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# Expression of CPLX1 in Different Degrees of Spinal Cord Injury in Subacute Stage

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**Abstract:** This study is to explore the expression of CPLX1 (Complexin 1) in spinal cord tissues with different degrees of injury in the subacute phase of spinal cord injury. Twenty-four SD rats were randomly divided into three experimental groups (light, medium and severe injury) and a sham-operated control group. The experimental group made acute spinal cord injury models according to the lisa method, and the spinal cord tissue were collected 72 hours after injury. The tissues of rats were taken for transcriptome high-throughput sequencing and proteomic analysis experiments to observe the expression of CPLX1 in the different degrees during the subacute phase of spinal cord injury. The mRNA and protein of CPLX1 were detect in rat spinal cord tissue, and they were expressed high in the sham operation group. The protein expression in the light injury group began to decrease, and with the increase of the degree of injury, the transcription level of CPLX1 and the amount of protein expression also gradually decreased. The change trend of mRNA transcription level and protein expression level was basically the same. When the spinal cord injury heavier, the less CPLX1 was expressed, the two showed a significant negative correlation. In the subacute stage of spinal cord injury, the translation and protein levels of CPLX1 decreased significantly. The more severe the injury, the lower the transcription level and protein expression level. CPLX1 may be used as a marker gene for the severity of spinal injury.

**Keywords:** CPLX1, Spinal Cord Injury, Subacute Phase, Degree of Injury

## 1. Introduction

With the development of modern society, the incidence of spinal cord injury due to traffic accidents, high falls and sports injuries has increased year by year [1]. Spinal cord injury is an extremely serious nervous system injury [2]. After spinal cord injury, there are corresponding changes in dystonia and positive pathological reflexes [3]. Various motor and sensory dysfunctions occur in the corresponding segments of the injury, and the disability rate is very high, seriously affecting the patient's quality of life, bringing a heavy psychological and economic burden [4]. Current methods for treating spinal cord injury include drugs, physiotherapy, and surgery, but have not been able to make breakthrough progress [5-7]. The main reason is that the molecular mechanism of cells after spinal cord injury is still unclear.

In the nervous system, changes in synaptic potentials and nerve impulses of neurons are the basic conditions for nerve function [8]. Complexin is an important neurotransmitter release regulator protein in the presynaptic structure [9, 10]. So far, four subtypes of Complexin 1-4 (CPLX 1-4) have been found. CPLX1 and CPLX2 are mainly distributed in the brain, and CPLX3 and CPLX4 are mainly expressed on the retina [10]. CPLX1 is a soluble protein with a molecular weight of 18-19 KDa. The biological function of CPLX1 has been controversial [11]. It may be a negative regulator of neurotransmitter release and a positive regulator of

calcium ions entering the synapse [11]. However, the characteristics of CPLX1 expression after spinal cord injury are unclear. The effect of CPLX1 on synaptic transmission after spinal cord injury needs further study. Therefore, in this study, the establishment of a rat spinal cord injury model to detect changes in CPLX1 expression during spinal cord injury, which may lay an experimental foundation for the study of neuro-chemical regulatory signaling mechanisms in spinal cord injury.

# 2. Materials and Method

### *2.1. Animal Model*

Twenty-four female SD rats weighing 220-250g were randomly divided into a sham-operated control group and three experimental groups, six in each group. Anesthetize with 10% chloral hydrate (300 mg/kg) intraperitoneal injection to expose the T10 segment spinal cord. According to the Lisa strike method, the experimental group had a light injury of 0.6 mm, a moderate injury group of 1.0 mm, and a severe injury group of 1.8 mm. The lower extremity of the rat was completely paralyzed by impacting the spinal cord, and the skin was sutured. After the operation, the room temperature was maintained at 23°C～28°C, and the bladder was squeezed to urinate twice a day. Drinking water, feed and light were not limited. The control group only exposed the T10 segment spinal cord without injuring the spinal cord, and the rest of the treatment was the same as the experimental group.

### *2.2. Transcriptome Sequencing*

RNA extraction was performed on the spinal cord tissue of model animals, and 3 biological replicates were set for each group. Total RNA was extracted by TRIzol method, RNA purity and concentration were determined by spectrophotometer, and RNA integrity was detected by bioanalyzer. After the RNA sample passed the test, single-stranded cDNA was synthesized in the M-MuLV reverse transcriptase system using mRNA as a template and random oligonucleotides as primers. Subsequently, RNA strand was degraded with ribonuclease H, and the second strand of cDNA was synthesized using dNTPs as raw material under the DNA polymerase I system. The purified double-stranded cDNA was subjected to end repair, A tail was added, and a sequencing adapter was connected. The purified magnetic beads were used to screen cDNA of about 250-300 bp for PCR amplification and the purified magnetic beads were used to purify the PCR product again, and the library was finally obtained. After the library construction is completed, use Qubit2.0 Fluorometer software for preliminary quantification, dilute the library to 1.5 ng/μL, use a bioanalyzer to detect the library inserts, and use RT-qPCR to accurately quantify the effective concentration of the library to ensure the library Quality, and finally sent to Annuo Gene Technology (Beijing) Co., Ltd. for RNA-seq sequencing.

### *2.3. Proteomics Mass Spectrometry*

Protein extraction was performed on the spinal cord tissue of model animals, and three biological replicates were set for each group. A 40 mg tissue sample was taken, minced, and ground with liquid nitrogen. After being completely ground into a powder, we put it into a 1.5 mL centrifuge tube and add the lysate at a ratio of 1g/10 ml. After lysis is complete, these samples were centrifuged at 4°C and 12 000 r/min for 30 min. Then we transferred the supernatant to a new centrifuge tube. The Braford method was used for quantification. After quantification, equal amounts of protein from each tissue were mixed.

We took 0.15 mg of the mixed protein sample, centrifuged at 12 000r /min for 15 min, added 0.15 mL SDT (4% SDS 0.1 mol /L DTT), incubated at 56°C water bath for 60 min, and centrifuged to discard the waste solution. Next, we added 0.2 mL of UA, discard the waste after centrifugation, and added 0.1 mL of IAA, incubated at room temperature in the dark for 20 minutes, then centrifuged and discarded the waste solution. Then, we added 0.1 mL UA, discarded the waste after centrifugation. Trypsin was added at a ratio of enzyme to protein 1/50 and enzymatic hydrolysis at 37°C overnight. The filtrate was collected by centrifugation at 12 000 r /min and lyophilized. The parameters of the Q Exactive mass spectrometer were as follows: spray voltage 2.5 kV; capillary temperature 275°C; collision energy 30% HCD; resolution setting is first level 70 000 @ m /z 200, second level 17 500 @ m /z 200; mother ion scan range is 300～ 1 800 m /z; the product ion scan range starts from 100 m /z. The raw data of mass spectrometry analysis was RAW file. Proteome Discoverer 1.4 software was used for database identification in UniProtKB Bovidae protein database.

# 3. Results

### *3.1. Expression of CPLX1 mRNA and Protein in the Subacute Phase of Spinal Cord Injury*

Transcriptome high-throughput sequencing of four differently injured spinal cord tissues in the subacute phase showed that the expression level of Cplx1 was higher in the sham operation group, and as the degree of injury deepened, the transcription level of Cplx1 became lower and lower. The results of protein profiling also showed that the transcription level of Cplx1 was higher in the control group, and as the degree of injury deepened, the transcription level of Cplx1 became lower. Moreover, the change trend of mRNA is basically consistent with that of protein.

The horizontal axis is the situation of rats in different groups (control, light injury, moderate injury and severe injury group), the left vertical axis is the relative value of protein expression (red) and the right vertical axis is the gene sequencing RNA expression Level (green).

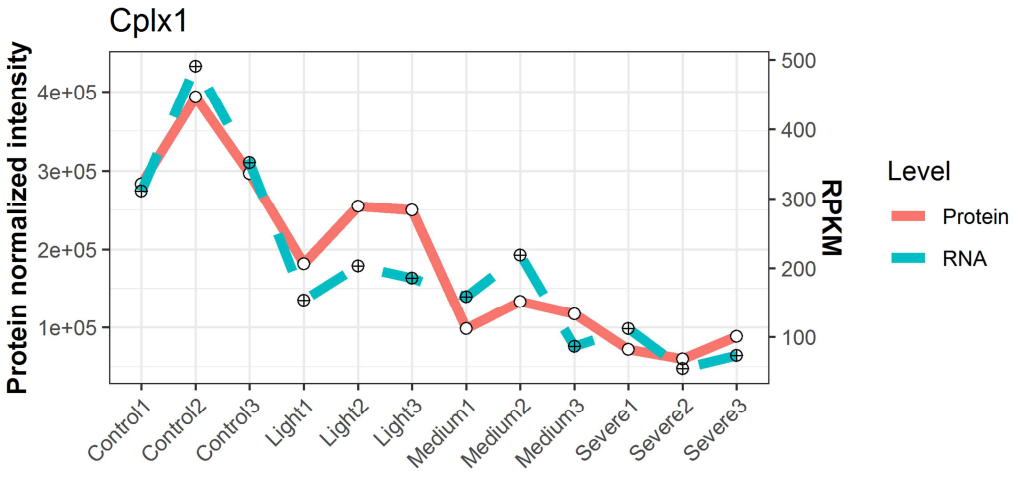


***Figure 1.*** *Expression of CPLX1 mRNA and protein in the subacute phase of spinal cord injury.*

### *3.2. Correlation Between CPLX1 mRNA and Protein Expression in the Subacute Phase of Spinal Cord Injury*

We analyzed the correlation between the change trend of mRNA and protein of CPLX1. The results showed that the correlation between mRNA and protein expression of CPLX1 in the subacute phase of spinal cord injury conforms to the formula Y=0.001X-2.795, and the r value is 0.911, which indicated that mRNA expression and protein 'S expression showed a clear positive correlation. With the aggravation of spinal cord injury, the translation level of CPLX1mRNA and protein expression level decreased significantly.

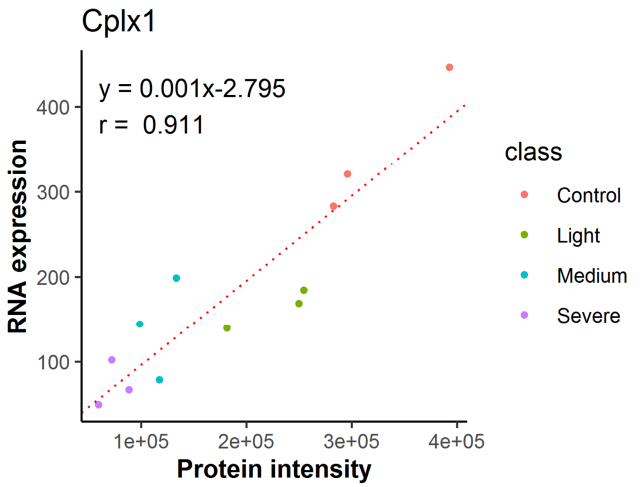


***Figure 2.*** *Correlation between CPLX1 mRNA and protein expression in the subacute phase of spinal cord injury.*

The horizontal axis is protein expressing level by proteomics analysis, and the vertical axis is the expression level of gene sequencing RNA. Different colored dots indicate different groupings (control, mild injury group, moderate injury group and severe injury group), each group has 3 animals. The R value represents the correlation between the two sets of data.

## 4. Discussion

CPLX1 was discovered by three laboratories at the same time in 1995 [12]. Complexin has four isomers, namely Complexin 1, Complexin 2, Complexin 3 and Complexin 4 [13]. CPLX1 is highly expressed in the nervous system such as the spinal cord [14], and is mainly distributed in the nerve-muscle junction and in the autonomic ganglion in the peripheral nervous system [15]. Complexin 1 (CPLX1) is a pre-synaptic small molecule protein that forms SNARE complexes in the central nervous system involved in axonal vesicle anchoring, pre-excitation and fusion [16]. And CPLX1 was first discovered due to its binding to the SNARES complex. However, after spinal cord injury, the expression characteristics of CPLX1 genes and proteins have remained unclear.

This study performed proteomics analysis and high-throughput sequencing of transcriptome to observe the expression characteristics of CPLX1 after spinal cord injury from the perspective of mRNA and protein. Compared with the sham operation group, the expression of CPLX1 in the experimental group in the subacute phase decreased. Furthermore, with the increase of the degree of injury, the expression of CPLX1 decreased more significantly. And even, the decrease in mRNA transcription level and protein translation level showed a significant positive correlation. It shows that after spinal cord injury, CPLX1 mainly affects the expression of protein levels by regulating the transcription level of mRNA. In addition, our results also show that the decreased level of CPLX1 expression is significantly negatively correlated with the degree of spinal cord injury. The deeper the degree of spinal cord injury, the less CPLX1 expression. Previous studies have found that CPLX1-/- rats exhibit severe ataxia, dystonia, movement and exploratory defects, and increase anxiety and sensory deficits, but normal cognitive function [17]. ReimK et al. knocked out CPLX 1/2

and found that the mice died a few hours after birth. The neuronal calcium-dependent neurotransmitter release was severely impaired, and this loss can be completely recovered by increasing the calcium ion concentration [18]. But other study found that CPLX1 promotes the exocytosis of synaptic vesicles. The results suggest that CPLX1 plays a dual role in regulation transmitters [19].

CPLX1 also acts as a protein activator in the process of synaptic release of neurotransmitters. Therefore, the above reports suggest that CPLX1 is very important for the generation of nerve impulses. When nerve impulses are transmitted to the presynaptic membrane, CPLX1 causes $Ca^{2+}$ influx to cause the vesicles to move to the presynaptic membrane and fuse with the presynaptic membrane to release neurotransmittersn [18]. The receptors on the membrane combine to cause the conformational changes of the ion channels on the membrane to cause changes in electrical potential, resulting in nerve impulses, and have a potential role in functional recovery after spinal cord injury.

## 5. Conclusion

In the subacute stage of spinal cord injury, the translation and protein levels of CPLX1 decreased significantly. CPLX1 expression reflects the extent of spinal cord injury, so that it may be used as a marker gene for the severity of spinal injury. These findings provide a new perspective for understanding the role of CPLX1.

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