Journal Club

Editor's Note: These short, critical reviews of recent papers in the Journal, written exclusively by graduate students or postdoctoral fellows, are intended to summarize the important findings of the paper and provide additional insight and commentary. For more information on the format and purpose of the Journal Club, please see http://www.jneurosci.org/misc/ifa_features.shtml.

Knocking Down Glycosaminoglycan Synthesis

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Review of Laabs et al. (http://www.jneurosci.org/cgi/content/full/27/52/14494)

The failure of axon regeneration after spinal cord injury is attributed in part to the upregulation of chondroitin sulfate proteoglycans (CSPGs) in the astroglial scar. CSPGs are a diverse class of complex macromolecules that consist of a protein core covalently attached to sulfated carbohydrate chains known as glycosaminoglycans (GAGs). Each GAG chain is made up of disaccharide units that are sulfated at various sites. CSPG GAGs with a 4,6-sulfation pattern are upregulated in the injured CNS, and are responsible for inhibiting axonal growth through the astroglial scar. To reduce the inhibitory effects of CSPGs, one approach is to degrade CSPG GAG using the enzyme chondroitinase ABC (cABC), which is derived from the bacterium *Proteus vulgaris*. Previous studies by Bradbury et al. (2002) demonstrated that cABC can stimulate robust axon regeneration through the glial scar. Despite the effectiveness of cABC treatment, however, a potential downfall is that cABC is a foreign molecule that may trigger an immune response in the nervous system. Furthermore, the short-lived activity of cABC and the rapid resynthesis of CSPG GAG *in vivo* may limit the time during which axon regeneration can occur.

In a study published recently in *The Journal of Neuroscience*, Laabs et al. (2007) developed a novel molecular approach to inhibit the synthesis of CSPG GAGs. Specifically, they have identified an enzyme, chondroitin polymerizing factor (ChPF), that mediates specifically CSPG GAG synthesis, and have produced a small interfering RNA (siRNA) that knocks down the expression of this enzyme via RNA interference (RNAi). Using primary rat astrocytes and an inhibitory astrocyte cell line Neu7, they demonstrated that siRNA-mediated knock-down of ChPF expression reduced CSPG GAG expression up to 70% [Laabs et al. (2007), their Figs. 2 (http://www.jneurosci.org/cgi/content/full/27/52/14494/F2), 4 (http://www.jneurosci.org/cgi/content/full/27/52/14494/F4)]. To determine whether the siRNA treatment could reduce axonal growth inhibition by Neu7 cells, they collected conditioned medium from both knock-down and control Neu7 cells. The conditioned medium was introduced to cerebellar granule neurons either as a substrate [Laabs et al. (2007), their Figs. 5 (http://www.jneurosci.org/cgi/content/full/27/52/14494/F5), 6*A*,*C* (http://www.jneurosci.org/cgi/content/full/27/52/14494/F6)] or in medium [Laabs et al. (2007), their Fig. 6*B*,*D* (http://www.jneurosci.org/cgi/content/full/27/52/14494/F6)]. The conditioned medium from ChPF-knock-down Neu7 cells was significantly less repulsive to axon outgrowth from cerebellar granule neurons than was the conditioned medium from control Neu7 cells [Laabs et al. (2007), their Figs. 5 (http://www.jneurosci.org/cgi/content/full/27/52/14494/F5), 6 (http://www.jneurosci.org/cgi/content/full/27/52/14494/F6)]. Overall, these *in vitro* studies strongly suggest that siRNA-mediated knock-down of ChPF may be an effective approach to block the synthesis of CSPG GAGs in the astroglial scar.

The RNAi approach to knocking down inhibitory molecules introduced by Laabs et al. (2007) has a number of advantages for *in vivo* applications. First, RNAi is extremely specific and can knock down synthesis of specific inhibitory epitopes without affecting the many other molecular players at work *in vivo*. Second, because siRNA is relatively simple to synthesize, multiple siRNAs could be developed to simultaneously target different enzymes that produce inhibitory molecules in the glial scar. Third, RNAi can effectively inhibit protein expression for up to 4 weeks *in vivo* (Bartlett and Davis, 2007). Therefore, RNAi should be able to knock down CSPG GAG synthesis for up to 4 weeks, during which regenerating axons would have sufficient time to make functional connections with their synaptic targets (Bradbury et al., 2002). Because a single cABC treatment can eliminate CSPG GAGs for only 1–2 d, further RNAi knock-down of CSPG GAG synthesis may be a more feasible approach to extend the time during which axons can regenerate to their targets without being impeded by the presence of CSPG GAGs. Last, the effect of RNAi endures only for a finite period, suggesting that after RNAi knock-down of ChPF expression, the adult spinal cord can restore the endogenous levels of

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CSPG GAG crucial for maintaining the integrity of existing axonal connections.

Despite possible advantages, however, a key issue with using siRNA to knock down CSPG GAGs is the potential effect of foreign siRNA on the mammalian immune system. Previous studies by Judge et al. (2005) demonstrated that certain siRNA sequence motifs can trigger an innate immune response *in vivo* by stimulating macrophage activation and the release of cytokines. Likewise, introducing foreign siRNAs into the injured spinal cord might further activate unfavorable immune responses that increase secondary damage and impede spinal cord repair. Therefore, further examination of the effects of siRNA on the immune system is crucial before RNAi can be safely implemented *in vivo*.

A more fundamental issue than the potential problems with the use of siRNA *in vivo* is our limited understanding of the role of CSPG GAGs *in vivo*. Previous studies have strongly suggested that CSPG GAGs are repulsive and that the degradation of CSPG GAGs by cABC reduces the repulsiveness of CSPGs (Bradbury et al., 2002). Therefore, a well accepted interpretation is that cABC treatment enhances axon regeneration by removing the inhibitory CSPG GAGs from the glial scar. Another possibility, however, is that the products of CSPG GAG degradation may enhance axon repair. This hypothesis has been supported by Rolls et al. (2004), who demonstrated that GAG disaccharide units released from cABC-mediated CSPG GAG degradation could stimulate axon repair by promoting axon outgrowth, overcoming the effects of strong repulsive factors, and activating the neuroprotective phenotype of endogenous microglia. Therefore, cABC treatment might stimulate axon regeneration by releasing GAG disaccharides. In this case, inhibition of CSPG GAG synthesis *in vivo* may not be beneficial, because it would hinder the release of GAG. Future *in vivo* characterization of the effects of ChPF siRNA-mediated knock-down may provide further insights into the role of CSPG GAGs in spinal cord repair.

In summary, Laabs et al. (2007) introduced a simple and effective strategy through which CSPG GAG synthesis could be specifically inhibited using RNAi. The data presented by Laabs et al. (2007) provide the first example that siRNA-mediated knock-down of a CSPG GAG synthesizing enzyme could reduce the repulsive nature of the inhibitory astrocyte cell line Neu7. This finding has a strong impact in the field of spinal cord research, because it is a first proof of principle that RNAi could be a feasible therapeutic tool to eliminate molecular obstacles that hinder axon regeneration.

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