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# Expression of CDc6 after acute spinal cord injury in adult rats☆

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ABSTRACT

The cell division cycle 6 (CDc6) protein has been primarily investigated as a component of the pre-replicative complex for the initiation of DNA replication. Some studies have shown that CDc6 played a critical role in the development of human carcinoma. However, the expression and roles of CDc6 in the central nervous system remain unknown. We have performed an acute spinal cord injury (SCI) model in adult rats and investigated the dynamic changes of CDc6 expression in spinal cord. Western blot have found that CDc6 protein levels first significantly increase, reach a peak at day 3, and then gradually return to normal level at day 14 after SCI. Double immunofluorescence staining showed that CDc6 immunoreactivity was found in neurons, astrocytes, and microglia. Additionally, colocalization of CDc6/active caspase-3 has been detected in neurons and colocalization of CDc6/proliferating cell nuclear antigen has been detected in astrocytes and microglial. In vitro, CDc6 depletion by short interfering RNA inhibits astrocyte proliferation and reduces cyclin A and cyclin D1 protein levels. CDc6 knockdown also decreases neuronal apoptosis. We speculate that CDc6 might play crucial roles in CNS pathophysiology after SCI.



## 1. Introduction

Spinal cord injury (SCI) is often seriously disabling, primarily affects young people, which is especially costly in personal, financial, and societal terms (Johnson et al., 1997). SCI causes tissue loss and associates with neurological dysfunction which attributes to mechanical damage and secondary biochemical and physiological responses (Dumont et al., 2001). Secondary injury mechanisms include neuronal apoptosis, reactive astrogliosis, scar formation, and so on (Tator, 1996). Neuronal apoptosis has a pivotal role in secondary injury, widely apoptosis of neurons has been found following SCI (Lu et al., 2000), which are cleared away by the immune system and form a cavity remains (Willyard, 2013). Proliferation of astrocytes and microglia is another typical characteristic after SCI which is associated with cell cycle activation (Becker and Bonni, 2004; Cernak et al., 2005). This causes gliosis, microglial activation, then, formation of a glia scar (Hoke and Silver, 1994; Raivich et al., 1999). The glia scar tissues surround the cavity area that stop axonal regeneration, as a result of that, hardly can electronic signals be transmitted, as loss of motor, sensory and autonomic functions (Liu et al., 2010b; Lu et al., 2014). Current treatment options for SCI are limited, but significant advances have been made to understand the mechanism of SCI (Wells et al., 2003).

CDc6 belongs to the member of the AAA + ATPase family which plays critical role in varieties of cell functions such as protein folding, unfolding and degradation, vesicle transport, organelle assembly, and DNA replication(Duderstadt and Berger, 2008; White and Lauring, 2007). CDc6 was firstly identified in a genetic screen targeted at finding mutations which arrested the budding yeast cell cycle (Hartwell, 1976). It spent many years to clone the corresponding gene and identify its homologs in human cells and fission yeast. Actually, CDc6 is existed in every eukaryotic organism. The first evidence for the role of CDc6 in DNA replication was a temperature-sensitive yeast CDc6 mutant that arrested cells at the G1-S transition (Borlado and Mendez, 2008).

Previous studies have indicated that CDc6 is essential for initiation of DNA replication. When CDc6 levels were downregulated in G1 phase, cells could not progress into S phase. Moreover, when CDc6 were downregulated by RNA interference (RNAi), it prevented cell proliferation (Petrakis et al., 2012). Previous studies have also indicated the aberrant expression of CDc6 in many processes of human malignancies, such as brain tumor (Ohta et al., 2001), cervical cancer (Wang et al., 2009),

*Abbreviations:* CDc6, Cell division cycle 6; SCI, Spinal cord injury; CNS, Central nervous system; PCNA, Proliferating cell nuclear antigen; NeuN, Neuronal nuclear antigen; GFAP, Glial fibrillary acidic protein; IBa1, Ionized calcium-binding adapter molecule 1; siRNA, Short interfering RNA.

☆ The authors declare no conflicts of interest.

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hepatocellular carcinoma (Xiong et al., 2008), prostate cancer (Liu et al., 2014), lung cancer (Zhang et al., 2014) and so on.

In this study, we first investigated CDc6 expression and its colocalization with active caspase-3 and PCNA in an acute SCI model on adult rats. The above data suggest that CDc6 is involved in pathophysiological and biochemical progression after SCI. Our experiments were conducted to gain deep insight into the functions of CDc6 and its roles in the cellular and molecular mechanisms underlying SCI and repair.

## 2. Materials and methods

### 2.1. Spinal cord injury

Experiments were accorded with the National Institutes of Health Guidelines for the Care and Use of Laboratory Animals; all animal experiments were permitted by the Department of Animal Center, Medical College of Nantong University. Surgery male Sprague–Dawley rats (n = 48) weighing 275–325 g and aged 2 months were subjected to contusive SCI. The rats were deeply anesthetized by sodium pentobarbital (65 mg/kg, i.p.), and the surgery was operated under aseptic conditions. A laminectomy was performed at the level of the 9th thoracic vertebrae to expose a circle of dura mater. The contusion injuries (n = 40) were used by the NYU impactor. The exposed rat's spinal cord was impacted by dropping a bar which was 10 g in weight and 2.0 mm in diameter from a height of 10 cm. Sham-injured animals (n = 8) received laminectomy without the weight drop. The animals were allowed to recover on a 30 °C heating pad. Post-operative treatments included saline (2.0 $cm^3$, s.c.) for rehydration and Baytril (0.3 $cm^3$, 22.7 mg/ml, s.c., twice daily) to prevent urinary tract infection. Bladders were manually expressed twice daily until the bladder-emptying reflex was re-established. The animals were killed at 6 h, 12 h, 1 day, 3 days, 5 days, 7 days, and 14 days after injury. All efforts were made to reduce the number of animals and their pain.

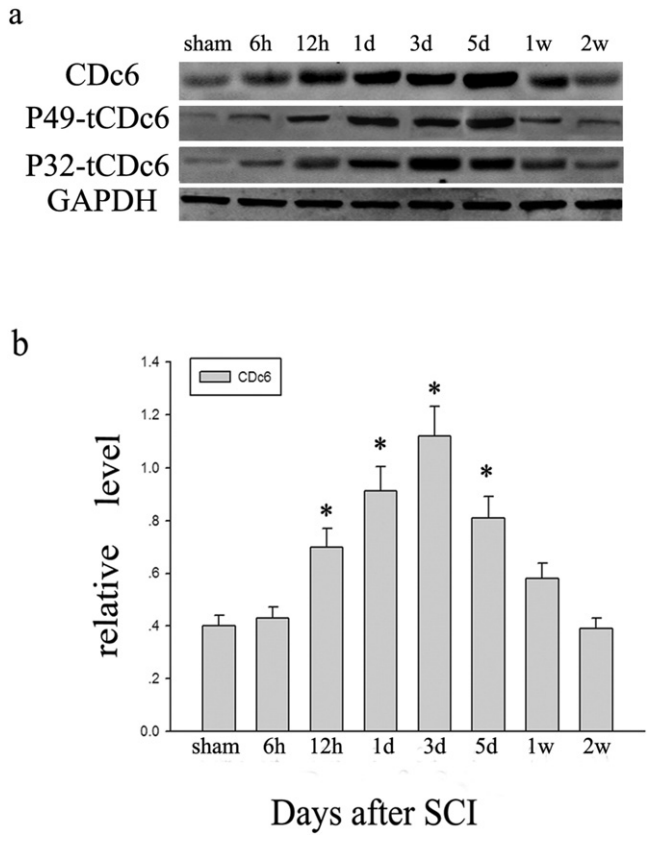


**Fig. 1.** Expression of CDc6 and cleaved CDc6 changes over time following spinal cord injury (SCI) in adult rats. (a) Spinal cord tissues from rats at various survival times after SCI were homogenized and subjected to immunoblot analysis. Samples immunoblots probed for CDc6 and loading control (GAPDH) are shown. (b) Expression levels of CDc6 were normalized against GAPDH, as estimated by optical density measurements. The data are means ± SEM (n = 3 rats per time point; *P < 0.05, significantly different from the sham-injured group).

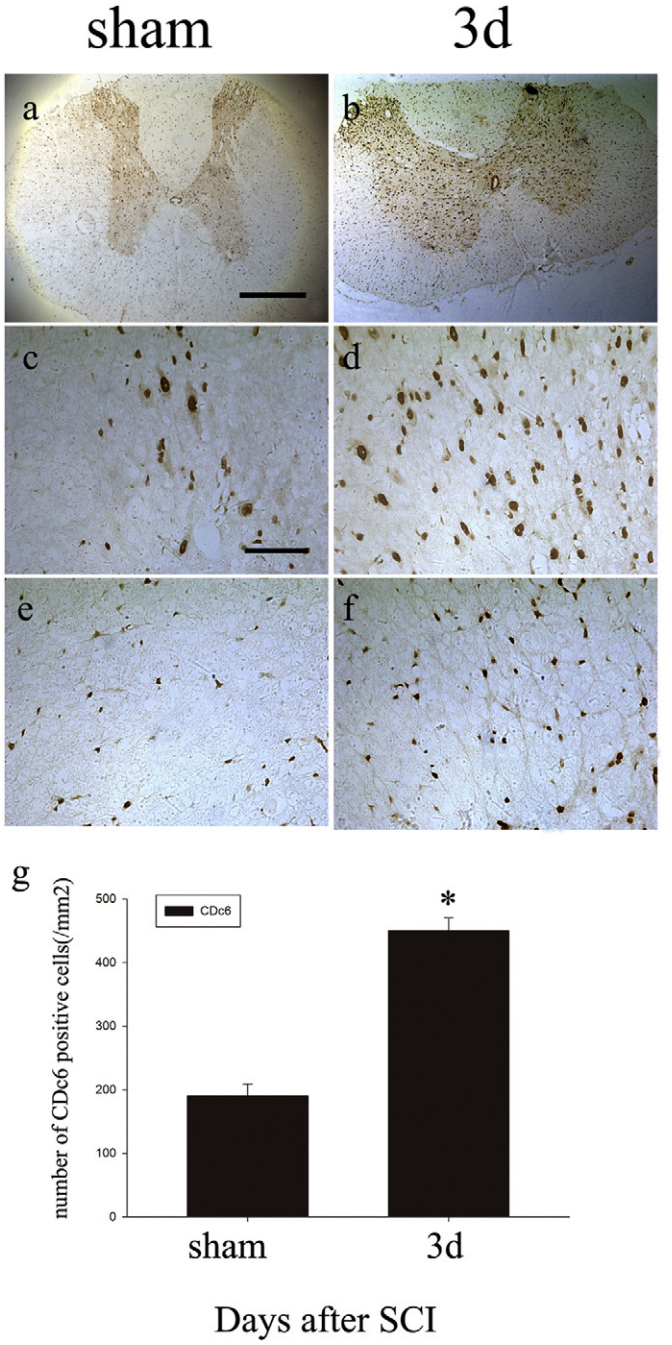


**Fig. 2.** Immunohistochemical expression of CDc6 in the adult rat spinal cord. Low-power views of transverse sections immunostained for CDc6 in sham-operated spinal cord (a) and 3 days after injury (b). High-power views in the gray matter (c, d) and white matter (e, f). Immunostaining of CDc6 shown that CDc6 expression increases in the gray matter (d) and white matter (f) after injury. *Bars* 200 μm (a, b), 20 μm (c–f). Quantitative analysis of CDc6-positive cells/$mm^2$ in sham-operated rats and 3 days after SCI (g), indicating an obvious increase after SCI compare with sham-operated animals (n = 3 in sham-operated group, n = 3 in injured group; *P < 0.05 indicates significant difference compared with sham-operated animals; *error bars* SEM).