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Transient activation of Wnt/β-catenin signaling reporter in fibrotic scar formation after compression spinal cord injury in adult mice

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ABSTRACT

After traumatic spinal cord injury (SCI), a scar may form with a fibrotic core (fibrotic scar) and surrounding reactive astrocytes (glial scar) at the lesion site. The scar tissue is considered a major obstacle preventing regeneration both as a physical barrier and as a source for secretion of inhibitors of axonal regeneration. Understanding the mechanism of scar formation and how to control it may lead to effective SCI therapies. Using a compression-SCI model on adult transgenic mice, we demonstrate that the canonical Wnt/β-catenin signaling reporter TOPgal (TCF/Lef1-lacZ) positive cells appeared at the lesion site by 5 days, peaked on 7 days, and diminished by 14 days post injury. Using various representative cell lineage markers, we demonstrate that, these transiently TOPgal positive cells are a group of Fibronectin(+);GFAP(−) fibroblast-like cells in the core scar region. Some of them are proliferative. These results indicate that Wnt/β-catenin signaling may play a key role in fibrotic scar formation after traumatic spinal cord injury.

1. Introduction

Wnt signaling is activated in neural stem cells and progenitors as well as in glial cells, and is critical for neurogenesis in the embryonic and adult CNS [1–6]. Wnt signaling also plays an indispensable role in neurite outgrowth and axon guidance during neural development and regeneration [7–11]. In the adult spinal cord, the expression of various Wnt signaling molecules is normally low, but is induced by spinal cord injury (SCI) in rodents or zebrafish [12–15]. Reinduction of repulsive Wnt/Ryk signaling molecules in the adult spinal cord has been shown to suppress axonal regeneration after SCI [16,17]. Conversely, Wnt3a administration enhances axonal regeneration and functional recovery after SCI in rats [18,19], while overexpression of an inhibitory ligand Dkk inhibits axon regeneration in zebrafish [15]. Nevertheless, the identities of the Wnt responsive cells in the injured spinal cord and the cellular mechanisms by which Wnt signaling influences recovery from SCI remain poorly understood.

The potential of axons to regenerate is likely conserved in the adult spinal cord, but it is blocked by various inhibitory factors generated at the lesion site [20–22]. Immediately after SCI, oligodendrocyte/myelin-derived axon growth inhibitors, such as Nogo, MAG, and OMG are produced [23–25]. Subsequently, a scar forms at the lesion site that becomes a physical barrier and also generates chemical inhibitors, such as chondroitin sulfate proteoglycans (CSPGs), further limiting the regenerative potential of the adult CNS axons [26–28]. The core scar region consists of dominantly fibroblasts that secrete extracellular matrix molecules, including Fibronectin, Laminin, and collagen type IV (Col IV) [29,30], but the cellular and signaling mechanisms responsible for fibrotic scar formation are not well understood.

In this study, we determined that the injury-induced Wnt responsive cells are neither neuronal nor glial lineages, but a group of fibroblasts at the lesion site. Many of them are proliferative. These results provide new insights into the function of Wnt/β-catenin signaling in fibrotic scar formation after adult neural injury and suggest Wnt signaling as a novel therapeutic target for control of scar formation and enhanced regeneration after SCI.

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2. Materials and methods

2.1. Animals

Wnt signaling reporter TOPgal transgenic mice (*Tg(Fos-lacZ) 34Efu/J*) [31] were obtained through the Jackson Laboratory and expanded/housed in the UC Davis vivarium. Two-to four-month-old adult mice were used in this study. All animal procedures were approved by the Institutional Animal Care and Use Committee at UC Davis and conformed to NIH guidelines.

2.2. Surgical procedures

All surgeries were performed under aseptic conditions. Spinal cord injury was carried out using the compression or crush method as described previously with minor modifications [32–34]. Animals were anesthetized with Ketamine/Xylazine (100/10 mg/kg) administered intraperitoneally (i.p.). Depth of anesthesia was assured by monitoring lack of response to a foot pinch prior to the surgery. The back of the mouse was shaved and disinfected with povidone-iodine (Betadine) and 70% alcohol. A skin incision was made above the lower thoracic vertebrae. Paravertebral muscles on both sides of the T10-T12 vertebrae level were cut, and the vertebrae were exposed. A laminectomy was then performed between T10-T12, and the spinal cord was compressed by inserting a sterilized No.5 Dumont forceps (Fine Science Tools) with or without 0.5mm spacer [32] for 30 s at the T11 level. After the compression injury, muscles and skin were sutured, and animals were placed on a heat pad until ambulatory. Each animal was given systematic postoperative analgesia (Buprenrphine, 0.05 mg/kg i.p.) to prevent pain. Animals were examined daily to record their body weight. After surgery, the mouse bladder was manually expressed twice daily until the recovery of spontaneous bladder function, which generally occurred between 7 and 14 days post-injury (dpi).

2.3. X-gal staining and immunohistochemistry

For X-gal staining to detect TOPgal activity, the injured mice were perfused transcardially with phosphate-buffered saline (PBS, pH7.4). The spinal cord was dissected out and immersed into X-gal (5-bromo-4-chloro-3-indolyl-beta-d-galactopyranoside) substrate solution (with 1 mg/ml X-gal in 5 mM $K_4Fe(CN)_6$, 5 mM $K_3Fe(CN)_6$, and 5 mM $MgCl_2$) for 12–24 h in the dark to detect β-galactosidase enzymatic activity. The stained sample was washed three times with PBS and post-fixed with 4% paraformaldehyde (PFA), then processed for histological analysis. For co-labeling of X-gal and

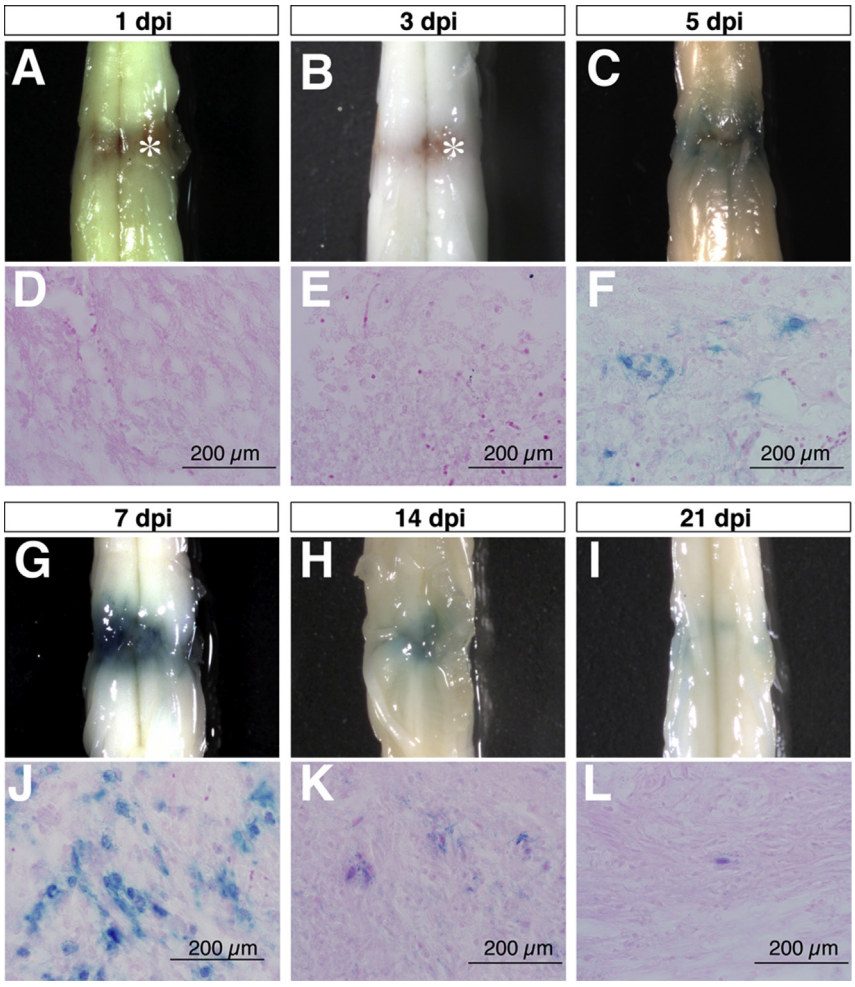

Fig. 1. Compression spinal cord injury activates the Wnt signaling reporter TOPgal in a time-dependent manner. The wholemount spinal cords (A-C,G-I) and respective transverse sections (D-F,J-L) show the X-gal staining results for the TOPgal activities in the adult TOPgal mice at 1, 3, 5, 7, 14, and 21 days post injury (dpi). Note that the blue X-gal staining appears at 5 dpi (C,F), peaks at 7 dpi (G,J), and diminishes dramatically at 14 (H,K) and 21 dpi (I,L). Asterisks (A,B) indicate the reddish-brown substance caused by hemorrhage in the lesion site at 1 and 3 dpi. The sections were counterstained with Eosin solution. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)