Inhibiting SHP2-mediated microglial subtype transition alleviates secondary inflammation following spinal cord injury

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INTRODUCTION: Spinal cord injury is a severe traumatic disorder of the central nervous system, with an incidence that is increasing each year. However, effective treatment methods are still lacking. Spinal cord injury can be divided into two phases: primary and secondary injury. The primary injury is unavoidable and untreatable, while the secondary injury is preventable and treatable. The primary injury can lead to the massive release of cytokines, triggering a cascade of inflammatory responses that exacerbate spinal cord damage. Regulating secondary inflammation can break the vicious cycle and has become one of the directions for treating spinal cord injuries. The protein tyrosine phosphatase SHP2, encoded by the PTPN11 gene, can regulate the activity of multiple signaling pathways through its dephosphorylation function and is involved in several key signaling pathways in immune inflammation. SHP2 mediates factors such as tumor necrosis factor and interleukin-1, affecting the occurrence and development of inflammatory responses. SHP2 is also involved in the production of inflammatory mediators and the regulation of inflammatory responses in the Toll-like receptor signaling pathway. However, the role of SHP2 in the immune-inflammatory microenvironment of spinal cord injury has not yet been reported.

METHODS: This study investigated SHP2's role in spinal cord injury-related inflammation and its impact on microglial polarization and metabolic reprogramming. Initially, SHP2 expression was assessed via immunohistochemistry and flow cytometry in microglia from injured mice, showing elevated levels at injury sites. The study employed siRNA and SHP2 inhibitors (SHP2i) to modulate microglial activity and evaluate their inflammatory response. In vitro, using the BV2 microglial cell line and primary microglia, changes in inflammatory cytokine secretion, M1 polarization, and metabolic pathways were measured following SHP2 interference. Additionally, glycolysis and oxidative phosphorylation were manipulated using 2-deoxy-D-glucose and oligomycin to study SHP2's regulatory effects on microglial metabolism and polarization. Data were validated through real-time PCR, Western blot, and metabolic assays, with statistical analysis to evaluate significance.

RESULTS SECTION: The results showed marked SHP2 upregulation in microglia at the injury sites. Using siRNA and SHP2 inhibitors significantly reduced lipopolysaccharide-induced microglial inflammation, decreasing cytokines like IL-6 and TNF- α . In vivo, SHP2-knocked-down mice displayed reduced inflammation and quicker motor function recovery. Further analysis indicated that M1 microglial polarization largely relies on glycolysis, and its inhibition substantially lowered inflammatory markers like iNOS and IL-1 β . Downregulation of SHP2 shifted microglial energy metabolism towards oxidative phosphorylation, enhancing M2-associated anti-inflammatory and repair functions. Intriguingly, inhibiting metabolic pathways had minimal impact on SHP2 expression, suggesting SHP2’ s role as an upstream metabolic regulator and a pivotal target in microglial metabolic reprogramming and polarization.

DISCUSSION: This study identifies SHP2 as a promising therapeutic target for spinal cord injury (SCI), crucial for reducing inflammation and aiding recovery by regulating energy metabolism. SHP2's role in modulating microglial activation is key to SCI pathophysiology, and ongoing developments in SHP2 inhibitors show potential for new treatments. The complexity of the SCI microenvironment and the limitations of in vitro models pose challenges to translating these findings. Elevated SHP2 may also trigger negative feedback affecting its therapeutic impact, necessitating further research. SHP2 inhibitors are still in preclinical development, requiring additional studies on biotoxicity and specificity. Clinical trials are essential to ensure their safety and efficacy in treating SCI. Thus, extensive research is necessary to fully determine the capabilities and limitations of SHP2 inhibitors in SCI therapy.

SIGNIFICANCE/CLIICAL RELEVANCE: This study identifies SHP2 as a potential therapeutic target for secondary inflammation in spinal cord injury.

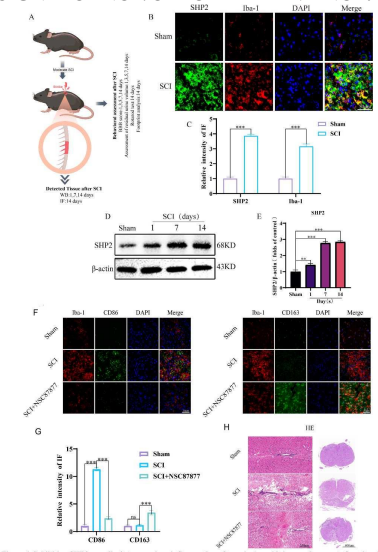

Figure 1. Inhibiting SHP2 can alleviate secondary inflammation after spinal cord injury and promote functional recovery. (A) Establishment of SCI mouse model. Created with Adobe Illustrator 2023. (B, C) Immunofluorescence staining for SHP2 (green-CoraLite488) and Iba-1 (red-CoraLite594) in the mouse spine 14 days after SCI. Quantitative analysis showing that SHP2 and Iba-1 expression levels increased significantly relative to the Sham group (n = 5 slices from three mice per group). Scale bar: 50 μm. (D, E) Western blotting was used to detect SHP2 expression 1, 7, and 14 days after SCI in mice. (F, G) Iba-1 (red-CoraLite594), CD163 (green-CoraLite488), and CD86 (green-CoraLite488) immunoreactivities were detected by immunofluorescence staining. Quantitative analysis showed that CD86 fluorescence intensity near the SCI site was significantly decreased in the SCI + NSC87877 group compared with the SCI group. Conversely, there was a significant increase in CD163 expression (n = 5 slices from three mice per group). Scale bars: 50 μm. (H) HE staining of mouse spinal cord sections 14 days after SCI. HE staining of transverse and longitudinal spinal cord sections showed that the injured area in the SCI + NSC87877 group was significantly smaller than that in the SCI group (n = 5 slices from three mice per group). Scale bars: 100 μm (left), 600 μm (right).

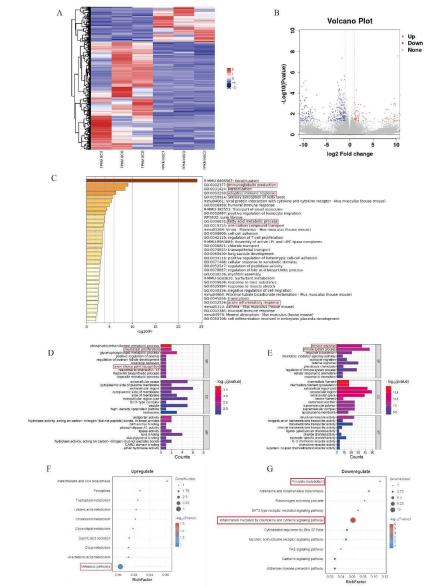

Figure 2. Transcriptome sequencing indicates that SHP2 is related to inflammation and energy metabolism. (A) Heatmap of the transcriptome sequencing results from SCI and SCI + NSC87877 mice 14 days after SCI (n = 3 mice per group). (B) Volcano map of the transcriptome sequencing results from SCI and SCI + NSC87877 mice 14 days after SCI. (C–G) GO term and KEGG pathway enrichment analyses of the transcriptome sequencing results from the SCI and SCI + NSC87877 mice 14 days after SCI. GO: Gene Ontology; KEGG: Kyoto encyclopedia of genes and genomes; NSC87877: SHP2 inhibitor; SCI: spinal cord injury; HP2: Src homology 2 domain-containing protein tyrosine phosphatase 2.

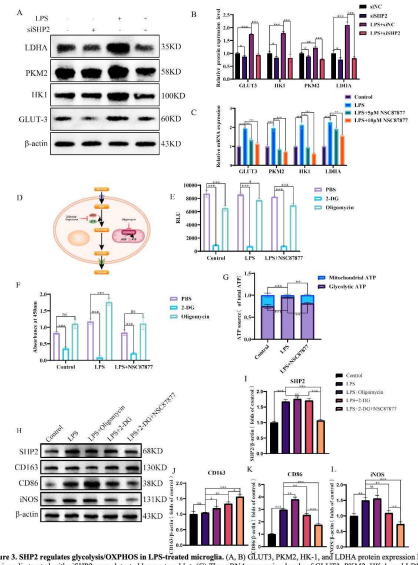

Figure 3. SHP2 regulates glycolysis/OXPHOS in LPS-treated microglia. (A, B) GLUT3, PKM2, HK-1, and LDHA protein expression levels in microglia treated with siSHP2 were detected by western blot. (C) The mRNA expression levels of GLUT1, PKM2, HK-1, and LDHA in microglia treated with NSC87877 were detected by qRT-PCR. (D) Schematic diagram illustrating blockade of the glucose metabolism pathway by 2-DG and oligomycin. (E–G) Intracellular ATP production and lactic acid production in microglia treated with LPS, oligomycin, 2-DG, and NSC87877, as detected using a glycolysis/OXPHOS kit. (H–L) SHP2, CD163, CD86, and iNOS expression levels in microglia treated with LPS, oligomycin, 2-DG, and NSC87877 were detected by western blot. Data are expressed as the mean ± SD. The experiment was repeated three times. *P < 0.05, **P < 0.01, ***P < 0.001 (one-way analysis of variance with Bonferroni's post hoc test). 2-DG: 2-Deoxy-D-glucose; CD163: cluster of differentiation 163; CD86: cluster of differentiation 86; GLUT3: glucose transporter 3; HK-1: hexokinase-1; iNOS: inducible nitric oxide synthase; LDHA: lactate dehydrogenase A; LPS: lipopolysaccharide; ns: not significant; NSC87877: SHP2 inhibitor; OXPHOS: oxidative phosphorylation; PKM2: pyruvate kinase M2; RLU: relative light unit; SHP2: Src homology 2 domain-containing protein tyrosine phosphatase 2; siNC: small interfering RNA negative control; siSHP2: small interfering RNA of SHP2.