Comprehensive analysis of m6A methylation modification in chronic spinal cord injury in mice

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Research Article

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Abstract

Chronic spinal cord injury (CSCI) is a catastrophic disease of the central nervous system (CNS), resulting in partial or complete loss of neurological function. N6-methyladenosine (m6A) is the most common form of reversible posttranslational modification at the RNA level. However, the role of m6A modification in CSCI remains unknown. In this study, we established a CSCI model using a water-absorbable polyurethane polymer, with behavioral assessment, electrophysiological analysis, and histochemical staining for validation. Methylated RNA immunoprecipitation sequencing (meRIP-seq) and mRNA sequencing (mRNA-seq) were jointly explored to compare the differences in CSCI spinal tissue and normal spinal tissue. Furthermore, qRT–PCR, western blotting, and immunofluorescence staining were used to analyze m6A modification-related proteins. We found that water-absorbable polyurethane polymer well simulated chronic spinal cord compression. BMS scores and electrophysiological analysis showed continuous neurological function decline after chronic compression of the spinal cord. meRIP-seq identified 642 differentially modified m6A genes, among which 263 genes were downregulated and 379 genes were upregulated. mRNA-seq showed that 1544 genes were upregulated and 290 genes were downregulated after CSCI. Gene Ontology (GO) terms and enriched Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways were also identified. qRT–PCR, western blotting, and immunofluorescence staining showed that Mettl14 was significantly upregulated after CSCI. Our study revealed a comprehensive profile of m6A modifications in CSCI and may provide a valuable tool for further research on CSCI.

Introduction

Chronic spinal cord injury (CSCI) is a catastrophic disease of the central nervous system (CNS) that usually causes partial or complete loss of neurological function; to date, there is no effective treatment1,2. The most common reason for CSCI is chronic spinal cord compression, such as intervertebral disc herniation, ligament sclerosis, and bone hyperplasia3,4. Although surgical treatment, such as decompression, can effectively relieve the symptoms of spinal cord compression, neuronal loss, apoptosis, and inflammatory factor infiltration persist due to the occurrence of secondary SCI5,6. Therefore, exploring the pathological changes and internal mechanisms of CSCI is critical for CSCI treatment.

Epigenetic modification is a widespread form of posttranscriptional modification in organisms and includes DNA modifications, histone modifications, chromatin remodeling, and RNA modifications7. Epigenetic regulation affects gene expression but does not alter the DNA sequence and plays a vital role in CNS diseases8. DNA methylation affects local chromatin structure to change gene expression and has been reported to regulate axon regeneration in CNS injuries9. Histone deacetylase 1 (HDAC1) is a crucial epigenetic factor that removes acetyl from histones. Inhibiting HDAC1 was found to promote neuronal loss and DNA damage, which further deteriorated behavioral outcomes after stroke10. It has been reported that knockdown of the epigenetic factor UTX can facilitate axon regeneration and angiogenesis after acute spinal cord injury by targeting microRNA-2411,12. Epigenetic modifications are also involved in angiogenesis after chronic compressive spinal cord injury through the Xist/miR-32-5p/Notch-1 axis13.

Among epigenetic modifications, N6-methyladenosine (m6A) is the most common and abundant mRNA modification14,15. m6A modification is a reversible and dynamic biological process that is mediated by methyltransferases (writers, mainly METTL3, METTL14 and WTAP), demethylases (erasers, mainly FTO and ALKBH5), and RNA-binding proteins (readers, mainly YTHDF1 and YTHDF3)16. m6A modifications have been reported to play an indispensable role in diverse biological processes. The development of various tumors is accompanied by m6A methylation modifications, including in colorectal cancer17, bladder cancer18, and prostate cancer19. m6A modifications are present in many CNS diseases15. m6A was found to promote the synthesis of a range of proteins in a YTHDF1-dependent manner. Knockout of YTHDF1 caused hippocampal deficits, resulting in damage to learning and memory20. Mettl14 deficiency in the substantia nigra suppressed tyrosine hydroxylase production in dopaminergic neurons, ultimately causing impairment of motor function21. Another study confirmed that microRNA-421-3p can directly target YTHDF1 to downregulate the expression of p65, a key component of the NF-kB signaling pathway, thus preventing inflammation in cerebral ischemia/reperfusion injury22. Recently, studies on m6A modifications have emerged in acute SCI. m6A modifications have been reported in acute spinal cord injury, especially in regulating neuronal apoptosis23,24. Xing et al. analyzed altered m6A modifications following acute SCI in zebrafish larvae25. However, a comprehensive analysis of m6A modification changes in mice with chronic compressive spinal cord injury has never been reported.

Here, we constructed a chronic compressive spinal cord injury model using a water-absorbable polyurethane polymer. meRIP-seq and mRNA-seq were used to explore m6A modifications in injured and normal spinal tissue. We jointly analyzed the differentially expressed m6A-modified genes and identified enriched GO terms and KEGG pathways. Moreover, we found that Mettl14 was highly expressed in the spinal cord after CSCI. Collectively, our research may provide new insights for the treatment of CSCI.

Methods And Materials

Animals and ethics statement

Eight-week-old C57BL/6 female mice (weighing approximately 20-23 g) were purchased from Hunan SJA Laboratory Animal Co., Ltd. (Changsha, China) and housed in the Department of Laboratory Animals, Central South University (Changsha, China). All animals had free access to food and water and lived in a temperature-suitable room under natural light. All procedures carried out were approved by the Animal Ethics Committee of Central South University.

Establishment of chronic spinal cord injury

Thirty-eight mice were randomly divided into two groups: a control group and a CSCI group (19 mice per group). Based on previous research, we applied a water-absorbable polyurethane polymer (Guangzhou Fischer Chemical Co., Guangzhou, China) to construct the CSCI model26. In brief, the mice were
anesthetized with 75 mg/kg sodium pentobarbital. The dorsal skin at the T10 level was incised, followed by fascia and muscle stripping to expose the T10 lamina. A portion of the lamina was removed, and a polyurethane polymer sheet (1.5 mm*1.0 mm*0.5 mm) was inserted into the epidural space. For the mice in the control group, after the T10 lamina was exposed, a portion of the lamina was removed without placing a polyurethane polymer sheet in the epidural space. Then, the muscles, fascia and skin were sutured sequentially. After the mice were awake, they were returned to their rearing cages. For the next three days, the mice were injected with penicillin daily to prevent infection.

### Histological and immunofluorescence staining

Four weeks after injury, the mice were anesthetized and sequentially perfused from the left ventricle with heparinized saline and 4% paraformaldehyde. Subsequently, a 10 mm length of spinal cord containing the injured area was harvested and dehydrated for sectioning. For histological staining, samples were dehydrated in gradient alcohol and embedded in paraffin. Spinal cord segments were transected into 8 µm thick slices and stained with an HE staining kit (Solarbio, China) according to the manufacturer's instructions.

For immunofluorescence staining, samples were dehydrated with sucrose solution and embedded with OCT compound (Sakura, United States). The spinal cord was sectioned longitudinally into 16 µm thick slices and incubated overnight at 4 °C with anti-Mettl14 antibody (1:100, Zen Bio, China) and anti-NeuN antibody (1:400, Abcam, United States). After washing with PBS, the slices were incubated at room temperature for 1 hour with secondary antibodies. 4’,6-Diamidino-2-phenylindole (DAPI, GeneTex, United States) was used to stain the cell nuclei.

### Quantitative Real-time PCR (qRT–PCR)

Total RNA was extracted using TRizol reagent (Invitrogen, United States). Complementary DNA (cDNA) was synthesized with a reverse transcription kit (Promega, United States). GoTaq® qPCR Master Mix (Promega, United States) and primers (Sangon Biotech, China, Table S1) were added to the cDNA mixture for qRT–PCR. CT values were detected using a quantitative PCR system (ABI, United States). GAPDH was used as an internal reference, and the relative gene expression was calculated using the 2^(-ΔΔCt) method.

### Western blotting

Total protein was extracted from the spinal cord using RIPA lysis buffer (Beyotime, China), and the protein concentration was detected with a BCA kit (Beyotime, China). The denatured proteins were separated via electrophoresis using 10% SDS-PAGE gels and then electrotransferred to PVDF membranes (Millipore, United States). Then, the membranes were blocked with 5% nonfat milk and incubated overnight at 4 °C with anti-Metttl14 antibody (1:1000, Zen Bio, China) and anti-β-actin antibody (1:2000, Cell Signaling Technology, United States). The next day, the membranes were washed with 0.1% TBST solution and incubated with secondary antibody for 90 minutes. Enhanced chemiluminescence (ECL) reagent was used to detect the membrane bands.

### Locomotor function assessment and neuroelectrophysiological analysis

Basso mouse scale (BMS) scoring was used to evaluate locomotor function as previously described27. The BMS score ranged from 0-9 (0 indicates complete paralysis, and 9 indicates normal locomotion). BMS scores were evaluated at 1, 3, 7, 14, 21 and 28 days postinjury. Neuroelectrophysiological analysis was performed at 28 days postinjury as described in our previous research28. Stimulating electrodes were placed on the surface of the skull (caudal/lateral to the bregma: 1 mm/0.5 mm, -4 mm/0.6 mm). Recording electrodes were inserted into the tibialis anterior muscle. The reference electrode was placed under the dorsal skin. Motor evoked potentials (MEPs) were documented to assess neurological connectivity.

### RNA extraction, meRIP-seq and mRNA-seq

meRIP-seq and mRNA-seq were carried out by Seqhealth Technology Co., Ltd. (Wuhan, China). Total RNA was extracted from the spinal cord area of CSCI and the same segments in the control group using TRizol (Invitrogen, United States) and enriched with Oligo d (T) magnetic beads. A total of 150 µl of each sample was taken, and a Nanodrop™ OneC spectrophotometer (Thermo Fisher, United States) was used to detect the concentration and purity of the RNA. An RNA Nano 6000 Assay Kit (Agilent, United States) was applied to detect the RNA quality number (RQN). The quality of the RNA library was evaluated using agarose gel electrophoresis. Finally, the qualified RNA library was quantified using Qubit 3.0 with a Qubit™ RNA Broad Range Assay kit (Life Technologies, United States). VAHTS mRNA Capture Beads (Vazyme, China) were used for polyadenylated RNA enrichment. Then, 20 mM ZnCl2 was added to the RNA to obtain fragmented RNA distributed in a range from 100 nt to 200 nt. Ninety percent of the RNA was used for m6A immunoprecipitation (IP) with anti-m6A antibody, and the rest was saved as “Input”. The RNA sequencing library was generated using a KC-DigitalTM Stranded mRNA Library Prep Kit for Illumina® (Catalog no. DR08502, Wuhan Seqhealth Co., Ltd. China) and finally sequenced on a NovaSeq 6000 sequencer (Illumina).

### Bioinformatics analysis

Raw reads acquired from the Illumina HiSeq sequencer were filtered using Trimmomatic (version 0.36) to remove low-quality and adaptor-contaminated beads. Clean beads were harvested through UID deduplication. Briefly, we performed cluster analysis on all sequences of the same UID to obtain different clusters. Then, sequence alignments were performed on the multiple sequences under each cluster, thereby obtaining a consensus sequence. The deduplicated clean data were mapped to the reference genome of mice using STAR software (version 2.5.3a). The specific region to which the protein binds (peak calling) was analyzed using exomePeak (version 3.8) software. After m6A peak annotation with bedtools (Version 2.25.0), the differentially methylated sites were identified with a Python script using Fisher's test. GO and KEGG pathway enrichment analyses were carried out using KOBAS software (version: 2.1.1). The threshold of significant enrichment was set as p < 0.05.

### Statistics

The threshold of significant enrichment was set as p < 0.05.
GraphPad Prism 8.0 was used to analyze the data. Data are presented as the mean ± standard deviation (SD). Unpaired Student's t test was applied to compare differences between two groups, such as in qRT-PCR, western blotting, and fluorescence quantitative analyses. Repeated-measures two-way ANOVA was used to analyze BMS scores. p < 0.05 was considered to indicate a significant difference (*p < 0.05, **p < 0.01).

**Results**

**Validation of the CSCI model in mice**

We implanted a water-absorbable polyurethane polymer to induce chronic compressive spinal cord injury according to previous studies. Since our experimental subjects were mice, we adjusted the size of the polyurethane polymer to 1.5 mm×1.0 mm×0.5 mm before implantation. Four weeks after implantation, the size of the water-absorbable material reached approximately 2.5 mm×1.65 mm×1.0 mm (Fig. 1A). The BMS scores showed that the locomotor function in the CSCI group continued to decline compared to that in the control group until the observation endpoint (Fig. 1B). Electrophysiological experiments showed that mice in the CSCI group displayed lower motor evoked potentials, indicating severely impaired neurological conduction capacity (Fig. 1C-D). To further explore the effect of chronic compression on the spinal cord, we performed HE staining. After compression, the spinal morphology was destroyed, and neurons were lost in large numbers (Fig. 1E).

**Overview of meRIP-sequencing results**

To explore the changes in m6A methylation modifications in spinal cord tissues after CSCI, we performed meRIP sequencing. The raw data showed that the average data size of the "IP" samples was 12.96 Gb and that of the "Input" samples was 10.77 Gb (Table 1). After removing low-quality and adaptor-containing reads through UID deduplication, the clean reads of "IP" samples reached approximately 76.82% of raw reads, and the "Input" samples reached approximately 69.81% (Table 1). The Clean_Q30 (%) value of each sample, which represents the quality of sequencing, exceeded 98.50%, and the GC base content of clean reads exceeded 52% (Table 1). Clean data were further aligned to the reference genome of mice to acquire comprehensive transcript information. The proportion of sequences for which each sample had a unique alignment position on the reference sequence reached 94% (Table 2). The numbers of reads mapped to the reference genome at both ends of the sequence were basically the same (Table 2).

**General features of m6A methylation in normal and CSCI spinal tissues**

We analyzed the read distribution in different regions of the reference genome and found that most of the reads were distributed in the CDS, 3'UTR and 5'UTR regions in both groups (Fig. 2). By searching for protein binding regions, we found 22798 m6A peaks in the control group and 22866 m6A peaks in the CSCI group (Fig. 3A). Compared to the control group, 263 m6A peaks were downregulated and 379 m6A peaks were upregulated in the CSCI group (Fig. 3B). The top 10 m6A hypermethylated or hypomethylated genes are listed in Table 3. The distribution of peaks in functional regions of the genome was similar in the control and CSCI groups. The peaks were mainly distributed in the CDS and 3'UTR regions (Fig. 3C-D). In the control group, m6A peaks were mainly distributed in CDSs (25.78%), 3'UTRs (30.89%) and introns (37.44%), while only 2.06% and 3.84% were distributed in exons and 5'UTRs, respectively (Fig. 3E). In the CSCI group, m6A peaks were distributed in CDSs (26.3%), 3'UTRs (29.94%) and introns (38.15%), while 1.9% and 3.71% were distributed in exons and 5'UTRs, respectively (Fig. 3F). Through peak annotation, we identified 10127 genes from 22798 m6A peaks in the control group and 10297 genes from 22866 m6A peaks in the CSCI group (Fig. 3G). Among them, 1353 genes were only detected in the control group, and 1183 genes were unique to the CSCI group (Fig. 3H).

**Functional analysis of differential m6A methylation and differentially expressed genes after CSCI**

We performed functional analysis to explore the effect of m6A modification on CSCI via GO analysis and KEGG pathway analysis. GO enrichment analysis revealed that hypomethylated m6A-peak genes were enriched in tissue development, system process and metabolic process in biological process; enriched in intrinsic component of the membrane, cell periphery, and integral component of the membrane in cellular component; and enriched in ion binding, metal ion binding and cation binding in molecular function (Fig. 4A). Genes with increased m6A methylation were enriched in developmental process, anatomical structure development and multicellular organism development in biological process; enriched in polymeric cytoskeletal fiber, supramolecular fiber and extracellular matrix in cellular component; and enriched in cytokine receptor binding, growth factor activity and cytokine activity in molecular function (Fig. 4B). We also used bubble diagrams to describe the top 15 GO terms specific to the control and CSCI groups (Fig. 5A-B). The KEGG pathway analysis showed that hypomethylated m6A-peak genes were significantly enriched in the wnt signaling pathway, β-alanine metabolism and neuroactive ligand–receptor interaction (Fig. 5C), while hypermethylated m6A-peak genes were significantly enriched in the PI3K-Akt signaling pathway, neuroactive ligand–receptor interaction, the NF-κB signaling pathway and other pathways (Fig. 5D).

Furthermore, mRNA sequencing was applied to analyze differentially expressed genes (DEGs, fold-change>2 and p value<0.05) after CSCI (Fig. 6A). We performed GO analysis and KEGG pathway analysis to explore the biological functions of the DEGs. The GO analysis indicated that downregulated genes were significantly enriched in cilium movement, integral component membrane and transmembrane transporter activity (Fig. 5E), while upregulated genes were significantly enriched in immune system process, immune response, and defense response (Fig. 5F). KEGG pathway analysis revealed that downregulated genes were significantly enriched in neuroactive ligand–receptor interactions, the cAMP signaling pathway and morphine addiction (Fig. 5G), while upregulated genes were significantly enriched in the TNF signaling pathway, osteoclast differentiation, the NF-κB signaling pathway and other pathways (Fig. 5H).

**Conjoint analysis of differentially expressed and methylated genes after CSCI**

The mRNA-seq results showed that there were 290 downregulated genes and 1544 upregulated genes (Fig. 6B-C) after CSCI, of which the top 10 upregulated and downregulated genes are listed in Table 4. To further investigate the association between m6A-methylated genes and differentially expressed genes after CSCI, we performed conjoint analysis of meRIP-seq and mRNA-seq data, and 55 genes were identified. Among the 34 m6A hypermethylated genes, 25 genes
showed upregulated mRNA expression, and 9 genes showed downregulated mRNA expression. Among the 21 m6A hypomethylated genes, 19 genes had upregulated mRNA expression, and 2 genes had downregulated mRNA expression (Fig. 6D).

**Mettl14 was significantly increased after CSCI**

To further explore changes in the mRNA expression levels of m6A-related enzymes, we performed qRT–PCR analysis to detect methylases (Mettl3, Mettl14, WTAP), demethylases (FTO and ALKBH5), and RNA-binding proteins (YTHDF1, YTHDF3). The results showed that mettl14, YTHDF1 and YTHDF3 were significantly upregulated after CSCI, while other proteins showed no significant differences (Fig. 7A). Western blotting results also verified that mettl14 expression was significantly increased in the CSCI group (Fig. 7C). Immunofluorescence costaining of NeuN (green) and Mettl14 (red) indicated that mettl14 was mainly expressed in neurons, with upregulated expression after CSCI.

**Discussion**

Chronic spinal cord injury (CSCI) is a catastrophic central nervous system disease for which there is currently no effective treatment. The pathological process of CSCI is complex, often resulting in vasculature damage and neuronal loss. Unlike acute SCI, which causes a rapid decline or loss of neurological function, CSCI tends to progress more slowly. Common modeling approaches for CSCI include using stainless steel screws, expanding polymers, silastic sheets and water-absorbing materials or using Tiptoe-walking Yoshimura (twy/twy) mice, which spontaneously deposit calcium at the C1-C2 level. In this study, we used a water-absorbable polyurethane polymer to construct the CSCI model and validated it with the BMS score, neuroelectrophysiology and HE staining.

m6A methylation is the most common posttranscriptional modification at the RNA level and is widespread in biological processes. An increasing number of reports have shown that m6A methylation plays an important role in central nervous system development and disease progression. In a cerebral ischemia/reperfusion (I/R) injury model, microRNA421-3p specifically targeted YTHDF1 to reduce its expression. YTHDF1 further recognized and bound to the p65 m6A site to promote p65 translation, thereby causing an inflammatory response. Another study comprehensively analyzed the m6A methylation profile after acute SCI in zebrafish and found that a bulk of neural regeneration-related genes were hypomethylated but highly expressed. However, the role of m6A modifications after CSCI has rarely been reported. In this study, we first performed meRIP-seq and mRNA-seq by isolating spinal cord tissues after chronic compression. We observed 22798 m6A peaks in the sham group and 22866 m6A peaks in the CSCI group. A total of 642 m6A differentially methylated peaks were identified, of which 263 peaks showed m6A downregulation and 379 showed m6A upregulation, indicating that m6A methylation changes did occur in spinal cord tissue after CSCI. We also found that the m6A peak distribution was similar in the two groups, with most peaks located in the CDS, 3’UTR and introns. Meanwhile, using mRNA-seq, we found that 290 genes were downregulated and 1544 genes were upregulated after CSCI. These results showed that m6A methylation modifications were likely to regulate the expression of a series of genes after CSCI to affect the pathological process of CSCI.

By association analysis of meRIP-seq and mRNA-seq, we found that 21 genes were hypomethylated, of which 19 genes were upregulated and 2 genes were downregulated; 34 genes were hypermethylated, of which 25 genes were upregulated and 9 genes were downregulated. Our data indicated that m6A regulation was complex. Both hypomethylated and hypermethylated m6A could induce or reduce mRNA expression, which was consistent with previous studies. The differentially expressed genes with altered m6A methylation included small proline-rich repeat protein 1A (sprr1a), c-type lectin domain family 4 member D (clec4d) and apelin receptor (aplnr). Sprr1a is highly expressed in injured neurons and thought to be a regeneration-associated gene (REG) that promotes spontaneous axon growth. Macrophage-inducible clec4d was found to promote neuroinflammation and neuronal damage after traumatic brain injury (TBI). Apelin and its receptor aplnr have been reported to alleviate neuropathic pain after compression spinal cord injury. Our study confirmed that these differentially expressed genes after CSCI were regulated by m6A modification, which may shed new light on CSCI pathophysiology for further studies.

Through GO analysis and KEGG pathway analysis, we found that m6A differentially methylated genes were enriched in developmental process, ion binding, the wnt signaling pathway, neuroactive ligand–receptor interaction, the PI3K-AKT pathway and the NF-kappa B pathway. Activation of the wnt signaling pathway is critical for neurological recovery after SCI. It has been reported that simulating the wnt signaling pathway can promote vascular regeneration and axon regrowth and reduce inflammatory responses. The PI3K-AKT pathway participates in many fundamental cellular processes, including cell growth, cell proliferation, and cell motility. Curcumin- and lipopolysaccharide-activated olfactory ensheathing cells were found to provide proangiogenic effects through the PI3K-AKT signaling pathway. Another study verified that activating the PI3K-AKT signaling pathway facilitated nerve reconstruction and attenuated neuropathic pain after peripheral nerve injury. The NF-kappa B pathway is a classical pathway involved in inflammatory and immune responses. miR-182 reduced the expression of the upstream target of the NF-kappa B pathway kB kinase B to inhibit apoptosis and the inflammatory response. Although these classical signaling pathways have been widely reported in SCI, little is known about whether m6A methylation regulates these pathways. Our study indicated that m6A modifications contributed to activation or blockade of these signaling pathways after CSCI; however, the specific role and mechanisms still need further exploration in the future.

The process of m6A modification requires the interaction of methyltransferases (writers), demethylases (erasers), and RNA-binding proteins (readers). Mettl3 and mettl14 are common methyltransferases in the central nervous system. Xing et al. found that mettl3 was increased after acute spinal injury in zebrafish and was mainly expressed in astrocytes and neural stem cells. Other studies have shown that mettl3 is significantly increased in a mouse model of chronic inflammatory pain and is mainly located in neurons. Downregulation of mettl3 was reported to alleviate inflammatory pain by regulating pri-miR-65-3p processing or ten-eleven translocation methylcytosine dioxygenase 1 expression. Mettl14 has been reported to increase neuronal apoptosis by inhibiting EEF1A2 expression or promoting miR-375 maturation after acute spinal cord injury. Another study demonstrated that mettl14 is essential for injury- or pten deletion-induced axon regeneration of DRG neurons. However, m6A modifications in chronic compressive spinal cord injury have rarely been reported.
study showed that mettl14 was significantly overexpressed after CSCI and mainly expressed in neurons, which was further validated by qRT–PCR, western blotting, and immunofluorescence.

Our study is not without limitations. First, we obtained whole spinal tissue for m6A-seq and mRNA-seq. Although the total m6A modifications were assessed, it was difficult to distinguish m6A methylation changes in specific cell types. In further in-depth research, extracting specific cells for verification of m6A modification and exploring its function would be meaningful. Second, we performed preliminary analyses of differentially expressed genes and enriched GO and KEGG pathways, but their roles in CSCI require further validation through extensive experiments.

Collectively, our study analyzed m6A methylation modifications in spinal cord tissue after CSCI. The results indicated that m6A plays an important role in the process of CSCI. Our research may provide new insights for further study of the CSCI pathological process and potential treatment options.

**Declarations**

**Author contributions**

JH, YC and HL designed the study. CL and JZ carried out most of the experiments and data analysis. YJ, TQ, CD and TW assisted in experiments and data analysis. CL drafted the manuscript, and JH and YC revised the manuscript. All authors contributed to the manuscript.

**Consent to Participate**

Not applicable.

**Consent for Publication**

The consent to publish this manuscript has been obtained from all authors.

**Data Availability**

Data are available from the authors upon request.

**Conflict of interest**

The authors have no conflicts of interest relevant to this article.

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**References**


Tables

Table 1. Detailed information of Raw data obtained by sequencing in the CSCI group and control group

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Table 2. Comparison of sequence alignment to the reference genome
Table 3. Top 10 genes of m6A hypermethylated or hypomethylated

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Table 4. Top 10 upregulated or downregulated genes
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Figures

Figure 1

Establishment and evaluation of CSCI model. A. Representative images of water-absorbable polyurethane polymer before and after implantation into epidural space. B. BMS scores at different time points in control and CSCI group. N=8 animals per group. C. Representative electrophysiological traces in control and CSCI group. D. Quantification of the amplitude of MEPs in control and CSCI group. N= 8 animals per group. E. Representative images of HE staining of control and CSCI group. Scale bar: 200um. N=4 animals per group. The data are presented as the means ± SD. **p < 0.01.
Figure 2

Distribution of samples in different regions of the reference genome.

A

B

Figure 3

The analysis and annotation of peak in control and CSCI group. A. Peak numbers of control and CSCI group. B. M6A down-regulated and up-regulated peak numbers. C-D. Peak count in 5'UTR, CDS and 3'UTR of genes of control and CSCI group. E-F. Peak distribution in different gene functional regions of control and CSCI group. G-H. Number of Peak-associated genes of control and CSCI group.
Figure 4

GO terms enriched in hypomethylated or hypermethylated m6A-peak genes. A. Major GO terms enriched in hypomethylated m6A-peak genes. B. Major GO terms enriched in hypermethylated m6A-peak genes.
Figure 5

GO and KEGG pathway analysis of differential m6A methylation and differentially expressed genes after CSCI. A. KEGG terms of hypomethylated m6A-peak genes. B. KEGG terms of hypermethylated m6A-peak genes. C. Top 15 GO terms of specific m6A peak-related genes of control group. D. Top 15 GO terms of specific m6A peak-related genes of CSCI group. E. Top 15 GO terms of down-regulated genes. F. Top 15 GO terms of up-regulated genes. G. Top 15 KEGG terms of down-regulated genes. H. Top 15 KEGG terms of up-regulated genes.
Figure 6

Analysis of differentially expressed genes and correlation analysis of meRIP-seq AND mRNA-seq of control and CSCI group. A. Cluster map of differential genes between control and CSCI group. B. Volcano plot of control and CSCI group. C. Down-regulated and up-regulated genes after CSCI. D. Four-quadrant diagram of correlation analysis of meRIP-seq AND mRNA-seq.
Figure 7

Relative expression of m6A-related proteins in control and CSCI group. A. Relative expression of METTL3, METTL14, WTAP, ALKBH5, FTO, YTHDF1 and YTHDF3. GAPDH was used as internal reference. N=4 per group. B. Representative fluorescent images of NeuN (green) and mettl14 (red) in control and CSCI group. N=4 per group. Scale bar: 50 um. Scale bar of magnified figure: 5um. C. Western blot analysis of mettl14 in control and CSCI group. N=4 per group. Data are presented as the mean ± SD. *p < 0.05, **p < 0.01, ns=not significant.

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