Integrin manipulation to improve regeneration

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After central nervous system (CNS) insults, such as spinal cord injury or traumatic brain injury, neurons encounter a complex microenvironment where mechanisms that promote regeneration compete with inhibitory processes. Sprouting and axonal re-growth are key components of functional recovery, but are often counteracted by inhibitory molecules. Several strategies are being pursued whereby these inhibitory molecules are either being neutralized with blocking antibodies, with enzymatic degradation or downstream signaling events are being interfered with.

Two recent studies\(^1\)\(^2\) show that activating integrin signaling in dorsal root ganglion (DRG) neurons renders them able to overcome inhibitory signals, and could possibly lead to new strategies to improve neuronal regeneration.

**Background**

The successful outcome of peripheral nerve regeneration is attributed both to the growth permissive milieu through which the neuronal growth cone advances toward its target and to the intrinsic ability of the neuron to initiate appropriate cellular responses such as changes in gene expression and cytoskeletal rearrangements. While injuries in the peripheral nervous system heal relatively well, after a CNS injury, various supporting cells start to produce growth-inhibiting molecules, including myelin-associated glycoprotein (MAG) oligodendrocyte-myelin glycoprotein (OMgp), Nogo and chondroitin sulfate proteoglycans (CSPGs), thus limiting regeneration.

**Manipulating Integrin Function to Improve Neuronal Regeneration**

Axonal growth cones interact with the extracellular matrix (ECM), by cell-surface receptors such as members of the integrin family. These molecules are essential to neuronal regeneration in the peripheral nervous system, reviewed in reference 3. Integrins are cell-surface receptors consisting of one \( \alpha \) and one \( \beta \) chain and elicit various intracellular signaling cascades upon activation that follows ligand binding ("outside-in" signaling). In addition, integrins have binding sites for divalent cations and the addition of manganese ions can activate integrins and has been shown to increase growth from retinal ganglions cells (RGC) in culture,\(^4\) showing that the developmental decrease in neurite growth capacity observed in RGCs could be reversed by integrin activation. This observation and the fact that the neurite growth inhibitor Nogo exerts at least part of its effect via inactivation of integrins,\(^5\) lead Tan and coworkers to investigate the role of integrin activation in relation to the growth inhibition effects by CSPGs.\(^2\)

Addition of CSPGs to dorsal root ganglion (DRG) (the pseudounipolar neurons responsible for conveying sensory information from the periphery to the CNS) cultures resulted in a reduction of activated integrins [as determined by a decrease in phosphorylated focal adhesion kinase (FAK)] and growth inhibition. By adding manganese to the cultures, both FAK phosphorylation and growth response was restored, indicating that integrin activation is sufficient to override the inhibitory effects of CSPGs.

In addition to the above-mentioned "outside-in" signaling, the affinity with...
which the integrin binds its ligand can be regulated from within the cell ("inside-out" signaling). This process is important to make sure that an appropriate level of adhesiveness is achieved. "Inside-out" signaling is regulated by the activity of proteins that interact with the intracellular domain of the integrin and one such group of proteins is the kindlin family (kindlin-1, -2 and -3). Inspired by the discovery that kindlin-1 can rescue the phenotype of keratinocytes from patients with Kindler syndrome (a hereditary disease caused by mutation in the kindlin-1 gene, leading to skin blistering and photosensitivity), by enhancing integrin activation, Tan and coworkers overexpressed this molecule in DRG neurons. The outcome was an increase in integrin activation (as determined by staining with an antibody specific for activated integrins) and an increase in phosphorylation of FAK in axons. Interestingly, knockdown of kindlin-2 (which, in contrast to kindlin-1, is expressed in the nervous system) in DRG neurons did not affect axonal growth and neither did overexpression of this molecule in DRG neurons. The outcome was an increase in integrin activation (as determined by staining with an antibody specific for activated integrins) and an increase in phosphorylation of FAK in axons. Finally, to examine if kindlin-1 overexpression also increases regeneration in vivo, the authors utilized the dorsal root crush model. In this injury paradigm, regrowing axons from the DRG neurons are unable to enter the spinal cord because of inhibitory molecules at the dorsal root entry zone (Fig. 1A). Injection of virus vectors carrying kindlin-1 constructs into the DRG lead to ingrowth of axons into the dorsal horn and dorsal column of the spinal cord (Fig. 1B). Importantly, this treatment also led to functional recovery of thermal but not pressure sensation in the forepaw. The reason for this is currently unclear. Possible explanations could be that the virus vector affects different subpopulations of neurons within the DRG differently, or that pressure-sensing DRGs regenerate at a slower rate or that integrin expression is different in subpopulations of DRG neurons. On a similar note, it should be noted that the number of regenerating nerve fibers was quite low and whether this was due to low efficiency of the viral construct (no quantification of the percentage of successfully transfected neurons was presented) or if kindlin-1 overexpression by itself is not enough to induce more robust regeneration in vivo is currently not known.

**Too Much of a Good Thing, Are There Causes for Concern?**

The authors did not see any signs of pain-related side-effects (a condition that can be caused by aberrant neuronal sprouting and can be ameliorated by integrin antagonists) and no other side effects were reported. However, it is currently not known if a more robust growth of axons would induce side effects. Given the relatively low number of regenerating axons and that the authors did not examine if the observed growth of neurites came from any particular subtype (one would probably expect more pain-related side effects if nociceptive DRGs were stimulated to grow vigorously), it seems premature at current to conclude that the strategy presented by Tan and coworkers is free from side-effects.

Moreover, a number of previous studies have shown that integrin activation can have deleterious effects on recovery after CNS injury. Fibrinogen that leaks into the CNS from the bloodstream after trauma binds to neuronal integrin αVβ3, which in turn activates the epidermal growth factor receptor (EGFR) and leads to growth cone collapse. Further, integrin β1 can bind the growth-inhibitory molecule MAG, and using an in vitro growth-cone turning assay, results from the same study showed that β1 integrin is essential for the repulsive signal from MAG. However, if integrin β1 is also responsible for MAG-induced repulsion and/or growth cone collapse in vivo is currently not known. Finally, when neurons are presented with a...
favorable substrate (laminin), combined with increased integrin activation (manganese administration) and elevated intracellular cAMP (which has been shown to increase the regenerative capacity of several neuronal types), the outcome is not an additive effect on growth, but rather the opposite: the combination of these manipulations instead activates Rho and leads to growth cone collapse. This raises concern that “overloading” the neurons with positive enhancement might actually trigger regeneration failure.

Conclusion and Future Directions

Although previous studies have shown that overexpression or activation of integrins can increase neurite growth on permissive substrates, such as laminin, fibronectin and tenascin-C, the studies by Tan and coworkers provide important new proofs-of-concept that enhancing integrin function, both “outside-in” (manganese administration) and “inside-out” (kindlin-1 overexpression) can overcome CSPG-mediated inhibition. It has been argued that growth inhibition by CSPGs is not the most likely mechanism underlying the regenerative failure after dorsal root crush model used by Tan and coworkers: Di Maio and colleagues claim that axons instead stop growing and form non-functional pre-synaptic terminals in the spinal cord and Zhang and coworkers did not detect substantial expression of CSPGs in the DREZ. However, other studies have claimed that CSPGs at the DREZ are contributing to the failed regeneration after dorsal root injury.

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Future studies will have to be performed to elucidate if the strategies developed by Tan and coworkers also function in other injury paradigms and on other neuronal types such as corticospinal neurons, responsible for much of our voluntary movement and thus crucial for recovery of locomotion after spinal cord injury. Much work is still ahead, but given the heterogeneous and wide-spread expression of integrin subunits in the CNS, the studies by Tan and coworkers has presented us with new promising possibilities for neuronal regeneration.

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