Optic Nerve Regeneration

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Retinal ganglion cells are usually not able to regenerate their axons after optic nerve injury or degenerative disorders, resulting in lifelong visual loss. This situation can be partially reversed by activating the intrinsic growth state of retinal ganglion cells, maintaining their viability, and counteracting inhibitory signals in the extracellular environment. Advances during the past few years continue to extend the amount of regeneration that can be achieved in animal models. These findings give hope that clinically meaningful regeneration may become a reality within a few years if regenerating axons can be guided to their appropriate destinations.


As in most central nervous system pathways, axons injured in the mature optic nerve cannot grow back, leaving patients who have traumatic nerve injury or degenerative diseases (such as glaucoma) with lifelong vision loss. Researchers have long studied the optic nerve for insights into the causes of regenerative failure in the central nervous system, focusing on such issues as the inhibitory effects of central nervous system myelin and the glial scar, the absence of appropriate trophic factors, the immune response to injury, cell-death pathways, and the decline in the intrinsic growth capacity of neurons. The past 10 to 15 years have witnessed major advances in understanding the reasons retinal ganglion cells (RGCs) normally fail to regenerate injured axons through the optic nerve and devising ways to reverse this situation. These findings give hope that functional repair might be possible.

AXON REGENERATION THROUGH THE OPTIC NERVE

Under normal circumstances, damaged axons show a transient sprouting response after optic nerve injury but no long-distance regeneration. Tello, a student of Ramon y Cajal, discovered that if the optic nerve is cut and sutured to a segment of peripheral nerve (PN), axons will grow for considerable distances into the graft. Aguayo et al extended these findings to show that some RGCs can regenerate axons all the way through a PN graft that extends from the eye to the superior colliculus and form synapses in the correct retinal recipient layers of the colliculus.

The ability of RGCs to regenerate axons through a PN graft is likely to be related in part to higher levels of growth-permissive molecules (eg, laminin) and lower levels of growth-inhibitory molecules (eg, Nogo-A) in PNs compared with the optic nerve. However, it is also possible that the optic nerve and PNs differ in their ability to provide essential trophic factors. To test this latter possibility, Berry and colleagues implanted a fragment of PN into the vitreous humor and found that this procedure stimulated RGCs to regenerate lengthy axons beyond the site of an optic nerve crush injury. Although this growth was initially attributed to trophic factors derived from Schwann cells, the grafts contained numerous macrophages, which can enhance axon regeneration when...
preactivated and placed in the optic nerve.\textsuperscript{7,8} Other methods that induce intraocular inflammation (ie, injuring the lens or injecting the proinflammatory agent zymosan into the eye) stimulate even greater regeneration than that stimulated by PN implants (Figure, A and B).\textsuperscript{9,10,14} This regeneration is associated with a marked change in the intrinsic growth state of RGCs, as evidenced by a marked upregulation of proteins such as GAP-43 and SPRR1A.\textsuperscript{13} Although PN implants secrete ciliary neurotrophic factor (CNTF),\textsuperscript{15} their primary effect in vivo is related to other factors associated with macrophages.\textsuperscript{16}

**ONCOMODULIN AS A POTENT MACROPHAGE-DERIVED GROWTH FACTOR FOR RGCs**

Using dissociated retinal cell cultures as a bioassay, 2 molecules present in the eye were found to stimulate mature RGCs to regenerate their axons. One is mannose, a simple sugar that is abundant in the vitreous. Mannose stimulates RGCs to extend moderately long axons if cells have sufficiently high levels of intracellular cyclic adenosine monophosphate (cAMP).\textsuperscript{17} The second growth factor is oncomodulin (Ocm), a 12-kDa, calcium-binding protein secreted by macrophages. Oncomodulin accumulates rapidly in the eye after intravitalre inflammation and exhibits cAMP-dependent, high-affinity binding to a cell surface receptor on RGCs.\textsuperscript{11,12} When released from polymeric beads placed into the vitreous, Ocm plus a cAMP analogue induce nearly as much optic nerve axon regeneration as intraocular inflammation\textsuperscript{11} (Figure, C). Conversely, an Ocm peptide antagonist or a neutralizing anti-Ocm antibody markedly suppresses inflammation-induced regeneration (Figure, D).\textsuperscript{11} Thus, Ocm appears to mediate most of the effect of intravitreal inflammation on optic nerve regeneration. However, additional factors derived from inflammatory cells or retinal glia also appear to play a role by causing an elevation of intracellular cAMP and by enhancing RGC survival.\textsuperscript{12} One group failed to detect an elevation of Ocm in the eye after inflammation\textsuperscript{18} and reported that an anti-Ocm antibody did not diminish inflammation-induced regeneration.\textsuperscript{19} The likely sources of these discrepant results are discussed elsewhere.\textsuperscript{12,20} Intraocular inflammation also enhances the ability of RGCs to regenerate their axons through a PN graft,\textsuperscript{10,21} and this effect is likewise blocked by an Ocm antagonist peptide.\textsuperscript{12}

**ALTERING INTRACELLULAR SIGNALING CAN PROMOTE OPTIC NERVE REGENERATION**

The signaling pathways that enable RGCs to regenerate their axons are beginning to emerge. The purine-sensitive protein kinase Mst3b plays a central role in the signal transduction pathway through which trophic factors induce axon growth.\textsuperscript{22,23} Suppression of Mst3b expression blocks the axon-promoting effects of Ocm in culture and of inflammation-induced regeneration in vivo,\textsuperscript{23} whereas expression of a constitutively active form of Mst3b enables RGCs to regenerate axons even in the absence of growth factors.\textsuperscript{23} The effects of Ocm can also be blocked by an inhibitor of calmodulin kinases or by combining inhibitors of the phosphatidylinositol 3-kinase–Akt pathway,\textsuperscript{11} and Janus kinase–signal transducer and activator of transcription pathways.\textsuperscript{11} Conversely, deleting genes that encode suppressors of these pathways stimulates axon regeneration in vivo. Appreciable optic nerve regeneration can be stimulated by deleting the gene for phosphatase and tensin homolog (PTEN), a protein and lipid phosphatase that suppresses signaling through the phosphatidylinositol 3-kinase (PI3K)/protein kinase B (Akt) pathway,\textsuperscript{24} and, to a somewhat lesser extent, by deleting the gene encoding SOCS3, a protein that suppresses signaling through the Janus kinase–signal transducer and
activation of transcription pathway. Deletion of PTEN or SOCS3 leads to phosphorylation of the S6 kinase, implying that activation of the mammalian target of rapamycin pathway plays an important role.

The intrinsic growth capacity of RGCs decreases in the early postnatal period and is accompanied by changes in the expression of Kruppel-like family (KLF) transcription factors. Overexpression of KLF-4 suppresses axon growth in immature RGCs, whereas diminished KLF-4 expression increases axon growth in mature RGCs and promotes a modest amount of regeneration in vivo. It is not yet known whether the effects of intraocular inflammation, PTEN deletion, or SOCS3 deletion are mediated through changes in the expression of any KLF transcription factors or whether manipulating multiple KLF members at once might produce more marked regeneration.

THE ROLE OF cAMP IN OPTIC NERVE REGENERATION

The second messenger cAMP augments axon regeneration in multiple ways, including altering the response of growth cones to inhibitory signals, stimulating the translocation of growth factor receptors to the cell surface, and altering gene expression programs. The latter effects include downregulation of SOCS3 expression and upregulation of arginase I, an enzyme involved in the biosynthesis of polyamines, which enhance the ability of neurons to extend axons over inhibitory substrates. Spermidine stimulates a modest amount of optic nerve regeneration, as does elevation of cAMP. As noted herein, cAMP strongly enhances the effects of oncomodulin and increases the effects of intraocular inflammation. A peptide that prevents Ocm from binding to its receptor eliminates the latter effects, showing that Ocm is the principal factor involved in inflammation-induced regeneration and in the enhancement of this phenomenon by cAMP.

RGC SURVIVAL AFTER OPTIC NERVE INJURY

A few days after their axons are injured, RGCs begin to die, particularly if damage occurs close to the eye. This death can be prevented almost completely by overexpressing the antiapoptotic Bcl family proteins Bcl-2 or Bcl-xL in RGCs. However, although axon regeneration clearly requires RGCs to remain viable, axon outgrowth and cell survival use different intracellular signaling pathways. This dissociation is exemplified by the failure of RGCs overexpressing Bcl-2 or Bcl-xL to regenerate axons without additional growth factors and by the persistent enhancement of RGC survival seen after intraocular inflammation even when regeneration is suppressed by Ocm-blocking reagents.

The death of RGCs can be slowed but not stopped with a number of trophic factors, including CNTF, BDNF, neurotrophin 3, BDNF, and neurotrophin 4/5 do not, although a combination of fibroblast growth factor 2, neurotrophin 3, and nerve growth factor has been reported to induce substantial regeneration. One group has argued that CNTF mediates the effects of intravitreal inflammation on optic nerve regeneration. This observation is based primarily on the outgrowth seen using concentrations of CNTF several orders of magnitude above the established median effective dose in culture and on the loss of axon regeneration and RGC survival seen when the genes encoding CNTF and leukemia inhibitory factor are deleted. However, physiologically relevant concentrations of CNTF do not promote strong regeneration in culture, and many laboratories have failed to find strong effects of CNTF in vivo. In addition, CNTF inhibitors have no effect or only a mild effect on inflammation-induced regeneration. Although CNTF enhances axon regeneration through a P38 MAPK-dependent pathway, this effect is associated with the proinflammatory effects of high CNTF concentrations and is eliminated when inflammation is suppressed. Thus, the direct effect of CNTF on optic nerve regeneration is weak, although it may contribute to maintaining RGC survival. The axon-promoting effect of CNTF becomes strong when the gene that encodes SOCS3, the negative regulator of the Janus kinase-signal transducer and activator of transcription pathway, is deleted.

GROWTH-INHIBITORY SIGNALS IN THE OPTIC NERVE

The mature optic nerve contains many molecules that suppress axon growth, including the myelin-associated inhibitors Nogo-A, myelin-associated glycoprotein, and oligodendrocyte-myelin glycoprotein; proteoglycans that accumulate in the scar at the injury site; and additional axon repellants (eg, semaphorins). Methods that counteract Nogo-A signaling do not lead to appreciable optic nerve regeneration, it has additive effects on survival, although the combination of BDNF and intraocular inflammation suppresses axon regeneration.

The death of axotomized RGCs can be slowed by preventing caspase cleavage, blocking the nuclear enzyme poly(adenosine diphosphate-ribose) polymerase (a substrate for caspases), blocking nitric oxide synthase, and eliminating reducing agents, or inhibiting cell death via caspase-independent pathways. Long-term prevention of RGC death after axotomy may require the development of long-term delivery systems or a combination of treatments.

EFFECTS OF OTHER TROPHIC FACTORS ON OPTIC NERVE REGENERATION

Fibroblast growth factor 2 stimulates some axon regeneration through the optic nerve. However, nerve growth factor, neurotrophin 3, BDNF, and neurotrophin 4/5 do not, although a combination of fibroblast growth factor, neurotrophin 3, and nerve growth factor has been reported to induce substantial regeneration. One group has argued that CNTF mediates the effects of intraocular inflammation on optic nerve regeneration. This observation is based primarily on the outgrowth seen using concentrations of CNTF several orders of magnitude above the established median effective dose in culture and on the loss of axon regeneration and RGC survival seen when the genes encoding CNTF and leukemia inhibitory factor are deleted. However, physiologically relevant concentrations of CNTF do not promote strong regeneration in culture, and many laboratories have failed to find strong effects of CNTF in vivo. In addition, CNTF inhibitors have no effect or only a mild effect on inflammation-induced regeneration. Although CNTF enhances axon regeneration through a P38 MAPK-dependent pathway, this effect is associated with the proinflammatory effects of high CNTF concentrations and is eliminated when inflammation is suppressed. Thus, the direct effect of CNTF on optic nerve regeneration is weak, although it may contribute to maintaining RGC survival. The axon-promoting effect of CNTF becomes strong when the gene that encodes SOCS3, the negative regulator of the Janus kinase-signal transducer and activator of transcription pathway, is deleted.
regeneration on their own. However, expression of a dominant-negative form of the Nogo receptor strongly amplifies the axon-promoting effects of intraocular inflammation. A more comprehensive way to counteract inhibition is by inactivating the small guanosine triphosphate hydrolase Ras homolog member A (RhoA), a part of the intracellular pathway through which multiple signals inhibit axon growth. Inhibition of RhoA results in modest levels of axon regeneration in the injured optic nerve but greatly increases the amount of regeneration associated with intraocular inflammation (Figure, E). Thus, although counteracting inhibitory signals is not sufficient to induce extensive optic nerve regeneration, treatments that simultaneously activate the growth state of RGCs and counteract inhibition can have notable effects.

Transforming RGCs into an active growth state enables axons to partially overcome inhibitory signals. The scar that forms at the injury site contains basement membrane components that are partially degraded by matrix metalloproteinases associated with growing axons. However, as noted herein, multiple other inhibitory signals remain in place, as evidenced by the notable effects seen when RhoA activity is suppressed in actively growing axons.

AXON GUIDANCE CUES DURING DEVELOPMENT AND REGENERATION

The initial development of retinal projections involves multiple cues that guide axons through the retina, optic disc, optic nerve, optic chiasm, diencephalon, and midbrain and enable them to form a topographically organized representation of visual space on central target areas. The guidance of retinal axons during development involves many types of axon-guidance molecules, including netrins, semaphorins, laminin, multiple members of the ephrtyropoietin-producing hepatocellular (Eph) receptor/Eph receptor–interacting protein (ephrin) families, the developmental protein Wnt, and slits. Because of the complex guidance mechanisms involved in the development of retinal projections, it will be important to determine whether the appropriate guidance cues are expressed in the mature brain to guide regenerating axons back to their correct destinations. There is evidence that at least some guidance cues remain in the mature central nervous system or become upregulated after optic nerve damage. However, whether these cues will suffice to guide regenerating axons to their proper target areas and re-form topographically organized maps remains to be determined.

ARE WE THERE YET?

In studies using the PN graft model, anterograde tracing and electrophysiologic responses reveal that a small number of axons can regenerate all the way back to the superior colliculus. In the case of axon regeneration through the optic nerve, one group reported a remapping of the retina on the superior colliculus, but the accompanying histologic findings raised questions as to whether the axons had been severed in the first place. Because of the scientific and clinical importance of successful regeneration, it will be important to apply strict criteria to proving one’s case, eg, showing that connections are forming gradually as time passes and are not owing to spared axons, and that any observed electrophysiologic changes correlate with clear anatomical evidence of regeneration. Despite these issues, the advances that have occurred during the past few years give hope for the possibility that at least some RGCs will be able to regenerate their axons all the way to their central targets. The next challenges will include finding ways to optimize this regeneration and testing whether they restore functionally meaningful levels of vision.

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