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# Increase in bFGF-responsive neural progenitor population following contusion injury of the adult rodent spinal cord

Yi Xu [a,*], Masaaki Kitada [a], Masahiro Yamaguchi [b], Mari Dezawa [a], Chizuka Ide [a]

[a] Department of Anatomy and Neurobiology, Kyoto University Graduate School of Medicine, Sakyo-ku, Kyoto 606 8501, Japan
[b] Department of Physiology, Graduate School of Medicine, University of Tokyo, Bunkyo-ku, Tokyo 113 0033, Japan

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**Abstract**

The number of neural progenitor cells, especially nestin$^+$ cells or BrdU-uptake cells is sparse in the normal adult rodent spinal cord. However, in the present study, we show that after spinal cord injury (SCI), many ordinarily quiescent cells were activated to become nestin$^+$ and undergo mitosis (BrdU$^+$) in the ependymal layer as well as in the parenchyma of the spinal cord. Nestin$^+$ cells and BrdU$^+$ cells were in most cases immunohistochemically GFAP$^+$, some of which displayed radial glial cell morphology and partly participated in the border formation of the lesion. The culturing of injured rat spinal cord tissues generated more neurospheres earlier than did the culturing of intact tissues, and these neurosphere cells were multipotent and bFGF-responsive. Immunohistochemical analysis showed that there existed many bFGF$^+$ cells after SCI, the number of which were almost 15 times greater than that in an intact spinal cord. Increased bFGF production after SCI might activate quiescent progenitor cells, and thus initiate their cell proliferation. Finally, SCI to the nestin-promoter green fluorescent protein (GFP) transgenic mice showed broad proliferation of progenitor cells that were induced in the injured spinal cord. The culturing of injured spinal cord tissues from these transgenic mice provides direct evidence that neurospheres can be generated by SCI-activated nestin$^+$ cells. Thus, the activation of bFGF-responsive progenitor cells and the concomitant increase in the population of bFGF$^+$ cells following SCI might be beneficial for spinal cord repair if these progenitor cells are properly manipulated.


*Keywords:* Spinal cord injury; Neural progenitor cell; bFGF; Radial glia; Glial scar

The spinal cord cannot usually regenerate itself after injury. Spinal cord injury (SCI) in mammals has a complex pathology including initial mechanical injuries and subsequent secondary processes [4]. In contrast, some lower vertebrates such as urodele amphibians were reported to regenerate their spinal cord tissues after injury [11,26]. Besides the relatively simpler pathology in urodele amphibians, endogenous progenitor cells were shown to play a key role in the regeneration. During the regeneration, the proliferative response of progenitor cells was shown to be regulated by bFGF [26].

The adult mammalian spinal cord was reported to host neural progenitor cells by both in vivo and in vitro evidence [12,18,22,25]. Neural progenitor cells were reported to remain quiescent in the adult mammalian CNS [14]. Whether these progenitor cells would be activated by injury like those of urodele amphibians remains unknown. In rodents, a significant increase of bFGF expression was found after many CNS diseases including SCI [7,9,13]. These findings prompted us to study how neural progenitor cells respond to contusion injury at an early stage after SCI, and the possible relationship between activated progenitor cells and the increase of bFGF-expressing cells in response to SCI in adult rodents.

The present study reveals that after SCI, many ordinarily quiescent cells are activated to express the immature progenitor marker nestin and to form a border-like structure around the injury site. In addition, neurosphere formation from injured spinal cord tissues is promoted after SCI and is further accelerated by the bFGF treatment. The increase in activated progenitor cells following SCI suggests that manipulating them by bFGF treatment might be useful for spinal cord regeneration.

Adult male Sprague–Dawley (SD) rats ($n = 152$) and nestin-promoter GFP-transgenic mice ($n = 26$) aged 8 weeks were used in this study. All the animal experiments were performed in

* Corresponding author. Present address: Department of Cancer Biology, Dana-Faber Cancer Institute, and Department of Neurobiology, Harvard Medical School, 1 Jimmy Fund Way, SM 1020, Boston, MA 02115, USA.
Tel.: +1 617 632 5032.
*E-mail address:* Yi_Xu@dfci.harvard.edu (Y. Xu).

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accordance with the Guidelines for Animal Experiments of Kyoto University.

Spinal cord injury was adopted from the previously described method [17]. Briefly, laminectomy was performed at the mid-thoracic T8–9 level of the spinal cord, where a standard spinal cord contusion was made using an NYU impactor. For rats, a 10 g metal rod (2.0 mm in diameter) was dropped from a height of 12.5 mm onto the exposed spinal cord. For mice, a 10 g metal rod (1.0 mm in diameter) was dropped from a height of 6.25 mm.

BrdU administration and detection was performed as previously described [23]. For the histological analysis, detailed protocols of using the primary antibodies and their final dilutions are available upon request.

Five days after SCI, injured T8–9 spinal cord tissues (5 mm in length) from rats or mice were dissected out for floating neurosphere culture. The experiments for primary culture, subculture, differentiation, quantitative analysis of primary neurospheres and examining the phenotype of differentiated cells were performed as previously described [23].

After contusion SCI, the increased expression of laminin was found within or at the border of the primary injury site (Fig. 1A1). We performed immunohistochemical staining for nestin, a marker for neural progenitor cells, to study the distribution of reactive cells in injured spinal cord tissues. In contrast to the intact spinal cord in which nestin$^+$ cells were sparsely found (data not shown), significantly increased nestin$^+$ cells were observed after SCI. Three days after SCI, many nestin$^+$ cells were found around the lesion to form a border-like structure (inset of Fig. 1A1), which became more defined two days later. Five days after SCI, tissues around the lesion (indicated by the boxes in Fig. 1A2) were examined in detail. In regions near the border of the lesion, many nestin$^+$ cells with a bipolar morphology were found, some of which were lined up along the border of the lesion (Fig. 1B), and most nestin$^+$ cells expressed

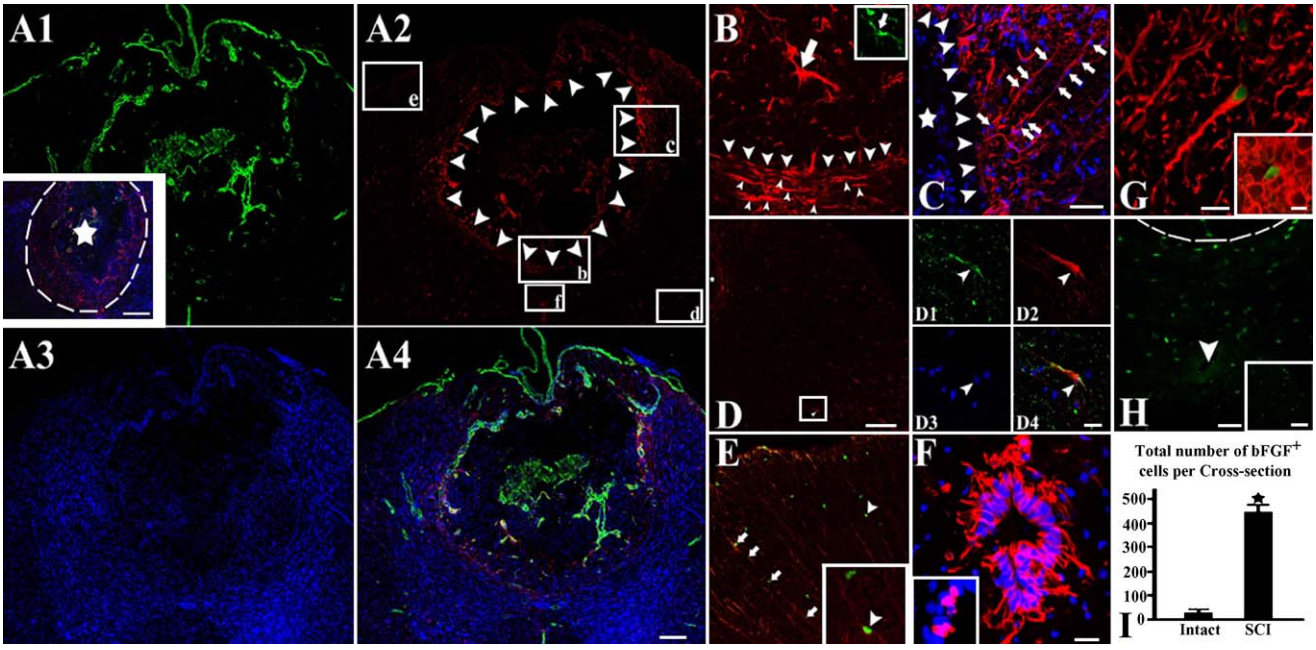


Fig. 1. Proliferation of progenitor cells and increase in the number of bFGF$^+$ cells at an early time point after contusion spinal cord injury (SCI). (A–F) cavity formation in a contusion SCI model, in which rats were injured by a 10 g weight dropped from a height of 12.5 mm. (A1–4) five days after SCI. The border between the lesion and surrounding intact area indicated by arrowheads in (A2) is vague. (A1) Laminin (green), (A2) Nestin (red), (A3) To-Pro-3, (A4) merged image. The areas corresponding to the boxes (b, c, d, e and f) in (A2) are shown in detail in micrographs (B), (C), (D), (E) and (F). Inset in (A1) three days after SCI. White lines delineate a border-like structure around the lesion (indicated by the asterisk). Red: nestin, green: laminin, blue: To-Pro-3. (B) The adjacent cryostat section to (A). This image was taken from a region near the border of the lesion indicated by Box b in (A2). Many nestin$^+$ cells with a bipolar morphology (red, small arrowheads) were found and were lined up along the border of the lesion (arrowheads). Most nestin$^+$ cells were GFAP$^+$ (inset: arrow, green). (C) The adjacent cryostat section to (A). This image was taken from a dorso-lateral region of the lesion indicated by Box c in (A2). Nestin$^+$ cells with their nuclei indicated by arrows are radially arranged with their processes extending from the pia mater to the border of the lesion (arrowheads). Red: nestin, blue: To-Pro-3. Asterisk: The site of the cavity, (D) The adjacent cryostat section to (A). This image was taken from a region distant from the lesion indicated by Box d in (A2). Some nestin$^+$ cells (red, arrow) were found with a radial process extending toward the border of the lesion. Asterisk: The site of cavity. (D1–4) High magnification of the box in (D). (D1) GFAP (green), (D2) Nestin (red), (D3) To-Pro-3 (blue), (D4) merged image. (E) the adjacent cryostat section to (A). This image was taken from a dorso-lateral region of the lesion indicated by Box e in (A2). After SCI, many nestin$^+$ cells (red) were BrdU$^+$ (green). BrdU$^+$ cells (green, arrows) were lined up and radially extended their nestin$^+$ processes (red) toward to the border of the lesion. Inset: High magnification of a BrdU$^+$ cell (arrowhead) with a nestin$^+$ radial process. (F) The adjacent cryostat section to (A). This image was taken from a region of the central canal indicated by Box f in (A2). After SCI, the ependymal layer of the central canal became full of nestin$^+$ cells (red). Inset: many ependymal cells were BrdU$^+$ (red). Blue: To-Pro-3, G: Five days after SCI. Some GFAP$^+$ cells (red) were bFGF$^+$ (green). Inset: some CD11b/c$^+$ cells (red) with macrophage-like morphology were bFGF$^+$ (green). (H) many bFGF$^+$ cells (green) were found in the region near the central canal indicated by the arrowhead. White lines delineate the lesion dorsal to the central canal. Inset: Many bFGF$^+$ cells (green) were found in the region below the pia matter. (I) the number of bFGF$^+$ cells was counted throughout the cross-sections of the spinal cord at T8–9, and was 15 times larger for SCI than in the intact rats ($P < 0.05$). The data shows the average ± S.E.M. Scale bar: 100 μm in (H)/inset, 50 μm in (A1–4), inset of (A1), (B), (C), (D), and (E), and 10 μm in the inset of (B), (D1–4), the inset of (E), (F)/inset, and (G)/inset.