

Optic Nerve Regeneration: Science and Progress

Research is shedding light on the degeneration of retinal ganglion cells and suggests possible means toward their recovery.

BY JEFFREY L. GOLDBERG, MD, PhD

Early in glaucoma, the axons of retinal ganglion cells (RGCs) are likely injured in the optic nerve.^{1,2} Their failure to recover or regenerate after such an insult leads to the irreversible loss of vision that is typical of glaucoma and, ultimately, to the RGCs' death.³ Why do RGCs fail to regenerate their axons through the optic nerve and back to their targets in the brain? Significant progress has been made toward understanding regenerative failure,⁴ and a number of approaches successful in animal models of optic nerve injury should be able to leap from the laboratory to the clinic. This article reviews a few principles underlying regenerative failure and their potential application to patients with glaucoma.

NEUROTROPHIC SIGNALS FOR AXONAL GROWTH

Neurotrophic factors are proteins that support RGCs' survival, axonal growth, and synaptic connectivity, both during development and throughout adult life. Some neurotrophic factors are made locally in the retina, and others come from RGCs' targets in the brain or from the glial cells in the optic nerve itself. Optic nerve injury disrupts connections between the RGCs' axons and the brain, resulting in a loss of target-derived neurotrophic factors that would normally be transported back to RGC bodies in the retina and help support the RGCs' function.

The delivery of a number of different neurotrophic factors has been shown to increase RGCs' survival and regeneration.⁵ For example, after optic nerve injury, intravitreal injections of brain-derived neurotrophic factor (BDNF), ciliary neurotrophic factor (CNTF), glial-derived neurotrophic factor (GDNF), neurotrophin 4/5

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(NT-4/5), nerve growth factor (NGF), or insulin-like growth factor 1 (IGF-1) at least temporarily increase RGCs' survival and enhance their axons' regeneration in the optic nerve.⁶⁻⁹ More recently discovered molecules like oncomodulin, which is expressed by macrophages that migrate into the retina after injury to the crystalline lens, also demonstrate efficacy in stimulating optic nerve regeneration in animal models.^{10,11} Could injuring the lens be a viable approach to treating glaucoma? The visual tradeoff between enhanced RGC function and cataract formation makes the molecular approach considerably more palatable. A number of neurotrophic factors have already been tested in other human neurodegenerative diseases, but problems with these proteins' delivery to the brain have limited their efficacy. Testing of neurotrophic factors for retinal neuroprotection has just begun in humans. The proteins' delivery to the eye using encapsulated cell technology, intravitreal micro- or nanoparticle carriers, or eye drops may increase the chance of therapeutic success in glaucoma.¹²⁻¹⁴

It remains unclear whether neurotrophic factors alone can elicit optic nerve regeneration. Shortly after optic nerve injury in animal models, RGCs' ability to respond to neurotrophic factors becomes minimal.¹⁵ Fortunately, this trophic responsiveness can be restored, and axonal growth can be enhanced by increasing the expression of

trophic factor receptors present on the surface of RGCs by gene therapy,¹⁶ elevating RGCs' intracellular cyclic-AMP (cAMP) levels pharmacologically, or electrically stimulating RGCs.^{15,17} In animal models—and likely in human glaucoma—RGCs are less electrically active after optic nerve injury.¹⁸ These findings suggest a two-pronged therapeutic approach to deliver neurotrophic factors in combination with electrical stimulation or cAMP elevation.¹⁷

OVERCOMING THE OPTIC NERVE'S INHIBITION

Developmentally, the optic nerve is an outgrowth of the forebrain, and like the rest of the central nervous system, it actively inhibits axonal regeneration in the adult. Bypassing the inhibitory optic nerve with peripheral nerve grafts,^{19,20} perinatal optic nerves,²¹ or various bridge matrices^{22–25} may provide a surgical approach to enhancing axonal regrowth to the brain. A more ele-

gant molecular approach, however, may be a more achievable goal.

Optic nerve cells (including meningeal cells, microglia, oligodendrocytes, and astrocytes) express a number of inhibitory molecules and proteins and thus create an unfavorable environment for optic nerve regeneration after injury.²⁶ In animal models, several approaches have proven useful in overcoming these inhibitory molecules. For example, treatment with a bacterial enzyme, chondroitinase ABC, can degrade inhibitory chondroitin sulfate proteoglycans (CSPGs),²⁷ and antibodies or peptides can block RGCs' response to other inhibitory proteins like Nogo.²⁸ The treatment of spinal cord injury with anti-Nogo antibodies has entered clinical trials in Europe and, if successful, should be followed by optic nerve regeneration clinical trials. Inside RGCs' axons, many inhibitory signals in the optic nerve activate signaling molecules, including Rho, the epidermal growth factor receptor (EGFR) and protein kinase C (PKC). Blocking the activity

DISCUSSION

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Blindness remains the devastating outcome of progressive optic nerve degeneration in the patients most severely affected by glaucoma. Because no current treatments can restore vision lost to this disease, the effective regeneration of the optic nerve remains a key challenge for glaucoma research. The article by Jeffrey Goldberg, MD, PhD, elegantly highlights some of the major issues involved in trying to restore the optic nerve and describes a number of approaches that may help us along this difficult route.

An important barrier to the optic nerve's recovery is the limited regenerative capacity of retinal ganglion cells (RGCs) in the adult human eye. Interventions such as the supplementation of neurotrophic factors, as discussed by Dr. Goldberg, can enhance the survival and recovery of the axons of a small proportion of RGCs immediately after acute optic nerve injury. Their ability to facilitate regeneration (as opposed to neuroprotection) in a chronic disease such as glaucoma, however, is far less clear. Thus, Dr. Goldberg's suspicions are likely correct that neurotrophic factors alone are unlikely to be the answer and that combined approaches should be explored. To this end, the emerging literature on the effects of electrical stimulation on RGCs' regeneration is of particular interest, and further work in this area is eagerly awaited.

The inhibitory nature of the optic nerve environment for regenerating axons is another obstacle, but perhaps here the route to progress is clearer. The inhibitory mechanisms involved are being elucidated successfully, and therapeutic interventions using treatments such as chondroitinase have

shown benefits in multiple different disease models.¹² The glaucoma community awaits the clinical trial results with interest.

Arguably, the most formidable hurdle of all is the re-establishment of functional connections between regenerating axons and the visual centers in the brain. Pioneering work by Aguayo and colleagues in the 1980s demonstrated that RGCs could regenerate through peripheral nerve conduits to re-establish the pupillary light reflex after transection of the optic nerve.³ Reformation of the exquisitely precise retinotopic map, which allows us to interact with our visual world, however, is a challenge on a different scale. Are the necessary axon-guiding signals still present in eyes with advanced glaucoma? If not, can they be reactivated or mimicked? Given the magnitude of the clinical need, this voyage into the unknown remains vital.

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of any of these or their downstream effectors increases regeneration,²⁹⁻³¹ more so when combined with CNTF and cAMP elevation.³² A number of drugs that undo these inhibitory responses of RGCs and other neurons in the central nervous system are now in clinical trials for spinal cord injury. The identification of targets of inhibitory signaling that are farther downstream will create more specific therapeutic strategies for future studies.

The immune system also interacts closely with RGCs and optic nerve glia in optic neuropathies such as glaucoma, and it has recently been the subject of clinical trials. For example, copolymer 1 (Cop-1) is a drug presently used to treat multiple sclerosis, and it may positively activate the immune system to decrease the degeneration of RGCs after optic nerve injury.³³ Clinical trials using Cop-1 (and others transplanting autologous activated macrophages into the injured spinal cord) will begin to address the role of the immune system in enhancing RGCs' regeneration.³⁴

ADDRESSING RGCs' INTRINSIC REGENERATIVE CAPACITY

During development, RGCs turn off their intrinsic capacity for rapid axonal growth.³⁵ Can RGCs' regenerative ability revert to embryonic levels? A search for developmentally regulated molecules that might control the ability of RGCs' axons to grow has yielded a number of promising targets, including the following:

- cAMP (discussed earlier)³⁶
- the mammalian target of rapamycin (mTOR) protein and its regulators
- phosphatase and tensin homolog (PTEN) and tuberous sclerosis complex 1 (TSC1)³⁷
- the ubiquitin ligase Cdh1-anaphase promoting complex (Cdh1-APC) and its regulators³⁸
- a family of transcription factors called *Krüppel-like factors* (KLFs) that change their expression through RGC development and regulate the growth of RGCs' axons³⁹

The discovery of *Krüppel-like factors* is the most recent. Blocking the expression of even one (ie, KLF4) in RGCs increases the growth of their axons in culture and, more importantly, increases the regeneration of these cells' axons after optic nerve injury.³⁹ Manipulating RGCs, either through gene therapy or with small-molecule drugs developed to target these proteins, represents an exciting new approach to enhancing RGC regeneration in diseases like glaucoma.

RECONNECTING RGCs' AXONS TO THEIR PROPER TARGETS

After enhancing the regeneration of RGCs through the injured optic nerve, will their axons find the lateral geniculate nucleus and other targets in the brain and re-create

functional vision? RGCs' axons will have to be guided back along the visual pathways to their proper targets, either through the re-expression of the cues they used during development⁴⁰ or with tissue grafts to direct them artificially.⁴¹⁻⁴³ In either case, preliminary data suggest that regenerating axons can reinnervate their targets if given the chance to reach them.⁴⁴ Thus, although the prospect of rebuilding the complex circuitry is daunting, even a small set of reconnections may restore some level of functional vision.

CONCLUSION

Today, vision lost due to glaucoma is gone permanently, but researchers have discovered a large number of targets for improving optic nerve regeneration. Success in enhancing the growth and regeneration of RGCs' axons in animal models of glaucoma and other optic nerve injuries has prompted the design of human clinical trials in the optic nerve. Moreover, analogous trials in the spinal cord will be followed closely by trials in optic neuropathies likely applicable to glaucoma. Thanks to a significant number of new targets and technologies, the prospect of real hope for patients with optic nerve disease draws closer. □

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