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An integrin approach to axon regeneration

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Abstract

Axon regeneration in the CNS is blocked by inhibitory molecules in the environment and by a developmental loss of regenerative potential in CNS axons. Axon growth is a specialized form of cell migration, and for any cell to migrate there must be an adhesion molecule at the growth tip that recognizes a ligand in the environment, and which is linked to signaling and cytoskeletal mechanisms. The reasons for this loss of regenerative ability in CNS axons are several, but important contributors are the developmental loss of integrins that recognize ligands in the mature CNS environment, and selective trafficking of integrins and other molecules to exclude them from axons and direct them to dendrites. Regeneration of sensory axons in the spinal cord can be achieved by expression of tenascin-binding α9-integrin together with the integrin activator kindlin-1. This works because integrins are transported into sensory axons. Transport of integrins into retinal ganglion cell axons is seen in the retina, but may become more restricted in the optic nerve, with a subset of axons containing expressed integrins. Transduction of ganglion cells with α9-integrin and kindlin-1 should promote regeneration of this subset of axons, but attention to transport may be required for regeneration of the remaining axons.
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Axon growth is a specialized form of cell migration, but all forms of cell migration must obey the same biological rules. For any cell to migrate, it must have on its surface a receptor that can bind to a molecule in the surrounding environment, and this receptor must be linked to intracellular signaling pathways and to the dynamic cytoskeleton. The cell surface receptors are cycled on and off the surface by trafficking endosomes. Regenerating axons must penetrate the glial extracellular matrix (ECM), and the key receptors for ECM molecules are integrins. Within the adult CNS the main ECM glycoprotein is Tenascin-C (TN-C), which is upregulated at sites of injury in the brain, spinal cord, and optic nerve. The integrin that allows cells to migrate on TN-C is alpha9 beta1 (α9β1), and this integrin is expressed in the CNS during embryonic development but then downregulated in adulthood and not upregulated after injury. When we started to investigate this field, our first question was whether expression of α9β1-integrin in adult neurons would promote axon regeneration. We found that neurons transfected *in vitro* grew axons for long distances on TN-C, but when the same idea was tried *in vivo* there was much less regeneration. An adeno-associated virus (AAV) vector was used to transduce sensory neurons with α9β1 and either the spinal cord or the dorsal roots were cut, in both cases seeing only a modest increase in regeneration. The reason for the difference between *in vitro* and *in vivo* regeneration turned out to be because the major inhibitory molecules of the adult CNS (not present in the *in vitro* model) all inactivate integrins, so that they can no longer bind to TN-C and signal. Integrin activation is regulated by talin and kindlin, which bind to the intracellular tail of β1-integrin. The next step was therefore to overcome the inactivation of integrins by chondroitin sulphate proteoglycans (CSPGs) and NogoA by transducing neurons with kindlin-1 to prevent integrin inactivation.[1] This, together with transduction with α9β1 allowed the axons to overcome inhibition by both NogoA and CSPGs, and to regenerate for long distances in the spinal cord.[2] The C6 and C7 dorsal root ganglia were transduced with α9β1 and kindlin-1 and the roots crushed. Regenerating sensory axons were able to grow all the way up the spinal cord to the medulla, making connections in the correct layers of the dorsal horn along the way. This is an unprecedented degree of regeneration, and the

regenerated connections were functional, with animals recovering pain and touch sensation and sensory-motor function.

If extensive regeneration of sensory axons in the CNS is achievable by transducing them with the correct integrin and activating it, would this same method work for retinal ganglion cells (RGCs) and corticospinal (CST) neurons? A requirement is that the neurons can transport integrins and kindlin down their axons to the site of axotomy in order that they can participate in the regeneration and progression of the axon growth cone. For dorsal root ganglion (DRG) neurons this is not a problem, because almost all molecules expressed in the cell bodies appear to be transported down axons. CNS neurons however are partitioned into a somatodendritic domain and an axonal domain, and there is a selective transport mechanism to direct some molecules to dendrites, others to axons. For instance very few of the many molecules that make up postsynaptic specializations are transported into axons. Integrins, trks, integrin-like growth factor receptors are three key receptor types that are excluded from the axons of cortical neurons,[3–5] while kindlin is transported for long distances. RGCs have some ability to transport integrins: when RGCs were transduced with α9β1-V5 many of their axons contained integrin within the retina, but transport into the optic nerve was more limited, although some axons contained integrin. Moreover RGCs in adulthood in mice continue to express some integrins at a level greater than cortical neurons but less than DRG neurons. A study of integrins in adult RGCs *in vitro* revealed that several integrins are expressed, and that they are present in all the cell processes.[6]

From the above studies it is clear that transport and trafficking of growth-promoting molecules is a key issue in axon regeneration. We therefore worked out how integrins are transported into regenerating axons through studying adult DRG neurons. In these cells integrins are found particularly in recycling endosomes marked by the small GTPases Rab11 and Arf6.[7] This implies that that integrins enter axons by first being inserted into the cell membrane in the somatodendritic compartment, then they are recycled by transcytosis in recycling endosomes and transported down axons. The control of endosomal transport is complex, but it depends on the state of activation of Arf6 and Rab11, which are linked together with the transport adaptor JIP3: when Arf6 is activated JIP3 links to the retrograde motor dynein so that all the Arf6/Rab11 cargos travel retrogradely and are excluded from axons.[4,8] The state of activation of Arf6 is controlled particularly by the GEF Efa6, which together with Arf6 is the master regulator of transport direction. In sensory neurons, activation of Arf6 decreases the amount of integrin in axons through an increase in retrograde transport and this inhibits axon growth and regeneration. The situation in cortical neurons is different, because their axons contain integrins during their developmental growth phase, but as they mature integrins are progressively excluded from the axons and at the same time the axons lose the ability to regenerate. We analyzed the factors that exclude integrins from cortical axons, and as for many complex biological controls, there are several influences. Chief amongst these is the developmental upregulation of the Arf6 GEF Efa6, which activates Arf6 and causes integrin transport to become predominantly retrograde, excluding integrins from the axons and reducing levels at the axon tip. However, the axon initial segment and postsynthetic modifications of tubulin also play a part.[4] Knockdown of Efa6 in mature cortical neurons causes large changes, with the axon now becoming full of Rab11 vesicles that contain integrin. Importantly these axons now have an increased ability to regenerate when they are cut by a laser *in vitro.*

From these findings it is now possible to construct methods for stimulating axon regeneration in the CNS. For sensory axons the situation is straightforward. Expression of α9β1-integrin and kindlin-1 through viral vector delivery will introduce these molecules into the axons, and they are then able to regenerate in the spinal cord. For cortical neurons the requirements are more demanding, because expression of α9β1 only introduces integrin into the somatodendritic compartment. It is now known that preventing Arf6 activation will allow some integrin transport, but it may be necessary to add a further intervention to ensure both transport and signaling sufficient to allow long-distance regeneration. For RGC axons the situation is not so clear. RGCs are able to transport integrins into their proximal axons, but only a subset may be able to transport them down the optic nerve where they are needed for regeneration. The first need is for experiments to be performed to find out whether expression of α9β1 and kindlin-1 will stimulate regeneration in the same way as in the sensory system. If this is not successful it will be necessary to add a treatment to increase long-distance anterograde transport of the integrin into the RGC axons, probably via knockdown of Efa6. In addition increasing PIP3 by knockdown of PTEN or overexpression of PI3Kδ stimulates axon growth and also enhances integrin transport.

Overall, axon regeneration research has progressed from the time when the mechanisms governing regeneration were very mysterious to the present situation, where there are several interventions that can promote regeneration in predictable and comprehensible ways. Researchers can therefore be somewhat optimistic that long-distance regeneration in the spinal cord and optic nerve is achievable. The challenge will then move on

to the next step, which is ensuring that the regenerating axons are guided to the correct targets and form appropriate functional connections. For this aim there is some comfort to be drawn from the appropriate guidance and target-finding that we saw in sensory axons expressing α9β1-integrin and kindlin-1.

Conflict of interest

JF is a paid consultant of Acorda Therapeutics Inc.

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