Journal Club

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Role of NG2 in Development and Regeneration

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Review of Yang et al. (http://www.jneurosci.org/cgi/content/full/26/14/3829)

Introduction

The chondroitin sulfate proteoglycan NG2 is expressed on the surface of NG2 glia and oligodendrocyte precursor cells. Several studies have indicated that NG2 is inhibitory to axonal regeneration. After CNS injury, NG2 glia become activated and contribute to the glial scar that is considered a barrier to axonal growth. However, de Castro et al. (2005) reported no difference in the extent of axonal regeneration after spinal cord transection in adult NG2-null mutant versus wild-type mice, challenging the view that NG2 is inhibitory. A recent paper in the *Journal of Neuroscience* by Yang et al. (2006) set out to further investigate whether NG2 is inhibitory for axonal growth. The authors examined the effects of NG2 cells, rather than the isolated NG2 proteoglycan, on axonal growth. In a well defined set of experiments, they show that NG2 cells derived from early postnatal rat cerebral cortices promoted axon outgrowth from early postnatal hippocampal neurons in coculture [Yang et al. (2006), their Fig. 4*A–H* (http://www.jneurosci.org/cgi/content/full/26/14/3829/F4)]. Furthermore, growth cones of hippocampal neurons preferentially formed contacts with NG2 cells, whereas coculture with MAG-expressing cells or mature oligodendrocytes led to growth cone retraction [Yang et al. (2006), their Figs. 1 (http://www.jneurosci.org/cgi/content/full/26/14/3829/F1) and 2 (http://www.jneurosci.org/cgi/content/full/26/14/3829/F2)]. The NG2 cocultures contained a small contamination with astrocytes. To evaluate whether these contaminating astrocytes might be responsible for the observed growth-promoting effects, the authors determined the approximate number of contaminating astrocytes and investigated their effects on axon outgrowth. Because the cocultured astrocytes promoted less axon outgrowth than NG2 cells, the authors suggested that NG2 cells are responsible for the growth-promoting effects. However, this still leaves open the question of whether factors released by contaminating astrocytes could alter the expression of growth-promoting molecules in NG2 cells.

Whatever growth-promoting factors may exist, they do not appear to be soluble in nature, because Yang et al. (2006) [their Fig. 4*I* (http://www.jneurosci.org/cgi/content/full/26/14/3829/F4)] show that the growth-promoting effects of NG2 cells appear to be contact-mediated or mediated via a slowly diffusible extracellular signal. Indeed they observed that NG2 cells (from postnatal rat cortex) express laminin and fibronectin at the cell surface [Yang et al. (2006), their Fig. 7 (http://www.jneurosci.org/cgi/content/full/26/14/3829/F7)], extracellular molecules that are known to exert growth-promoting effects. The expression of these extracellular molecules may also be responsible for the extensive contacts between growth cones and NG2 cells that were observed in the developing corpus callosum *in vivo* [Yang et al. (2006), their Fig. 3 (http://www.jneurosci.org/cgi/content/full/26/14/3829/F3)], although the authors did not test whether NG2 cells in the corpus callosum actually express laminin and fibronectin.

In an interesting part of this study, the authors tested the hypothesis that negative effects of the NG2 proteoglycan on axon outgrowth (Chen et al., 2002) could be attributable to higher levels of purified NG2 compared with the physiological levels on NG2 cells. However, neither elevation of NG2 levels by at least fivefold nor knockdown of NG2 using RNA interference altered the growth-promoting effects of NG2 cells on hippocampal neurons [Yang et al. (2006), their Figs. 5 (http://www.jneurosci.org/cgi/content/full/26/14/3829/F5) and 6 (http://www.jneurosci.org/cgi/content/full/26/14/3829/F6)]. The reasons for the differential effects of NG2 cells versus NG2 proteoglycan on neurite outgrowth between studies remain unclear. The authors suggest that the epitope on NG2 that causes growth cone collapse may be masked on the surface of NG2 cells or that NG2 cells may express potent growth-promoting molecules that override the negative effects of the NG2 proteoglycan, theories that can be tested in the future.

Together, the authors provide convincing evidence that NG2 cells facilitate growth of early postnatal neurons *in vitro* and *in vivo*, suggesting a novel role for

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NG2 cells in development. The authors also suggest that this mechanism could occur during regeneration, but no *in vivo* axon lesioning model was used to investigate NG2 in regeneration. It should be noted that the neurons used for the *in vitro* models are from neonatal animals. Neonatal neurons possess an elevated intrinsic growth potential compared with adult neurons and are able to grow on myelin, an effect lost with maturation. This difference appears to be attributable, at least in part, to high levels of endogenous cAMP (Cai et al., 2001). In fact, most of the studies performed on the effects of NG2 on neurite/axon growth have been performed in neonatal animals. However, there are differences in the molecular expression patterns between NG2 cells in the neonatal and adult CNS (Horner et al., 2002). These differences could affect the impact of NG2 on outgrowth. To establish a role for NG2 cells in regeneration, it will be necessary to use an adult *in vivo* regeneration model to investigate the effects of altering NG2 levels. Coincidentally, a recent issue of *Journal of Neuroscience* carries an article reporting that neutralizing the NG2 proteoglycan with anti-NG2 antibodies enhances the regeneration of sensory axons after spinal cord transection in the adult rat. This effect was markedly augmented when neurons were preconditioned by a peripheral nerve lesion 1 week before spinal cord transection and NG2 neutralization (Tan et al., 2006). This prerequirement for neurons to be in a growth state, to fully reveal the role of NG2 in regeneration, may also partly explain why de Castro et al. (2005) did not see an effect of NG2 knock-out on axon regeneration after spinal cord injury in adult mice.

In summary, the present study nicely shows that NG2-expressing cells do not inhibit outgrowth from neonatal neurons; however, the majority of studies still point to an inhibitory role of the NG2 molecule, both in isolation and in the mature CNS. Successful regeneration in the adult CNS will require not only overcoming inhibitory molecules but also activating the intrinsic growth state of neurons.

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