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NG2 expression in rats with acute T_{10} spinal cord injury☆

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Abstract

Rat models of T_{10} spinal cord injury were established with a clamp method. NG2 expression was detected with immunohistochemical staining and western blot. Ten days after spinal cord injury, the number of NG2-positive cells in the damaged areas and NG2 absorbance were both significantly increased. The findings indicate that acute T_{10} spinal cord injury in rats can lead to upregulation of NG2 protein expression in damaged areas.

Key Words: NG2 protein; spinal cord injury; immunohistochemistry; western blot; neural regeneration

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INTRODUCTION

Spinal cord injury triggers glial cell proliferation, migration and differentiation, thus leading to formation of glial scar tissue[1-5]. Inhibitory molecules synthesized by cellular components of glial scar tissue have an inhibitory effect on some nervous processes, and these molecules are called proteoglycans. Chondroitin sulfate is an important inhibitory proteoglycan subfamily expressed in glial progenitor cell membranes, and includes NG2, polysaccharides and phosphorus-containing polysaccharides. An increasing number of studies have confirmed that NG2 expression is increased in post-traumatic spinal cord nerve tissue and brain ischemic penumbra, and the number of NG2-positive cells is also increased, however, there is little evidence regarding changes in NG2-positive cells during spinal cord injury[6-9].

In this study, we sought to elucidate the altered expression of NG2 in rat models of acute T_{10} spinal cord injury, verified by magnetic resonance imaging (MRI).

RESULTS

Quantitative analysis of experimental animals

A total of 32 rats were randomly divided into a model group (n = 16; T_{10} spinal cord injury in rats was established by segmental clamping and sewing) and a sham-surgery group (T_{10} segment was only partially cut and then sutured, with no damage to the spinal cord). All 32 rats were involved in the final analysis with no drop-out due to death or infection.

MRI after spinal cord injury

MRI showed that the rat spinal cord was smooth and uniformly dense, and the structure of the spinal cord and dura mater was clearly visible 1 day before injury (Figure 1A). The spinal cord structure was disordered in the damaged areas and the signal abnormalities were diffuse and ill-outlined 10 days after injury. T1WI showed low signal intensity and T2WI showed high signal intensity like spots. A small range of low-intensity signals with an indistinct outline was seen in the central areas of damage, and high-intensity signals were observed in the dorsal dura, indicating local exudative effusion (Figure 1B).

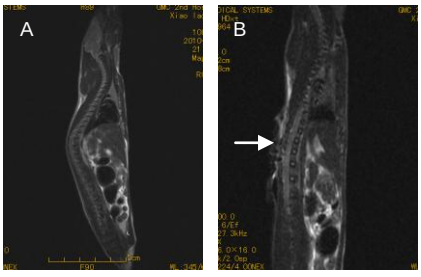

Figure 1 MRI sagittal image of rat spinal cord.

(A) Sham-surgery group: Normal spinal MRI sagittal image shows the uniform density and smooth boundary of the spinal cord, with no spinal cord signal abnormalities.

(B) Model group: T2WI signal was significantly enhanced and the arrow represents the injury foci at the dorsal T_{10} segment.

Morphology of NG2-positive cells in damaged areas in rats with spinal cord injury

Immunohistochemical staining showed that NG2 cells in the damaged areas were relatively short, thin and small at 10 days after spinal cord injury. Compared with the sham-surgery group, more NG2-positive cells were seen in the damaged areas, such as the spinal cord gray matter anterior and posterior horns. The number of positive cells was highest in the spinal cord white matter (Figure 2).

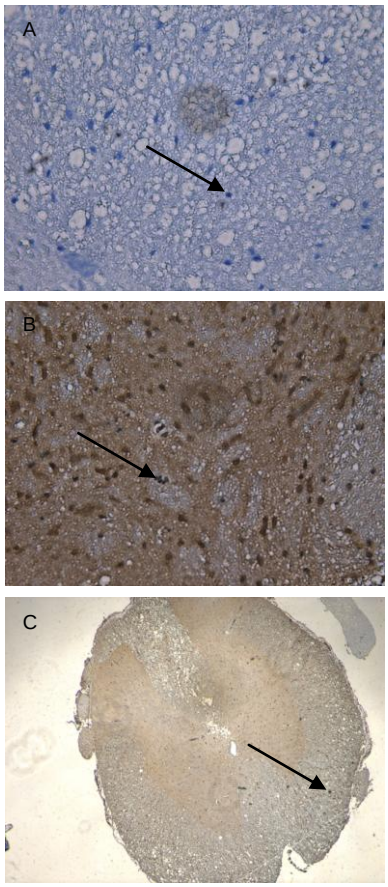

Figure 2 Morphology of NG2-positive cells in T_{10} segment of rat spinal cord 10 days after injury (immunohistochemical staining, optical microscopy).

(A) Sham-surgery group: A small number of NG2-positive cells (arrow) in spinal cord white matter (× 40).

(B) Model group: NG2-positive cells (arrow) in spinal cord white matter were significantly increased (× 40).

(C) Model group: Cross-section of spinal cord tissue (T_{10} spinal cord, including gray and white matter); the number of NG2-positive cells (arrow) in white matter was slightly higher than that in gray matter (× 5).

Number and expression of NG2-positive cells

NG2 protein absorbance (detected by western blot) and cell density (detected by immunohistochemistry) in the injured spinal cord were significantly higher than in the sham-surgery group at 10 days after spinal cord injury ($P < 0.05$; Table 1).

Table 1 Average density (cells/mm^2) of T_{10} spinal cord cells and NG2 protein relative absorbance in model and sham-surgery groups before and after spinal cord injury (western blot detection)

Group	Cell density		Absorbance value	
	1 day	10 days	1 day	10 days
Model	60±12	178±35[a]	0.172±0.023	0.383±0.056[a]
Sham-surgery	62±11	58±9	0.169±0.021	0.169±0.021

[a]$P < 0.05$, *vs*. sham-surgery group at 10 days after injury. Data are expressed as mean ± SD, $n = 16$, two-sample *t* tests.

Immunohistochemical analysis showed that the number of NG2-positive cells in the model group was higher than that in the sham-surgery group at 10 days after spinal cord injury ($P < 0.01$; Table 2).

Table 2 Changes in the number of NG2-positive cells in the model and sham-surgery groups at 10 days after spinal cord injury (cells/40-fold visual field)

Group	Gray matter	White matter
Model	93.5±6.2[a]	62.8±5.1[a]
Sham-surgery	5.5±1.8	7.3±2.4

[a]$P < 0.01$, *vs*. sham-surgery group. Image analysis of immunohistochemically stained NG2-positive cells was conducted and number of cells at 40 × magnification was calculated from the same sections in each group. Data are expressed as mean ± SD, $n = 16$, two-sample *t* test.

DISCUSSION

Monkeys, pigs, dogs and other mammals are ideal to establish models of spinal cord injury, but they are too expensive to be widely used, because the methods for model preparation are complex and require strict experimental conditions, the models show poor reproducibility, and they incur a high cost for the long experimental period required[10-11]. In the present study, rats were utilized to establish a spinal cord injury model by using a clamping and sewing technique. The models obtained maintain the integrity of the spinal dura mater, and the anatomical structure and neurological function are similar to the changes in humans after spinal cord injury. This model is simple, less invasive, reproducible, and has a high success rate, clear observed targets, and less strict experimental requirements.

MRI is a high-resolution and multi-dimensional imaging technique and is extremely sensitive for lesion morphology and the biochemical environment, and it can be used to reveal the pathological characteristics of spinal cord injury[12-15]. In the present study, MRI findings corresponded with intramedullary hemorrhage, the injury was widely visible, and spinal shock occurred; all of which was consistent with the MRI findings after spinal cord injury in humans.

Immunohistochemical analysis showed that NG2-positive cells were widespread in the anterior and posterior horns of the grey and white matter in rats with spinal cord injury, especially in the white matter. The imaging findings of spinal cord injury in rats are similar to those in humans[16-17]. This provides evidence that the rat spinal cord injury model established with the clamp method is consistent with spinal cord injury in humans, indicating that rat models of spinal cord injury are reliable.

In the present study, NG2-positive cell density and NG2 absorbance in models of spinal cord injury were higher than in the sham-surgery group at 10 days after injury, which supports the conclusion that NG2 cells are significantly increased in humans after spinal cord injury[18].

Other studies have revealed that NG2 is a specific marker of spinal cord injury in all mammals[19].

Moreover, we also found that the NG2-positive cell distribution in white matter was greater than that in gray matter.

We speculated that this occurs possibly because the proliferative capacity of glial cells in spinal cord white matter was greater than that in gray matter, or there were inhibitory factors affecting glial cell proliferation in gray matter.

Further studies are required to determine conclusively the blood flow distribution in the spinal cord gray and white matter after glial cell proliferation[20].

In summary, the present study confirms that T_{10} spinal cord injury can lead to upregulation of NG2 protein expression in rats.

MATERIALS AND METHODS

Design

A randomized controlled animal experiment.

Time and setting

Experiments were implemented from March 2008 to June 2010 in the Second Affiliated Hospital of Guangzhou Medical College, China.

Materials

A total of 32 Wistar purebred rats, aged 5–6 months, weighing 330–360 g were obtained from Animal Breeding Center of Guangzhou Medical College, China (license No. SCXK (Yue) 2008-0008).

All rats were allowed free access to a standard adequate diet, and were housed in separate cages in an air-conditioned room at constant temperature and humidity, under a 12-hour light-dark cycle. All experiments were conducted in strict accordance with the *Guidance Suggestions for the Care and Use of Laboratory Animals*, issued by the Ministry of Science and Technology of China[21].

Methods

Establishment of clamp-type spinal cord injury models

Models of spinal cord injury were established using a clamping and sewing method in rats. A linear incision was made under anesthesia in the middle of the backs of the rats, which were fixed in the ventral decubitus position, to expose the T_{9-11} spinal cord and lamina. The aneurysm clip (calibrated force 20 g) was opened with a clamp holder and crossed through the T_{10} spinal cord, and then the clip was suddenly released to induce a sudden violent injury in the spinal cord.

The aneurysm clip was removed 3 minutes later, and the incision was disinfected and sutured layer by layer.

MRI findings of rats with spinal cord injury

Rats were scanned with a GE 2.0T MRI scanner and GE 1.5T superconducting MRI scanner (GE Healthcare, Fairfield, CT, USA) under anesthesia, and rat spinal axis line corresponding with the MRI machine was positioned with the assistance of sandbags squeezing on both sides. Using the smallest upper arm coil, T1W1 and T2W1 images were collected with the SE and FSE sequences, respectively, and gradient-recalled echoes were obtained. Axial images were acquired through general scanning, while sagittal and/or coronal scans were conducted in some rats. Scanning parameters were slice thickness 2 mm and spacing 1 mm.

Immunohistochemical detection of NG2 protein

The cross sections of the injured T_{10} spinal cord were cut into serial coronal frozen sections, and free floating sections were subjected to immunohistochemical staining and blocked with goat serum at room temperature. Sections were incubated with mouse anti-NG2 (1: 100; Chemicon, Billerica, MA, USA) at 37°C for 2 hours, and at 4°C for an additional 48 hours, and then treated with biotinylated goat anti-mouse IgG (1: 200; Beijing Zhongshan Biotechnology Co., Ltd., Beijing, China) at 37°C for 1 hour and with horseradish-labeled affinity avidin (1: 200; Beijing Zhongshan Biotechnology) at 37°C for another 1 hour. After diaminobenzidine staining, samples were observed under an optical microscope (Olympus, Tokyo, Japan).

The number of positive cells was counted with Image Pro-plus image analysis software (Media Cybernetics, Silver Spring, MD, USA) and the number of positive cells within a 1-mm^2 area was calculated.

Western blot analysis of NG2 protein

NG2 protein was extracted from the injured spinal cord on ice with RIPA buffer and protein concentration was determined with Coomassie brilliant blue. Samples (60 μg) were subjected to 8% sodium dodecyl sulfate polyacrylamide gel electrophoresis. NG2 protein relative molecular mass in target protein was 28 000, total protein (80 μg per well) was separated through sodium dodecyl sulfate polyacrylamide gel electrophoresis under constant pressure (60 V), and protein in the gel was wet transferred to polyvinylidene fluoride membrane (4°C) under 100 mA constant current for 5 hours. Tissues were blocked with Tris-buffered saline Tween-20 containing 5% skimmed milk (50 mM Tris-HCl, 150 mM NaCl, 0.1% Tween-20, pH 7.5) at room temperature for 6 hours, and incubated with rat NG2 monoclonal antibody (1: 1 000; Sigma, St. Louis, MO, USA) at room temperature for

12 hours and with horseradish peroxidase-labeled anti-rat IgG (1: 20–1: 50; Beijing Zhongshan Biotechnology) for an additional 2 hours at 37°C, followed by diaminobenzidine staining. β-actin (Sigma) served as the internal reference of total protein.
The integral absorbance of NG2 protein bands to the internal reference was measured with an SX-300 image analysis system (Second Affiliated Hospital of Guangzhou Medical College, China), and NG2 expression in each group was represented by integrated absorbance ratio of NG2 protein to the internal reference.

Statistical analysis

Measurement data were expressed as mean ± SD and analyzed with SPSS 10.0 statistical software (SPSS, Chicago, IL, USA). Differences between the groups were compared with a two-sample *t* test. $P < 0.05$ was considered as significant.

Author contributions: Haoran Lv had full access to the data and integrated the data, was responsible for the study concept and design, and wrote the manuscript. Jinshun Yang was in charge of funding, research instructor, and checked the manuscript. Yu Zhao performed the data analysis. Zhuangwen Liao was responsible for statistical analysis. Yan Huang provided technical support or information.
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REFERENCES

[1] Giger RJ, Hollis ER 2nd, Tuszynski MH. Guidance molecules in axon regeneration. Cold Spring Harb Perspect Biol. 2010;2(7): a001867.
[2] Li Q, Brus-Ramer M, Martin JH, et al. Electrical stimulation of the medullary pyramid promotes proliferation and differentiation of oligodendrocyte progenitor cells in the corticospinal tract of the adult rat. Neurosci Lett. 2010;479(2):128-133.
[3] Jiang S, Ballerini P, Buccella S, et al. Remyelination after chronic spinal cord injury is associated with proliferation of endogenous adult progenitor cells after systemic administration of guanosine. Purinergic Signal. 2008;4(1):61-71.
[4] Tu J, Liao J, Stoodley MA, et al. Differentiation of endogenous progenitors in an animal model of post-traumatic syringomyelia. Spine (Phila Pa 1976). 2010;35(11):1116-1121.
[5] Almad A, McTigue DM. Chronic expression of PPAR-delta by oligodendrocyte lineage cells in the injured rat spinal cord. J Comp Neurol. 2010;518(6):785-799.
[6] Liu H, Shubayev VI. Matrix metalloproteinase-9 controls proliferation of NG2+ progenitor cells immediately after spinal cord injury. Exp Neurol. 2011;231(2):236-246.
[7] Wu J, Leung PY, Sharp A, et al. Increased expression of the close homolog of the adhesion molecule L1 in different cell types over time after rat spinal cord contusion. J Neurosci Res. 2011;89(5): 628-638.
[8] Tripathi RB, Rivers LE, Young KM, et al. NG2 glia generate new oligodendrocytes but few astrocytes in a murine experimental autoimmune encephalomyelitis model of demyelinating disease. J Neurosci. 2010;30(48):16383-16390.
[9] Ulrich R, Seeliger F, Kreutzer M, et al. Limited remyelination in Theiler's murine encephalomyelitis due to insufficient oligodendroglial differentiation of nerve/glial antigen 2 (NG2)-positive putative oligodendroglial progenitor cells. Neuropathol Appl Neurobiol. 2008;34(6):603-620.
[10] Nakajima H, Uchida K, Yayama T, et al. Targeted retrograde gene delivery of brain-derived neurotrophic factor suppresses apoptosis of neurons and oligodendroglia after spinal cord injury in rats. Spine (Phila Pa 1976). 2010;35(5):497-504.
[11] Hossain-Ibrahim MK, Rezajooi K, Stallcup WB, et al. Analysis of axonal regeneration in the central and peripheral nervous systems of the NG2-deficient mouse. BMC Neurosci. 2007;27(8): 80.
[12] Kang SH, Fukaya M, Yang JK, et al. NG^{2+} CNS glial progenitors remain committed to the oligodendrocyte lineage in po stnatal life and following neurodegeneration. Neuron. 2010;68(4):668-681.
[13] Busch SA, Horn KP, Cuascut FX, et al. Adult NG^{2+} cells are permissive to neurite outgrowth and stabilize sensory axons during macrophage-induced axonal dieback after spinal cord injury. J Neurosci. 2010;30(1):255-265.
[14] Lytle JM, Chittajallu R, Wrathall JR, et al. NG2 cell response in the CNP-EGFP mouse after contusive spinal cord injury. Glia. 2009; 57(3):270-285.
[15] Sundberg M, Skottman H, Suuronen R, et al. Production and isolation of NG2+ oligodendrocyte precursors from human embryonic stem cells in defined serum-free medium. Stem Cell Res. 2010;5(2):91-103.
[16] Lytle JM, Chittajallu R, Wrathall JR, et al. NG2 cell response in the CNP-EGFP mouse after contusive spinal cord injury. Glia. 2009; 57(3):270-285.
[17] Busch SA, Horn KP, Cuascut FX, et al. Adult NG^{2+} cells are permissive to neurite outgrowth and stabilize sensory axons during macrophage-induced axonal dieback after spinal cord injury. J Neurosci. 2010;30(1):255-265.
[18] Wu J, Yoo S, Wilcock D, et al. Interaction of NG2(+) glial progenitors and microglia/macrophages from the injured spinal cord. Glia. 2010;58(4):410-422.
[19] Cizkova D, Cizek M, Nagyova M, et al. Enrichment of rat oligodendrocyte progenitor cells by magnetic cell sorting. J Neurosci Methods. 2009;184(1):88-94.
[20] Huang X, Kim JM, Kong TH, et al. GM-CSF inhibits glial scar formation and shows ong-term protective effect after spinal cord injury. J Neurol Sci. 2009;277(1-2):87-97.
[21] The Ministry of Science and Technology of the People's Republic of China. Guidance Suggestions for the Care and Use of Laboratory Animals. 2006-09-30.

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