

● PERSPECTIVES

Extrinsic inhibitors in axon sprouting and functional recovery after spinal cord injury

Summary: The limited axonal growth after central nervous system (CNS) injury such as spinal cord injury presents a major challenge in promoting repair and recovery. The literature in axonal repair has focused mostly on frank regeneration of injured axons. Here, we argue that sprouting of uninjured axons, an innate repair mechanism of the CNS, might be more amenable to modulation in order to promote functional repair. Extrinsic inhibitors of axonal growth modulate axon sprouting after injury and may serve as the first group of therapeutic targets to promote functional repair.

Introduction

Recovery from injury to the CNS is limited in the mammalian adult. This limitation is due in large part to the failure of CNS axons to grow after injury. There are two principal types of injury-induced axonal growth: regeneration, the growth from injured neurons, and sprouting, the growth from uninjured neurons. Many factors are thought to play a role in restricting axon growth after injury, including the poor intrinsic axon growth ability of CNS neurons, the presence of growth inhibitory molecules and a lack of growth-promoting factors in the CNS environment. While many attempts have been made to encourage regeneration of damaged axons by modulating these factors, few experimental manipulations have led to robust, functionally meaningful regeneration. Meanwhile, extensive literature indicates that targeting various inhibitory molecules present in the CNS environment such as myelin-associated inhibitors and chondroitin sulfate proteoglycans may improve functional recovery in models of spinal cord injury, first shown with the IN-1 antibody (Bregman et al., 1995) and later with chondroitinase ABC (Bradbury et al., 2002). Subsequent studies raised the question of how robustly targeting these extrinsic inhibitors improves axon regeneration (Bartus et al., 2012; Lee and Zheng, 2012). Instead, a consistent theme has emerged that manipulating these extrinsic inhibitors alters the axonal sprouting response of intact axons (Figure 1). Promoting uninjured axon sprouting may be an alternative approach to improve recovery from spinal cord injury. This mini-review evaluates the evidence that modulation of extrinsic inhibitors of axon growth can increase sprouting of uninjured axons, which can mediate functional recovery from spinal cord injury. In particular, we will discuss the sprouting of corticospinal tract axons across the midline as an example to illustrate this point.

Sprouting of the corticospinal tract: the unilateral pyramidotomy model

The corticospinal tract (CST), a major descending tract, is important for voluntary motor control and for functional recovery from spinal cord injury in humans. Sprouting of the CST in rodents can be readily assessed after experimental unilateral pyramidotomy. In this injury model, one side of the CST is lesioned as it travels through the medullary pyramids en route from the cortex to the contralateral spinal cord, with the lesion placed just above where the tract crosses the midline. Pyramidotomy allows for a “cleaner” injury to one side of the CST than a lateralized spinal cord injury (e.g., lateral hemisection) in rodents because the two main corticospinal tracts are far apart in the pyramids but adjacent to each other around the midline in the spinal cord.

In order to assess sprouting of the intact CST after injury, the intact side of the CST is labeled and sprouting of fibers across the

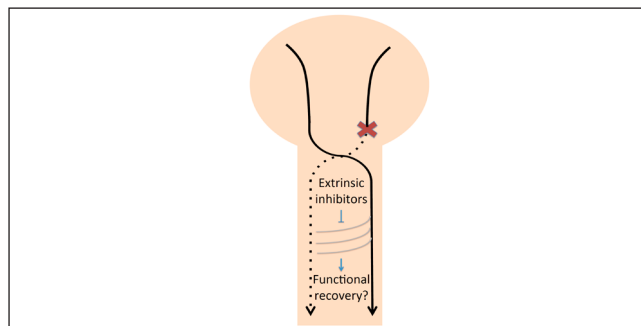


Figure 1 Extrinsic inhibitors attenuate anatomical and functional recovery from injury.

After an axonal tract in the central nervous system is lesioned (bold X), the distal segments degenerate (dotted line). Uninjured axon fibers sprout into the denervated side of the spinal cord after injury (horizontal curved lines), which is attenuated by extrinsic inhibitors. This sprouting may contribute to functional recovery from spinal cord injury. Arrows denote the direction of the two descending axonal tracts (one on each side) within the spinal cord.

midline of the cervical spinal cord into the denervated side is evaluated (note that these axons have now re-crossed the midline). In rodents, there is a small but significant amount of spontaneous CST axon sprouting across the midline after unilateral pyramidotomy (Thallmair et al., 1998; Lee et al., 2010; Shim et al., 2012). Therefore, it is important to take into consideration this baseline level of spontaneous sprouting when assessing the effect of any molecular manipulation. Interestingly, the level of spontaneous CST sprouting appears to be much more extensive in non-human primates than rodents after a lateral cervical injury, implicating the translational importance of CST sprouting in anatomically incomplete spinal injuries (Rosenzweig et al., 2011).

Extrinsic inhibitors suppress sprouting after injury

Various molecules expressed in the CNS are inhibitory to axon growth, many of which originate from mature oligodendrocytes. The prototypical myelin inhibitors Nogo, myelin-associated glycoprotein (MAG), and oligodendrocyte myelin glycoprotein (OMgp) are expressed on the surface of myelin and have been extensively characterized as inhibitors of neurite outgrowth in vitro. In addition, various pharmacologic and genetic studies have shown that inhibition of Nogo signaling leads to axon sprouting after spinal cord injury. One such study using the unilateral pyramidotomy model demonstrated that administration of an antibody that recognizes Nogo-A increased sprouting of the intact CST and functional recovery as assessed by food pellet retrieval, sticky paper removal, and rope climbing (Thallmair et al., 1998).

Interestingly, although all three of the prototypical inhibitors Nogo, MAG, and OMgp signal through common receptors, genetic deletion of Nogo but not MAG or OMgp increased sprouting of the intact CST after pyramidotomy (Lee et al., 2010). Surprisingly, MAG deletion was found to decrease axon growth after injury in this model (Lee et al., 2010), suggesting that MAG and perhaps myelin inhibitors in general have a dual role in modulating axonal sprouting after injury.

In addition to the prototypical myelin inhibitors, oligodendrocytes also express axon guidance molecules such as ephrins and semaphorins that may inhibit axon growth after injury. For instance, Sema6A is highly expressed by mature oligodendrocytes and inhibits neurite outgrowth from dorsal root ganglion neurons in vitro via its receptor PlexinA2 (Shim et al., 2012). Genetic deletion of PlexinA2 leads to increased sprouting of the intact CST on



both sides of the cervical spinal cord after pyramidotomy, as well as increased functional recovery in a pellet-reaching assay (Shim et al., 2012). The extent to which various axon guidance molecules modulate spinal axon sprouting after CNS injury remains to be fully explored.

Chondroitin sulfate proteoglycans (CSPGs) present in the extracellular matrix of the glial scar have also been shown to inhibit axon growth *in vitro* and *in vivo*. The bacterial enzyme chondroitinase ABC (ChABC) degrades side chains from CSPGs, attenuating their inhibitory nature. In the pyramidotomy model, ChABC treatment has been shown to increase CST sprouting and functional recovery of paw preference for weight support during rearing (Starkey et al., 2012). In contrast to the bilateral sprouting observed after PlexinA2 deletion, ChABC treatment increased sprouting on the denervated side of the spinal cord only, suggesting distinction in the mechanisms involved.

Sprouting and functional recovery

In studies using the pyramidotomy model, the increased CST sprouting achieved by manipulating extrinsic growth inhibitors was often associated with improved functional recovery, as assessed by pellet retrieval (Thallmair et al., 1998; Shim et al., 2012), sticky paper removal (Thallmair et al., 1998; Shim et al., 2012), paw preference for weight support during rearing (Starkey et al., 2012), or rope climbing (Thallmair et al., 1998). Furthermore, the ability of these sprouted CST axons to form functional synapses has been implicated by their co-localization with a variety of pre- and post-synaptic markers including vGlut1 (Starkey et al., 2012), synaptophysin, SV2, and PSD-95 (Shim et al., 2012), suggesting the possibility that these sprouted fibers mediate functional recovery.

Yet the question remains whether the observed sprouting of CST axons in the cervical spinal cord is directly responsible for functional recovery. Indeed, performance in these behavioral tasks may be partially mediated or compensated for by plasticity of other tracts involved in motor functioning, such as the rubrospinal tract or the reticulospinal tract. For instance, with IN-1 antibody treatment the rubrospinal tract has been shown to innervate motor neurons denervated by bilateral CST transection (Raineteau et al., 2002). It is also possible that growth of CST axons rostral to the lesion into subcortical nuclei important for motor functioning may be involved in behavioral recovery (Z'Graggen et al., 1998).

Supporting evidence for a role of CST sprouting in functional recovery came from experiments where the spared CST axons were transected following a recovery period after experimental spinal cord injury. For example, spontaneous functional recovery after dorsal column lesion to disrupt the dorsal main CST was abolished when a second lesion was performed to eliminate the ventral CST (a more prominent minor CST tract in rats) (Weidner et al., 2001). Similarly, after unilateral lesion of the sensorimotor cortex, mice show spontaneous sprouting of intact CST fibers into denervated cervical spinal cord and gradual partial spontaneous improvement in fine motor control; this functional recovery is attenuated by subsequent pyramidotomy on the uninjured side (Ueno et al., 2012). These experiments demonstrate that the spared CST, which is a source of axon sprouting, is important for behavioral recovery after unilateral lesion to the CST system. However, definitive demonstration that the sprouted fibers mediate functional recovery remains a challenge.

Prospects and challenges

Investigation into sprouting of the CST after pyramidotomy has revealed that various extrinsic inhibitors may be important targets for improving anatomical and behavioral outcomes from spinal cord injury. Nevertheless, important questions remain before findings from these studies can move toward the goal of providing clinical benefit to patients with spinal cord injury. In particular, definitive evidence of what exactly mediates the functional recovery is still lacking. Is axonal sprouting necessary and sufficient to achieve functional improvement with various interventions in models of

spinal cord injury? What are the axonal tracts involved and what are the underlying mechanisms? These questions are important to consider in order to determine where to target therapeutics and to evaluate what clinical injuries are likely to see benefit from potential therapies targeting extrinsic inhibitors of axon growth. Finally, while the pyramidotomy model discussed here allows relatively straightforward assessment of CST sprouting, this model is not a spinal cord injury model per se. Assessing CST sprouting in experimental spinal cord injury would be a necessary step in translating findings from the pyramidotomy model to the clinics.

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