● PERSPECTIVE

Promoting functional recovery by inhibition of repulsive guidance molecule-a after spinal cord injury

Spinal cord injury (SCI) leads to permanent disability with motor and sensory dysfunctions. The mature mammalian central nervous system (CNS) possesses a limited capacity to regenerate/regrow after injury. Research works on functional restoration from SCI *via* enhanced sprouting of injured/spared fibers based on molecular mechanisms have greatly increased in recent years, especially using small animal models such as anamniotes and rodents. These results allow us to understand the advanced mechanisms of axonal remodeling after SCI. However, for a successful translation of the mechanisms into human patients with SCI, we have to consider certain anatomical and physiological differences in neural circuits between small animals and humans. For example, the corticospinal tract (CST) has been focused as a therapeutic target to enhance motor functions after SCI (Oudega and Perez, 2012). The CST fibers originating from the motor cortex directly connect with spinal interneurons and/or motoneurons, to transmit motor commands to them for achieving voluntary movement. Less dexterous animals such as rodents and cats do not physiologically have a direct connectivity of the CST fibers with spinal motoneurons (Isa, 2017). Thus, it is generally considered that the CST closely relates to the development of manual dexterity through evolution. Recently, at least part of the CST fibers originating from the contralesional primary motor cortex (MI) in a primate SCI model have been reported to extend into the spinal medial gray matter and the motoneuron pool in conjunction with spontaneous recovery from impaired manual dexterity (Nakagawa et al., 2015) (**Figure 1A**). The relative number of sprouting CST fibers increased in the motoneuron pool as compared to a normal control (Nakagawa et al., 2015). However, such an event is not observed in a rodent SCI model (Hata et al., 2006) (**Figure 1A**). This indicates that the reorganization pattern of CST fibers below the lesioned site in primates differs from that in rodents.

To promote axonal sprouting and regrowth as the strategy for functional recovery in human patients with SCI, the CST fibers are needed to extend over a long distance beyond the lesioned site and connect properly with target spinal neurons. For considering such a therapeutic strategy for human patients, it is essential to understand the mechanisms underlying neural network remodeling to achieve functional recovery in primate animals who are characterized by manual dexterity using precision grip.

Repulsive guidance molecule-a (RGMa) was originally cloned from chicken embryos and identified as a guidance molecule for retinal axons during developmental (Monnier et al., 2002). It has been reported in rodent models that RGMa expressed in glial cells is specifically upregulated around the damaged/lesioned sites, and that suppression of its action promotes axonal sprouting and regrowth (Hata et al., 2006; Siebold et al., 2017). According to previous works (Siebold et al., 2017), Neogenin interacts with RGMa to lead to growth cone collapse, and several downstream signaling for RGMa-neogenin has been identified in an *in vitro* assay. So far, the suppression mechanisms underlying axonal regeneration and regrowth *via* RGMa-neogenin signaling after injury have become gradually clear using *in vitro* and *in vivo* experiments (Siebold et al., 2017).

In our recent study (Nakagawa et al., 2018), we investigated the effects of a neutralizing antibody against RGMa on functional recovery and axonal sprouting in macaque monkeys whose manual dexterity was impaired after SCI (**Figure 1B**). The anti-RGMa antibody was directly delivered around the lesioned site through an osmotic pump with a catheter for four weeks following SCI. The recovery process of a skilled motor behavior was analyzed by using multiple behavioral tasks and enhanced extremely by treatment with the anti-RGMa antibody. The CST fibers derived from the contralesional MI extended below the lesioned site at the cervical cord in the monkeys treated with the anti-RGMa antibody, compared with normal control monkeys. In human patients with CNS injuries and diseases, hand and digit functions are readily left impaired. In many cases, the functional restoration is correlated with the increased regrowth of CST fibers (Raineteau and Schwab, 2001). Several studies have examined the possible contribution of sprouting CST fibers originating from the ipsilesional MI to restoration of motor functions after SCI (Rosenzweig et al., 2010; Baker et al., 2015). In monkeys with a complete unilateral hemisection at the C_7 level, spontaneous recovery from impaired manual dexterity is observed in parallel with the increased density of CST fibers, including recrossing fibers originating from the ipsilesional MI and extending below the lesioned site (Rosenzweig et al., 2010). By contrast, it has been reported that there is no evidence that fibers sprouting from the ipsilesional CST physiologically contribute to motor functions at a late stage in a monkey pyramidotomy model (Baker et al., 2015). We also observed that the digit region in the ispilesional MI did not participate in functional recovery at a late stage in the unilateral hemisection model at the border between the C_6 and the C_7 segment (Nakagawa et al., 2018). These results might be ascribable to the variability in CNS injuries and subsequent remodeling of neural networks for restoration of motor functions. Thus, it is critical to understand the differences in the remodeling mechanisms in individual CNS injury models to promote recovery of motor functions effectively and efficiently. To translate some treatment strategies into human patients with SCI, we should take the extent of spinal lesions into consideration. Many SCI patients generally undergo traumatic damages to the spinal cord with a fracture. In other words, the spinal cord is hardly injured in clinical cases in a manner similar to that in the hemisection model. It is, therefore, important to understand the mechanisms underlying functional recovery in a primate contusion model in future works.

Most of the human SCI cases are anatomically and functionally incomplete, leaving part of the CST intact, and the functional restoration takes place in many incomplete SCI cases as well as in animal SCI models (Raineteau and Schwab, 2001). Hence, neural network remodeling for recovering motor functions after incomplete SCI is more likely to occur in human patients (Oudega and Perez, 2012).

Our studies have provided strong evidence that the effect of the neutralizing antibody against RGMa promotes recovery from impaired manual dexterity after SCI in primate animals possessing neural circuitry which is anatomically similar to that in humans. We are currently testing the validity of intravenous administration, instead of intrathecal infusion *via* the osmotic pump, of the RGMa antibody for easier clinical application. The most critical factor achieving manual dexterity is the ability to move the digits individually, and impaired manual dexterity severely affects daily living activity (Schieber and Santello, 2004). A recent study has shown in rodents that treatment of thoracic cord lesions with the neutralizing antibody against RGMa enhances cell survival around the lesioned site compared with a control group (Mothe et al., 2017). Growing evidence for RGMa suggests that inhibition of its effect after SCI may not only promote axonal sprouting and regrowth, but also bring about other important outcomes, such as prevention of neuronal death and neurodegeneration due to secondary damages, to restore motor functions.

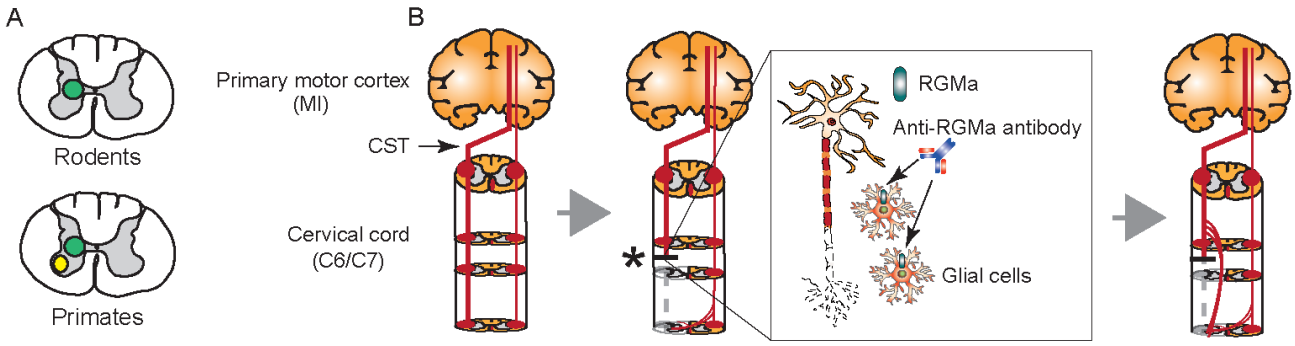

Figure 1 Schematic diagram showing corticospinal tract (CST) fiber sprouting after spinal cord injury (SCI) with anti-repulsive guidance molecule-a (RGMa) antibody treatment.
(A) The CST fibers after SCI extend into the medial gray matter (green circle) below the lesioned site in rodents, while they extend into the motoneuron pool (yellow circle) as well as into the medial gray matter (green circle) in primates. (B) The unilateral cervical cord is lesioned at the C_6/C_7 level and the CST connecting primary motor cortex (MI) neurons to spinal interneurons and motoneurons is fully disrupted. Following SCI, RGMa expressed in glial cells is upregulated around the lesioned site. Sprouting of the CST fibers is promoted by treatment with anti-RGMa antibody. The asterisk denotes the lesioned site.

Following lateral CST lesions at the border between the C_4 and the C_5 level in monkeys, not only the motor-related cortices, but also the mesolimbic structures were activated and involved in the recovery from impaired manual dexterity (Isa, 2017). In addition, several studies have reported that combined treatment of molecular mechanisms, rehabilitative training, and growth factors effectively promotes recovery in motor functions after CNS injuries (Zhao and Fawcett, 2013). Other descending fibers, such as those through the reticulospinal and rubrospinal tracts, may participate in recovery of manual dexterity after SCI (Baker et al., 2015).

Eventually, it would be critical to confirm that the mechanisms and strategies for functional recovery have certain advantageous effects on human patients with CNS injuries and diseases. We assume that understanding of neural mechanisms characteristic of primates from both the normal and the abnormal viewpoints is crucial to provide effective and efficient treatment strategies. It should be noted here that the mechanisms underlying axonal sprouting and neural circuit changes to enhance functional restoration after CNS injuries and diseases in primates remain largely unclear. These efforts are critical to lead to the development of effective and efficient therapies for human patients in future studies.

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***Comments to authors:** This article reviews the groups research into the discovery of the inhibitory role of RGMa and its role in limiting regeneration and plasticity in spinal cord injury first in rodents and more latterly in primates. The title talks about translational medicine but there is little discussion of how this is to be translated into human studies, especially as the mode of administration of the antibody is via intrathecal pumps. Another limitation of this work is that the animal models that have been used have been surgical hemi-section and the effects of RGMa inhibition in other models of SCI such as contusion have not been performed.*

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