 7 days (Fig. 1C) [15,16]. Direction of current conduction in the SCI area was orientated from head to tail.

2.3. BBB score

To assess the recovery of hindlimb motor function after SCI in the open field, rats were evaluated using the BBB score [17], which assesses detects several aspects of locomotion, including joint movement, walking ability, trunk stability, limb coordination, tail position, and fine movement of the paws. The BBB scale was evaluated by two investigators, who were blinded to the groups and adequately trained, at 1, 3, 7, 14, 21, 28, 35, and 42 dpi after SCI. If there was inconsistency between the investigators, a consensus score was agreed upon after discussion.

2.4. Transcriptome sequencing

Twelve rats were used for transcriptomic analysis at 7 dpi after SCI. For the ES group and SCI group, six rats were used with three biological replicates. The 5-mm spinal cord tissue (from 2.5 mm rostral to 2.5 mm caudal from the lesion core) of different groups of rats were sent to LC-Bio Technology (Hangzhou, China) for sequencing and analysis. After cleaning and quality control, the data were analyzed using bioinformatics as detailed in the Supplementary Information.

2.5. Proteome sequencing

A total of 10 samples were used for proteomics at 7 dpi after SCI, including six samples from the SCI group and four samples from the ES group. Strict data filtering was adopted; the accuracy of peptide-level identification false discovery rate (FDR) was set to 1 % for the identification protein needed to contain at least one unique peptide (Supplementary Information Table 1). These methods are described in detail in the Supplementary Information.

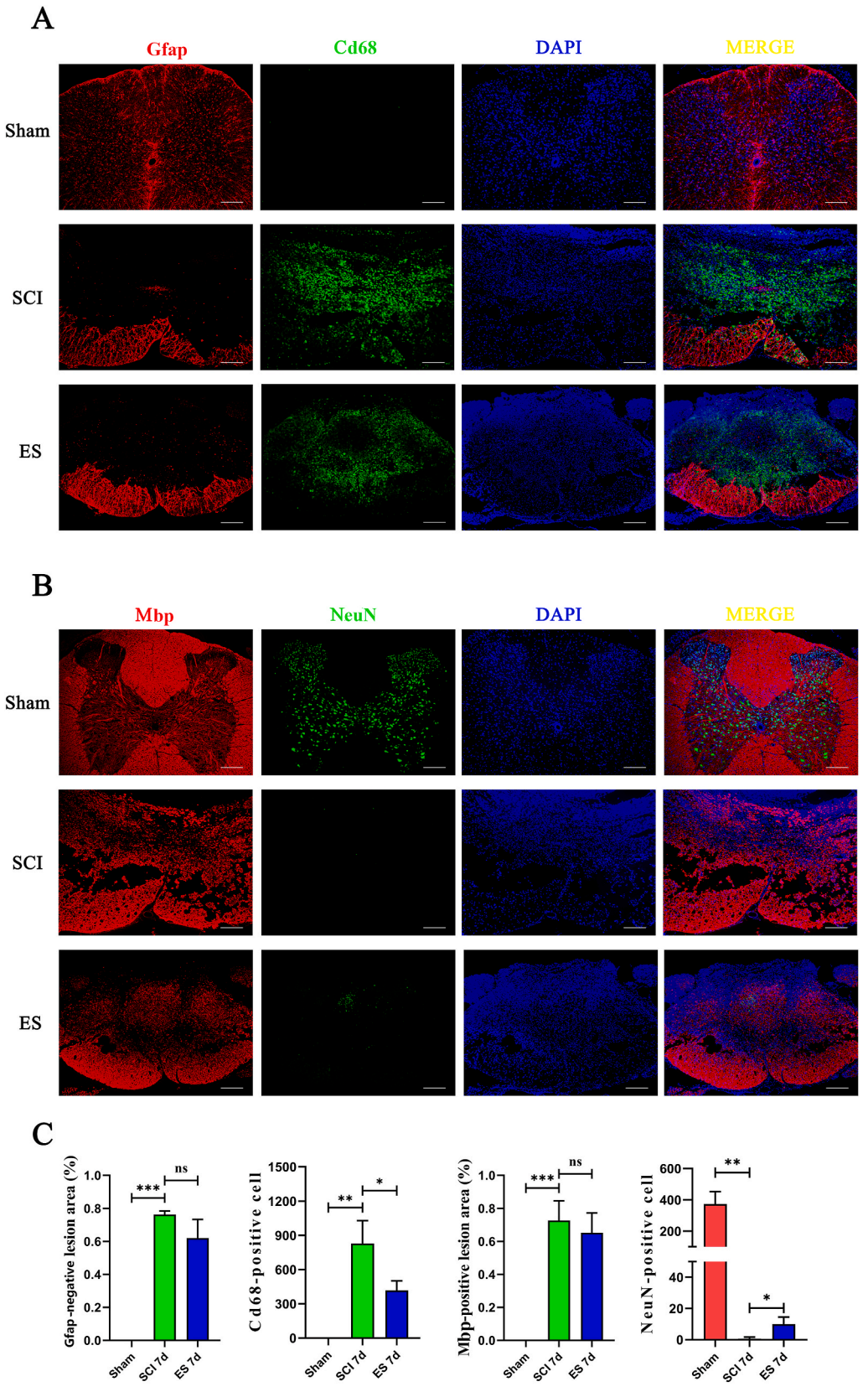

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Fig. 2. ES promotes neuronal regeneration and anti-inflammatory effects (n = 4). A, Representative images of Gfap (red) and Cd68 (green) immunofluorescence staining in the different groups. B, Representative images of Mbp (red) and NeuN (green) immunofluorescence staining in the different groups. C, Comparison of cell counts between the three groups. Scale bar: 200 μm. The positive area was used to evaluate the expression of Gfap and Mbp; cell count was used to evaluate the expression of NeuN and Cd68. *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$. NS, not significant. ES: electrical stimulation, Mbp: myelin basic protein, Gfap: glial fibrillary acidic protein.

2.6. Western blot

The rats were euthanized by an overdose of 3 % sodium pentobarbital (100 mg/kg) at 7 dpi after SCI, and T9 spinal cord tissues (5 mm) at the lesion site, approximately in the middle, were rapidly removed and frozen in liquid nitrogen. Total protein was extracted using a Minute Total Protein Extraction Kit (Inventbiotech, Plymouth, MN, USA) according to the manufacturer's protocol. Protein concentration was immediately determined using a BCA protein assay kit (Beyotime Biotechnology, Shanghai, China). Details regarding the antibodies (including concentrations) and procedures are provided in the Supplementary Information.

2.7. Immunofluorescence staining

Immunofluorescence staining has been described in detail in previous studies [14]. Details regarding the antibodies, staining conditions, and scoring methods are provided in the Supplementary Information.

2.8. Statistical analysis

Data are presented as the mean ± standard deviation. SPSS (version 22.0; IBM Corp., Armonk, NY, USA) and GraphPad Prism (version 8.30; GraphPad Prism Inc., San Diego, CA, USA) were used for data analysis and visualization of the results. BBB scores were analyzed using repeated-measures two-way analysis of variance (ANOVA) followed by Tukey's post hoc test for multiple comparisons. Western blotting and immunofluorescence results were compared between multiple groups using one-way ANOVA with Tukey's post-hoc comparisons. The level of statistical significance was set at $P < 0.05$.

3. Results

3.1. ES can effectively improve hindlimb motor ability after SCI in rats

To assess general motor recovery in the hindlimbs of rats, the BBB score was evaluated within 42 days of SCI (Fig. 1D). The behavioral results showed that T9 contusive SCI had a devastating effect on hindlimb motor function in rats. Compared to the sham group, the BBB scores were significantly lower in the SCI and ES groups at all time points ($P < 0.0001$). Despite severe hindlimb functional impairment, BBB scores continued to improve over time. The trend for improvement was more pronounced in the ES group. Seven days after SCI, the BBB score of the ES group was significantly higher than that of the SCI group ($P < 0.05$), and this improvement remained significant until 42 days after SCI. In summary, ES is a highly promising method for effectively improving hindlimb motor ability after SCI in rats.

3.2. ES adjusted Cd68, NeuN, Mbp, and Gfap expression after SCI in rats

We further explored protein expression changes after SCI in rats. Cd68 protein is a pan-microglia/macrophage marker and a sign of inflammation [18]. In this study, the results showed that ES effectively reduced the expression of Cd68 ($P = 0.0306$). This aligns with the results of previous studies. Further, the expression of inflammatory cytokines in microglia increases significantly after SCI, and the production of inflammatory cytokines in filtered monocytes and neutrophils decreases during treatment [19]. Our results showed that ES reduced the expression of Cd68 in the injured area (Fig. 2A, green, C). Mature differentiated neurons express the neuronal marker NeuN [20]. Reduced NeuN expression represents acute neuronal death [21]. Our results suggest that compared with the sham group, the expression of NeuN in local tissue sharply decreased after SCI ($P < 0.01$). Subsequently, ES effectively increased the expression of NeuN after SCI in rats ($P = 0.0268$) (Fig. 2b, green, C). Interestingly, although there was a trend for a decrease in the positive area of glial fibrillary acidic protein (Gfap) in the ES group at 7 dpi, no significant differences were found between the ES and SCI groups (Fig. 2A, red, C). Myelin basic protein (Mbp) is the specific marker of oligodendrocytes [22]. Mbp expression followed a similar pattern (Fig. 2B, red). The reason for these results is not clear but could be related to the stimulation period.

3.3. Transcriptomic and proteomic sequencing to explore the therapeutic mechanism involved in the use of ES to restore spinal cord function after injury

To identify the mechanisms of ES in SCI, we first performed a transcriptome analysis. The data have been deposited in the Gene Expression Omnibus database under accession number GSE 214291. All samples within and between groups had good parallelism, which met the requirements of quality control for follow-up analysis (Supplemental Information Figure S1 A-B). In total, we obtained 1026 differential genes. There were 274 upregulated and 752 downregulated genes (Supplemental Information Figure S1 C-E). Gene

Ontology (GO) analysis showed enrichment for signal transduction (GO:0007165), transmembrane transport (GO:0055085), and protein phosphorylation (GO:0006468) of biological process (BP), membrane (GO:0016020) of cellular component (CC), and protein binding (GO:0005515) of molecular function (MF) (Fig. 3A). The Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis showed that the PI3K−Akt signaling pathway, p53, and hedgehog signaling pathways may be potential pathways of action (Fig. 3B).

To definitively identify the role of ES proteins in SCI, proteomic analysis is needed. Proteomics is the study of proteomes through a combination of approaches, such as expression proteomics, structural proteomics, and protein-protein interaction analysis [23]. A total

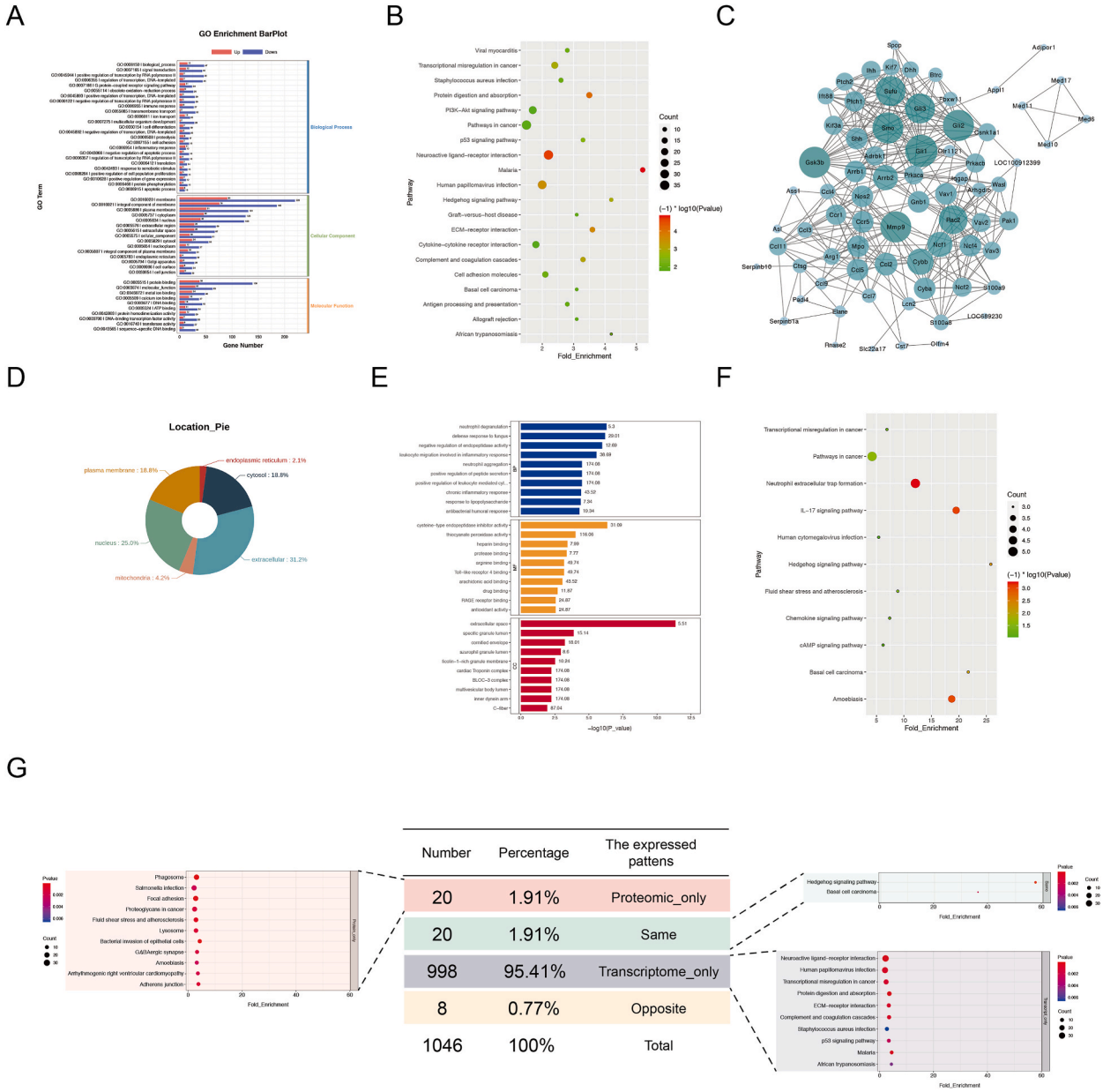

Fig. 3. Transcriptomic and proteomic sequencing was performed to explore the therapeutic mechanism underlying the usefulness of ES to restore spinal cord function after injury. A, GO enrichment of transcriptome sequencing. B, KEGG pathway enrichment of transcriptome sequencing showed that hedgehog signaling pathways were upregulated after ES on SCI. C, Major hub proteins in the PPi network; the higher its degree, the larger its circle, and color. D, All the subfine positioning information measured using the white matter of the target. E, GO enrichment of proteomic sequencing. F, KEGG pathway enrichment of proteomic sequencing showed that hedgehog signaling pathways were upregulated after ES on SCI. G, Overview of the expressed patterns of genes and proteins; KEGG pathway enrichment analyses of expressed genes and expressed proteins. ES, electrical stimulation; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; Gfap: glial fibrillary acidic protein; GO, gene ontology; KEGG, Kyoto Encyclopedia of Genes and Genomes; PPi: protein-protein interaction; SHH, Sonic hedgehog; SMO, Smoothened.

of 10 samples were obtained, including four from the ES group and six from the SCI group. Previous experimental results showed that all samples within and between groups had good parallelism, meeting the quality control requirements for follow-up analysis (Supplemental Information Table 1, Supplemental Information Fig. S2). A total of 48 differential proteins were identified, including 45 upregulated and three downregulated proteins (Supplemental Information Fig. S3). To assess the interactions between differentially expressed proteins, a protein-protein interaction (PPi) network was constructed based on the protein-protein interaction data screened from the *STRING* database (https://www.string-db.org/) (Fig. 3C). Subcellular localization is critical for protein function [24]. In the present study, the protein was located in the extracellular space (31.2 %), nucleus (25 %), plasma membrane (18.8 %), cytosol (18.8 %), mitochondria (4.2 %), and endoplasmic reticulum (2.1 %) (Fig. 3D). GO analysis revealed enrichment for neutrophil degranulation (BP), positive regulation of peptide secretion (BP), chronic inflammatory response (BP), protease binding (MF), and extracellular space (CC) (Fig. 3E). KEGG pathway enrichment analysis showed that neutrophil extracellular trap formation, the IL-17 signaling pathway, and the hedgehog signaling pathway may be potential pathways of action (Fig. 3F).

A combined transcriptomic and proteomic approach provides deeper insights into the molecular changes underlying ES in SCI. In this study, 95.41 % expressed only the transcriptome, and the main mechanism was neuroactive ligand-receptor interaction; 1.91 % only expressed proteomics, and phagocytosis was the main mechanism; 0.77 % expressed the opposite trend of proteomics and transcriptomics, and 1.91 % expressed the same trend of proteomics and transcriptomics, and the hedgehog signaling pathway was the main mechanism (Fig. 3G).

3.4. ES mediates functional repair after SCI through the SHH signaling pathway

The mammalian hedgehog family is comprised of SHH, desert hedgehog, and Indian hedgehog, which diverge in their expression patterns but all utilize common transduction mechanisms [25]. Upon binding of the SHH ligand to its receptor, Smoothened (Smo) accumulates in the cilium to activate GLI-1 transcription factors [26]. We illustrated the expression of SHH, Smo, and Gli-1 proteins expression in all three groups. In the SCI and ES groups, SHH, Smo, and Gli-1 protein expression levels were higher than those in the sham group. Seven days after SCI, the expression levels of SHH ($P < 0.001$), Smo ($P = 0.0338$), and Gli-1 ($P < 0.01$) proteins in the ES treatment group were significantly increased compared with those in the SCI group. Further at 14 days after SCI, the protein expression of SHH ($P < 0.001$), Smo, and Gli-1 ($P = 0.0423$) was higher in the ES group than that in the SCI group, and the difference was statistically significant except for Smo. Furthermore, the protein expression levels of SHH, Smo, and Gli-1 in the same group showed an increasing trend over time (Fig. 4A and B).

In healthy tissues, astrocytes normally provide sustenance and a fabric on which neural circuits form and function [27]. In SCI, reactive astrocytes can mitigate tissue loss and motor dysfunction [28]. We further validated whether ES attenuates motor dysfunction after SCI by activating the astrocyte SHH signaling pathway in rats. Immunofluorescence staining was used to visualize the expression of SHH (Fig. 5A), Smo (Fig. 5B), and Gli-1 (Fig. 5C). Compared to the SCI group, the ES group showed significantly increased expression of SHH (P = 0.0181), Smo ($P = 0.021$), and Gli-1 ($P = 0.0126$) (Fig. 5D). In addition, the immunofluorescence results clearly visualized the distribution of SHH, Smo, and Gli-1 at the center of the injury site. These results suggest that ES mediates functional repair after spinal cord injury by activating SHH signal pathway.

4. Discussion

This study describes the positive effects of ES in the treatment of motor dysfunction after SCI in rats. Based on our results, we can confirm that the proper administration of ES is efficient in the treatment of motor dysfunction after SCI in rats. This is consistent with the findings of previous studies showing that extracellular electric fields may have beneficial effects on subsequent tissue regeneration [29] and rebuild hindlimb motor function [30].

In cases of injury to the central nervous system, such as SCI, a key pathological event is the severing of long-projection axons,

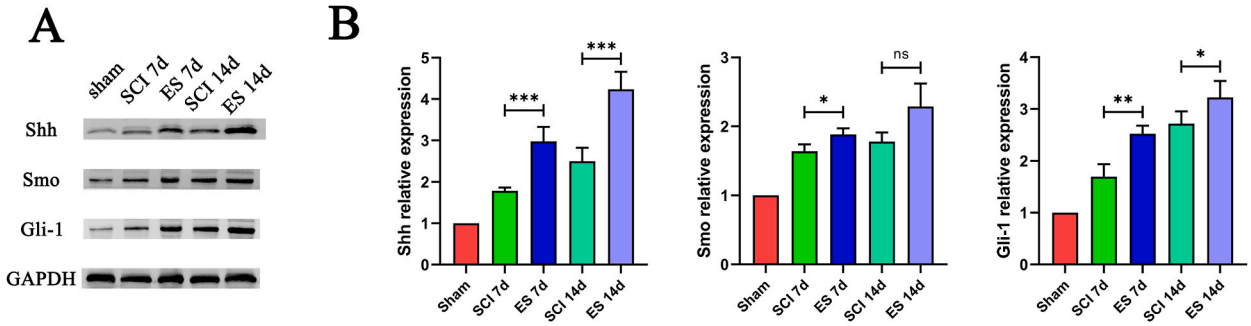

Fig. 4. Western blot analysis of the relative expression of SHH, Smo, and Gli-1 in the different groups at 7 dpi and 14 dpi ($n = 3$). A, Western blot bands of SHH, Smo, Gli-1 and the internal reference GAPDH are shown separately. B, Intergroup comparison of the relative expression of Gfap, SHH, Smo, and Gli-1 in the different groups, with the relative protein expression in the sham group defined as 1. $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$. ns, not significant. dpi: days post-injury; Gfap: glial fibrillary acidic protein; SHH, Sonic hedgehog; Smo: Smoothened.

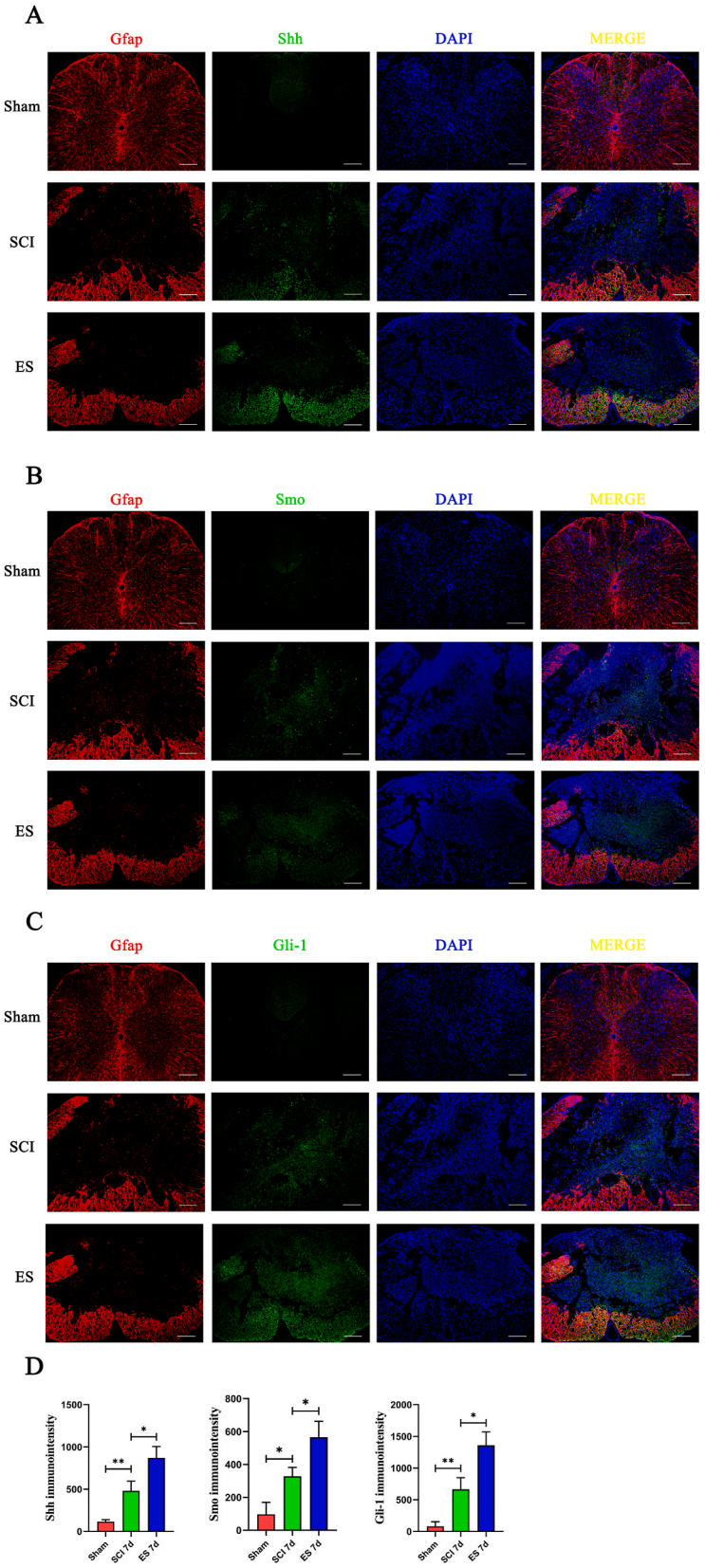

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Fig. 5. Double immunofluorescence staining for hedgehog signaling pathway related protein at 7 dpi (n = 4). A, Representative images of Gfap and SHH immunofluorescence staining at 7 dpi in the different groups. B, Representative images of Gfap and Smo immunofluorescence staining in the different groups. C, Representative images of Gfap and Gli-1 immunofluorescence staining at 7 dpi in the different groups. D, Comparison of cell counts between the three groups. Scale bar: 200 μm $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$. ns, not significant. dpi: days post-injury; Gfap: glial fibrillary acidic protein; SHH, Sonic hedgehog; Smo: Smoothened.

leading to the disruption of relevant circuits [31] and motor function defects [32]. Functional restoration of the corticospinal motor system after SCI is of principal importance because it is essential for the recovery of voluntary motor control recovery [33]. Early recovery of motor function after SCI is of great significance not only from an economic point of view, but also from the physical and mental point of view of patients. ES has been shown to activate motoneurons presynaptically through the recruitment of proprioceptive feedback circuits [34]. ES leads to motor pattern formation through the recruitment of proprioceptive feedback circuits [35]. The BBB score is a stable 1–20 scoring system that can be used to evaluate the progress of rats from rapid loss of walking ability after injury to moderate improvement in motor recovery [36]. In our study, the BBB score was significantly improved, no severe wound infection occurred throughout the survival period, and no adverse reactions to the ES treatment were observed. In addition, we observed beneficial effects of ES on the health status of rats, including earlier urination after surgery and a better mental state.

Previous studies have highlighted the facilitatory effect of ES on neuron regeneration [37,38] and its association with neuroprotection [39]. Likewise, our study showed that ES can significantly promote neuron regeneration (marked by NeuN) following SCI. ES of the vagus nerve is thought to regulate neural communication with T cells and macrophages, block the production of inflammatory cytokines, and promote an anti-inflammatory state [40]. Our data suggest that ES suppresses the expression of Cd68 and exerts anti-inflammatory effects.

To clarify the specific mechanism of ES in the treatment of motor dysfunction in rats after SCI, we used proteomics combined with transcriptomic techniques. Transcriptomics have been applied to a variety of SCI models, including zebrafish [41], rats [42], and mice [43]. However, studies on the transcriptome of SCI tissues are insufficient or biased. As the main executors of cell function, proteins are the main effector molecules that ultimately participate in cell biology. Proteomics is the study of the composition, distribution, and interactions of proteins throughout a cell or organism [44]. In this study, we proposed for the first time that the hedgehog signaling pathway may be the key mechanism of ES in the treatment of motor dysfunction after SCI.

In mammals, hedgehog signaling is initiated by one of three spatiotemporally confined ligands: SHH, Indian hedgehog, and desert hedgehog [45]. The SHH signaling pathway consists of four main components: 12-transmembrane domain receptor protein Ptch1; 7-transmembrane domain protein Smo as an activated membrane component; Gli1 transcription factor; and Gli negative regulatory factor fusion protein inhibitor [46]. The binding of the ligand protein Hedgehog secreted to the extracellular cell with the receptor protein Ptch1 on the surface of the target cell triggers the activation of this pathway. The downstream molecule of Ptch1 protein is G protein coupled receptor protein Smo. In the absence of Hedgehog, Ptch1 indirectly inhibits Smo. However, the binding of hedgehog and Ptch1 releases this inhibition, thus activating Smo and its downstream pathway and inducing the expression of development-related proteins [47]. SHH is a glycoprotein molecule that is expressed throughout the central nervous system, and regulation of the sonic hedgehog signal cascade may play a potential role in improving the effects of SCI [48]. SHH has emerged as a potential avenue of therapy for enhancing regeneration following SCI [49]. In the spinal cord, deletion of Shh and Smo result in gliosis in subsets of astrocytes in the cortex and spinal cord, suggesting that Shh signaling suppresses their proliferation [50], and may reduce scar formation. Gli1 induce motor neurons [51], which is consistent with the results of our study. The results of the western blotting and immunofluorescence results showed that, compared with the SCI group, the expression of SHH, Smo, and Gli-1 increased significantly after ES.

Neuroglial cells are a group of stromal cells that perform critical functions in the nervous system [52]. We previously showed that glial cells play an important role in the spinal cord of rats using single-cell sequencing [12]. Targeting astrocytes results in an additive increase in motor function by delaying both onset and progression [53]. The SHH/Gli-1 signal activated in astrocytes plays an important role in locomotor recovery after SCI [54]. Our results show that ES activates the SHH signaling pathway in astrocytes.

The present study had some limitations. We failed to study the changes in cell spatial localization and in the local microenvironment of the spinal cord tissue after ES at the single-cell level. Future studies should analyze these microenvironmental changes.

5. Conclusion

In summary, ES had a positive effect on motor dysfunction and anti-inflammatory effects after SCI in rats. Transcriptomic and proteomic sequencing provided unique perspectives on the complex relationships between ES on SCI, where the SHH signaling pathway plays a critical role. Our study provides an important theoretical basis for ES clinical conversion therapy in patients with SCI.

Funding information

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Data availability statement

The data have been deposited in the Gene Expression Omnibus (GEO) database under accession number GSE 214291.

CRediT authorship contribution statement

Erliang Li: Writing – review & editing, Writing – original draft, Visualization, Methodology, Investigation, Data curation. **Rongbao Yan:** Visualization, Software, Data curation. **Huanhuan Qiao:** Software, Data curation. **Jin Sun:** Data curation. **Peng Zou:** Data curation. **Jiaqi Chang:** Data curation. **Shuang Li:** Software. **Qiong Ma:** Software. **Rui Zhang:** Supervision, Resources, Project administration, Conceptualization. **Bo Liao:** Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix ASupplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2023.e23960.

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