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# Differential Expression of Tubulin Acetylase and Deacetylase Between the Damaged Central and Peripheral Branch of Dorsal Root Ganglion Neurons

Authors' Contribution:
Study Design A
Data Collection B
Statistical Analysis C
Data Interpretation D
Manuscript Preparation E
Literature Search F
Funds Collection G

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**Background:** The differences between the peripheral and central branches of the dorsal root ganglion (DRG) have not been fully elucidated. This study aimed to explore the expression of tubulin post-translational modifications (acetylation and deacetylation) between damaged peripheral and central branches of DRG neurons.

**Material/Methods:** Fifty Sprague-Dawley rats were randomly assigned to five groups with 10 rats in each group. These five groups consisted of spinal nerve ligation (SNL) at 24 hour and 48 hour, and cauda equina compression (CEC) at 24 hour and 48 hour, and a sham group. SNL injury in rats was induced by ligating L5 and L6 spinal nerves with 1-0 silk thread outboard the DRGs. CEC injury in rats was induced by a piece of silicone (10×1×1 mm) placed under the laminae of the L5–6 vertebra. Sham-operated rats underwent a simple laminectomy in L4, but silicone was not implanted. The expression profile of acetylase and deacetylase was examined by real-time PCR, Western blotting, and immunohistochemistry.

**Results:** In the experimental groups, rats presented increased expression of acetylase (NAT1 and MEC-17) and decreased expression of deacetylase (Sirt2 and HDAC6) levels. Additionally, the expression of NAT1 and MEC-17 was gradually increased in DRG neurons following peripheral axonal injury compared to central axonal injury in a time-dependent manner. Conversely, the expression of Sirt2 and HDAC6 was gradually decreased in DRG neurons following peripheral axonal injury compared to central axonal injury in a time-dependent manner.

**Conclusions:** Our study indicated that insufficiency of acetylase and upregulation of deacetylase in DRG neurons after central axonal injury may contribute to the pathogenesis of cauda equine syndrome.

**MeSH Keywords:** Acetylation • Cauda Equina • Ganglia, Spinal • Histone Deacetylases • Protein Processing, Post-Translational

**Full-text PDF:** http://www.medscimonit.com/abstract/index/idArt/902829







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## Background

Dorsal root ganglion (DRG) neuron is a pseudo-unipolar neuron. The posterior ramus of the lumbar spinal nerves consist of peripheral axon branches of DRG neurons, which can readily regenerate after injury caused by lumbar disc herniation. Lower limb pain and numbness can be significantly relieved after lumbar decompression surgery. In contrast, the sensory branches of cauda equine consist of central axon branches of DRG neurons. The consequences of cauda equine syndrome (CES), such as sensory disturbance of the perineal region, is hard to recover from after an injury caused by central lumbar disc herniation [1]. However, the central axon branches, critical components of cauda equine, do not easily regenerate after DRG neurons injury [2,3]. Such differences between the peripheral and central branches of DRG have not been fully elucidated.

Microtubules are a component of the cytoskeleton, and are important in a broad range of cellular processes, such as organelle movement, macromolecular assemblies, and chromosome separation [4]. Moreover, extensive investigations of the neuronal microtubules have revealed that they play critical roles in axon growth, dendritic arborization, and neuron migration [5,6]. It has been shown that these diverse roles of microtubules are dependent on post-translational modifications (PTM) such as the non-reversible removal of the conserved penultimate glutamate residue of α-tubulin, the removal and subsequent addition of the C-terminal tyrosine in α-tubulin, and poly glutamylation of α- and β-tubulin [7,8]. Additionally, several types of PTM have been discovered that include acetylation, phosphorylation, poly glutamylation, and poly amination [7]. In the current study, we explored the expression pattern of tubulin post-translational enzymes (acetylase and deacetylase) in the damaged peripheral and central branch of DRG neurons in rat models of spinal nerve injury and cauda equine injury.

## Material and Methods

### Animals

Fifty male SD rats, 6–8 weeks old, weighing 200–250 g, were used for surgical intervention. The surgical interventions for animal experiments were approved by the Institutional Animal Committee, and the animals were cared for in accordance with the Guide for the Care and Use of Laboratory Animals after the surgery.

### Experiment design

All the rats were randomly divided into five groups with 10 rats in each group. These five groups consisted of spinal nerve ligation (SNL) at 24 hours and 48 hours, and cauda equine compression (CEC) at 24 hours and 48 hours, and a sham group. For establishment of the SNL model, rats were anesthetized with 10% chloral hydrate (3 mL/kg, i.p.). After anesthetization, a midline approach was made, and a laminotomy was performed at the L5-6 level of the vertebra. The L5 and L6 DRGs were exposed by one side. The L5 and L6 spinal nerves were ligated with 1-0 silk thread outboard the DRGs. To achieve the CEC, a laminotomy was performed with L4 lamina under anesthesia. A piece of silicone block (10×1×1 mm) was placed under the laminae of the L5–6 vertebra. The dural sac should be avoided to prevent damage during the surgical operation [9]. Sham-operated rats underwent a simple laminectomy in L4, but silicone was not implanted. To prevent urinary retention, the rats' bladders were massaged daily three times.

### Real-time PCR

Total RNAs were isolated by TRIZol reagent and 1 μg of RNAs were reversely transcribed into cDNA. Real-time PCR was performed in a total volume of 20 μL reaction mixture. Relative expression of each gene was calculated using $2^{\Delta\Delta Ct}$ methods.

### Western blot

Tissues were harvested and lysed with lysis buffer for 20 minutes on ice and the protein concentration was quantified using a BAC assay kit (Beyotime Biotech, China). Proteins were electrophoresed through a 12% sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and transferred to a PVDF membrane. The membrane was incubated with anti-NAT1 (1: 200), anti-MEC-17 (1: 300), anti-Sirt2 (1: 200), and anti-HDAC6 (1: 200) antibodies (Santa Cruz, USA) overnight at 4°C. After washing, the membrane was incubated with appropriate horseradish peroxidase-conjugated secondary antibodies for one hour at room temperature. Protein bands were detected with an enhanced chemiluminescence kit.

### Immunofluorescence

Tissue sections were placed in EDTA solution and heated in an oven for antigen retrieval. The section was blocked with 2% skim milk for one hour at room temperature. The sections were then incubated overnight at 4°C with the indicated primary antibodies followed by an appropriate secondary antibody. The slices were stained with DAPI for 10 minutes at room temperature and images were captured using an inverted fluorescence microscope (Nikon, Japan).

### Statistical analysis

For statistical analysis of the results, the SPSS version 19.0 software was used. Experimental data are presented as mean





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**Table 1.** Transcriptional expression of acetylase and deacetylase in DRG neurons.

| Groups | Relative expression of mRNA levels of acetylase and deacetylase in DRG | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | NAT1 | | MEC-17 | | Sirt2 | | HDAC6 | |
| | 24 h post-injury | 48 h post-injury | 24 h post-injury | 48 h post-injury | 24 h post-injury | 48 h post-injury | 24 h post-injury | 48 h post-injury |
| SNL group | 1.033±0.185 | 2.729±0.675 | 6.335±1.406 | 12.613±3.466 | 4.271±0.702 | 1.011±0.317 | 0.993±0.176 | 0.495±0.115 |
| CEC | 0.797±0.253 | 2.572±0.636 | 1.531±0.528 | 4.121±1.364 | 6.510±1.249 | 5.016±1.440 | 8.583±2.737 | 5.692±1.556 |
| *F* | 1.69 | 0.09 | 30.69 | 15.59 | 7.33 | 22.14 | 22.97 | 33.30 |
| *P* | >0.05 | >0.05 | <0.01 | <0.05 | >0.05 | <0.01 | <0.01 | <0.01 |

±SD. One-Way ANOVA was used for comparison of different groups. Results were considered statistically significant when the *p* value was less than 0.05.

## Results

### Transcriptional expression of acetylase and deacetylase in DRG neurons

First, we evaluated the transcriptional expression of acetylase (NAT1 and MEC-17) and deacetylase (Sirt2 and HDAC6) in DRG neurons by real-time PCR. As shown in Table 1, the transcripts of MEC-17 were significantly higher in SNL group than the CEC group at 24 hours ($p<0.01$) and 48 hours ($p<0.05$) post-injury. The mRNA levels of HDAC6 were markedly lower in SNL group than the CEC group at 24 hours and 48 hours post-injury ($p<0.01$). Sirt2 transcripts presented decreased expression in SNL group comparing with the CEC group at 48 hours post-injury ($p<0.01$). Additionally, compared with the sham group, surgical intervention increased the transcriptional expression of acetylase (NAT1 and MEC-17) and decreased the mRNA levels of deacetylase (Sirt2 and HDAC6).

### Protein expression of acetylase and deacetylase in DRG neurons

Furthermore, Western blotting was performed to detect the protein expression of acetylase (NAT1 and MEC-17) and deacetylase (Sirt2 and HDAC6) in DRG neurons. Results showed that the protein levels of NAT1 and MEC-17 were obviously higher in the SNL group than the CEC group at both 24 hours and 48 hours post-injury. Conversely, the expression of deacetylase including Sirt2 and HDAC6 was lower in the SNL group than the CEC group at 24 hours and 48 hours post-injury (Figure 1). Moreover, we found that surgical intervention increased the protein expression of acetylase (NAT1 and MEC-17) and reduced deacetylase (Sirt2 and HDAC6) levels.

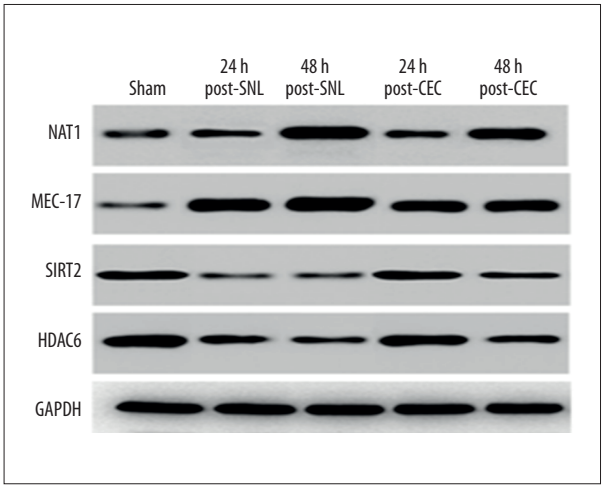


**Figure 1.** Protein expression of acetylase and deacetylase in DRG neurons. Two acetylase including NAT1 and MEC-17 and two deacetylase including Sirt2 and HDAC6 were determined by Western blotting.

### Positive expression rates of acetylase and deacetylase in DRG neurons

Immunofluorescence analysis method by Pro-Plus Image 6 software: we picked three microscopic vision fields (200) at random each group and choose red or green for positive expression, blue as the nucleus. The percentage of positive cells was calculated under microscope. We further explored the positive expression rates of acetylase (NAT1 and MEC-17) and deacetylase (Sirt2 and HDAC6) in DRG neurons by immunofluorescence staining. Representative images were shown in Figure 2 and positive DRG neurons for acetylase and deacetylase were listed in Table 2 and Figure 3. We found that NAT1 expression was increased in the SNL group compared with the CEC group at 48 hours post-injury ($p<0.01$). MEC-17 levels were higher in the SNL group than the CEC group at 24 hours post-injury ($p<0.01$). For the deacetylase, HDAC6 protein expression was significantly lower in the SNL group than the CEC group at 24 hours ($p<0.01$) and 48 hours ($p<0.05$) post-injury. The Sirt2





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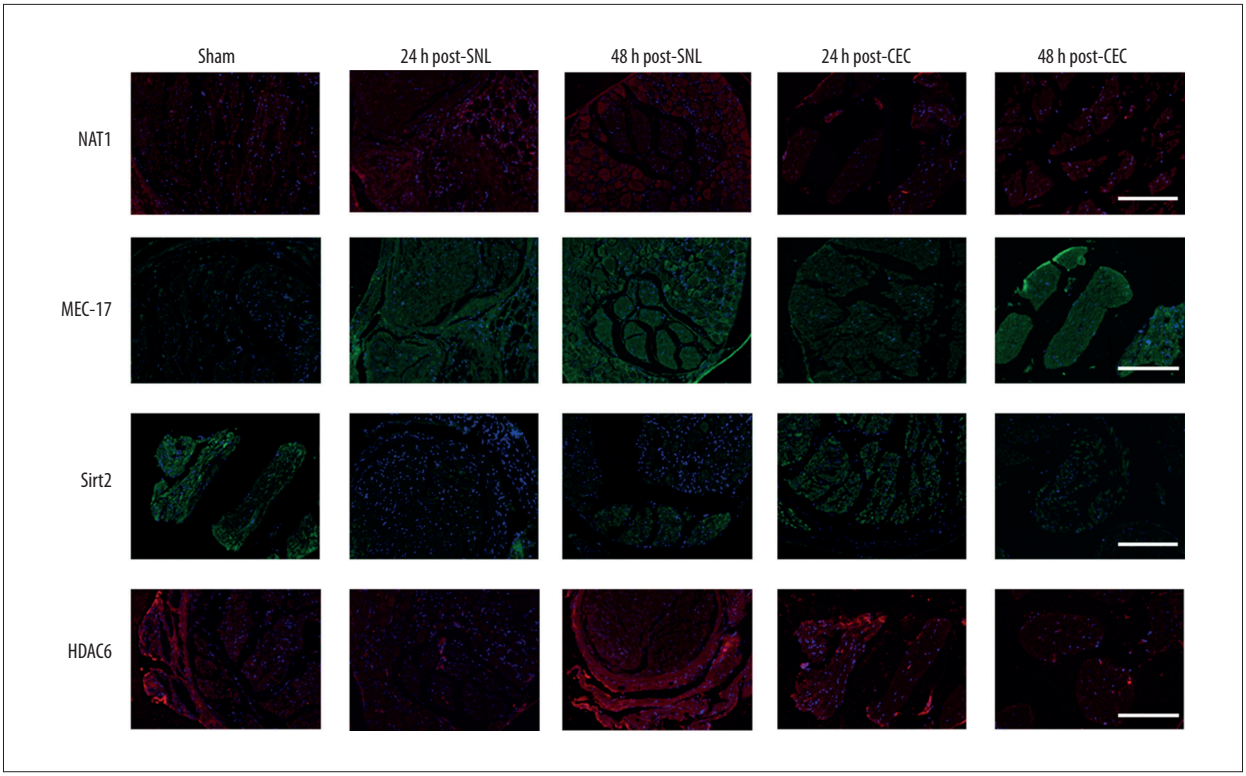


**Figure 2.** Immunofluorescence staining of acetylase and deacetylase in DRG neurons. Magnification 200×. Red or green for positive expression, blue as the nucleus. Scale bar=25 μm.

**Table 2.** Positive DRG neurons for acetylase and deacetylase.

| Groups | Positive DRG neurons | | | |
|---|---|---|---|---|
| | NAT1 | MEC-17 | Sirt2 | HDAC6 |
| Sham | 0.2503±0.2177 Aa | 0.2223±0.0174 ABa | 6.6748±1.1325 Ac | 5.3641±0.5658 Aa |
| 24 h post-SNL | 1.5583±0.5384 Aac | 2.4153±0.2304 Bb | 2.1063±0.3461 Bd | 2.2116±0.2723 Bb |
| 48 h post-SNL | 8.1627±1.1315 Bb | 5.2652±0.9221 ABb | 0.8982±0.1498 Bb | 1.9725±0.0785 Bb |
| 24 h post-CEC | 1.7479±0.8319 Aac | 0.8112±0.1799 Aa | 5.3475±0.7317 Aa | 3.8334±0.5151 Cc |
| 48 h post-CEC | 2.0402±1.1326 Ac | 1.8179±0.2107 ABb | 1.9069±0.1783 Bbd | 2.8151±0.3336 Bd |

Pairwise comparison in multiple groups was conducted with SNK method. There was a very significant difference with no same capital letters between the two groups ($P<0.01$). There was a significant difference with the same capital letters but no same small letters between the two groups ($0.01<P<0.05$). There was no significant difference with the same capital and small letters between the two groups ($0.01>0.05$).

expression in the SNL group was decreased compared with the CEC group at 24 hours post-injury ($p<0.01$).

## Discussion

Axon regeneration after the sensory branches of cauda equina injury in the mature mammalian central nervous system is extremely limited, which differs from that in the mammalian peripheral nervous system. The peripheral axonal branch of DRG neurons readily regenerates after nerve injury, whereas the central branch does not [2,3]. The main clinical manifestations of CES include saddle sensory disorder and motor dysfunction. After surgical operation, saddle sensation is hardly recovered but the motor function is markedly improved, which could be caused by the incapability to repair the central axon branch of DRG neurons [10]. Therefore, it is critical to elucidate the molecular mechanism underlying the axon





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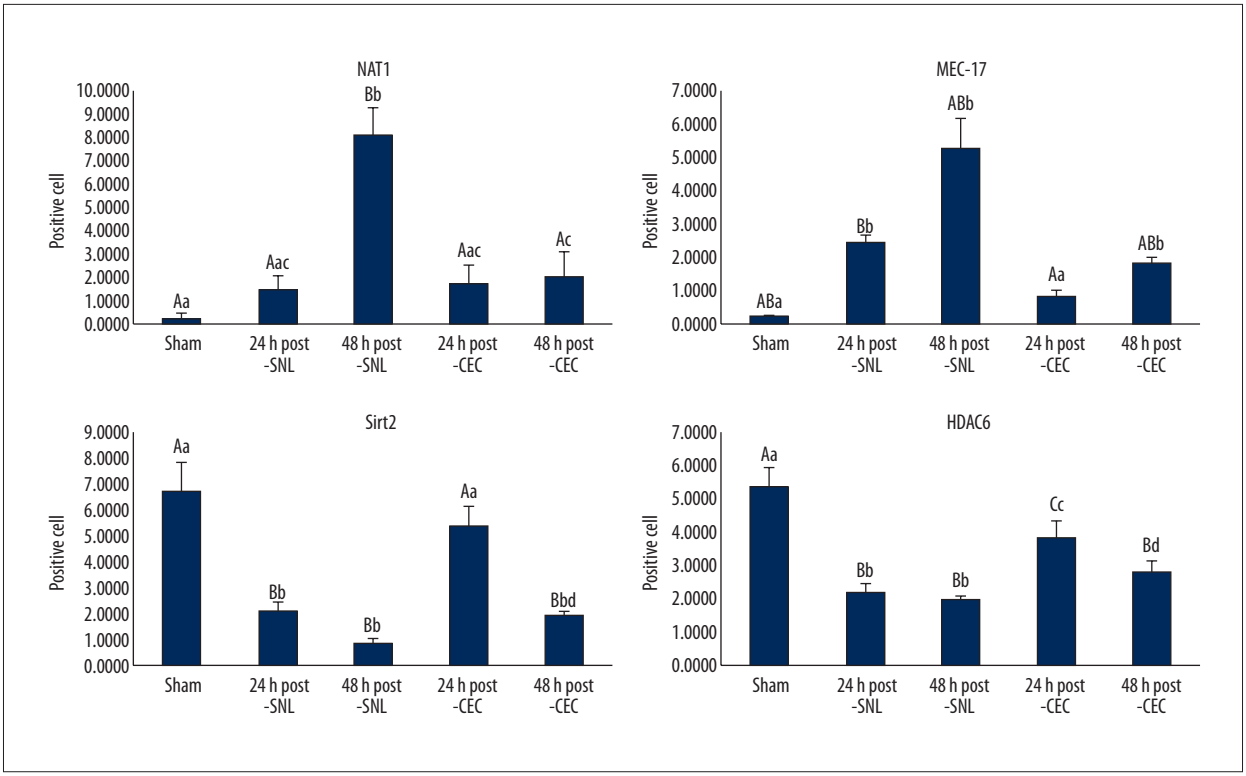


**Figure 3.** Positive DRG neurons for acetylase and deacetylase.

growth and regeneration of DRG neurons, especially the central axon branch.

Microtubules, an important component of the cytoskeleton, are critically implicated in diverse cellular processes, including neuron morphogenesis and maturation, axon regeneration, and transportation [4]. In addition, microtubules are finely controlled by a serial of molecules to maintain their dynamics and stability [11,12]. Recently, numerous studies have reported that the diverse roles of microtubules are modulated by the PTM including acetylation, poly-glutamylation, and poly amination [13,14].

In our study, we found that both central and peripheral axonal injury promoted the acetylase expression and decreased deacetylase levels in DRG neurons. Such changes in the transcriptional and protein expression of these enzymes could probably contribute to the repair of injury nerves. It has been reported that microtubule stability plays an important role in axon growth and regeneration [15,16]. And the dynamic stability of microtubules is dependent on the post-translational modification of microtubules, including acetylation and deacetylation [17]. Moreover, elevated tubulin acetylation promotes the vesicular transport of brain-derived neurotrophic factor and kinesin-1 [18,19]. HDAC6 inhibitors increase tubulin acetylation, reverse axonal loss and axonal transport deficits in a mouse model of mutant HSPB1-induced neuropathies [20]. Li et al. reported that MEC-17, which regulates the acetylation of tubulin, appears to control the migration and morphological transition of cortical neurons [21]. Knockdown of MEC-17 leads to reduction in a-tubulin acetylation, impaired migration of cortical neurons [21], and induces abnormal structure of flagella in mouse sperms, and decreases the contact inhibition during cell proliferation [22,23]. Our study confirmed that tubulin acetylation and deacetylation are involved in the repair of injury nerves.

Furthermore, the expression of acetylase (NAT1 and MEC-17) was time-dependently increased in DRG neurons after peripheral axonal injury compared to central axonal injury. Conversely, the expression of deacetylase (Sirt2 and HDAC6) was gradually decreased after peripheral axonal injury compared to central axonal injury. These results suggested that different types of axonal injury affected the expression of acetylase and deacetylase in DRG neurons. It has been shown that peripheral target tissues, instead of central tissues, maintain the DRG neuron survival. Peripheral axonal injury in the sensory neurons leads to DRG neuron death, while, central axonal injury dose not. In addition, Ni et al. reported that peripheral and central axonal injury increased the expression of BNDF and NT-3 in DRG neurons; and the effect of peripheral axonal injury was greater than that of central axonal injury. Therefore, we concluded that the peripheral branch of DRG neurons is more important to maintain cell survival. Thus there is a high system value for repair of the peripheral axonal injury of DRG neurons.





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## Conclusions

Our study indicated that insufficiency of acetylase and upregulation of deacetylase in DRG neurons following central axonal injury may contribute to the pathogenesis of cauda equine syndrome. Our study may provide novel therapeutic targets for cauda equine syndrome therapy. In the future studies, we will explore the effects of tubulin post-translational enzymes (acetylase and deacetylase) on axon growth and regeneration of DRG neurons *in vitro*.

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