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The Cell and Molecular Biology of Glaucoma: Axonopathy and the Brain

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Many ophthalmologists and members of the lay public alike view glaucoma in its historical context—that is, as exclusively a disease of the eye. This view has been (and remains) overwhelmingly pervasive because the etiology of the disease involves so many factors associated with the eye: intraocular pressure (IOP), corneal thickness, optic disc morphology, and so on. Glaucoma is associated with sensitivity to IOP, and lowering IOP through either topical application of hypotensive drugs or surgery is the only form of treatment. Studies of animal models of glaucoma generally incorporate elevations in IOP as a way of mimicking the sort of injury that seems relevant to the human disease. However, although managing IOP can slow disease progression, it is not a cure, and vision loss in glaucoma often continues despite hypotensive regimens.¹

What is most damaging about glaucoma occurs outside of the eye, with degeneration of the optic nerve and its connections to the brain. This process involves the progressive loss of retinal ganglion cell (RGC) axons, some 1.5 million of which comprise the optic nerve in humans. Smaller eyes in experimental models have correspondingly fewer axons. Axon loss is followed eventually by apoptotic elimination of the RGC soma population in the retina. The rate at which degeneration progresses depends on many factors, including treatment efficacy. In the worst case, the loss of RGC neurons and their connections in the optic projection represents a substantial injury to the brain. Some 50% to 60% of the cerebral cortex spread over 40 to 45 distinct visual areas processes information from the retina via the optic nerve. In this sense, glaucoma is the premier age-related optic neuropathy and is becoming more prevalent as the population ages. Since the retina and optic nerve are part of the central nervous system, they lack the limited but intrinsic capacity of peripheral nervous system neurons for self-repair. Thus, identifying new neurocentric therapies for glaucoma is important, not only for preserving vision as the population ages, but also for translation to new treatments for other age-related brain diseases such as Alzheimer's and Parkinson's

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disease. Much of what we learn in glaucoma research from a neurobiological standpoint is now informing research for these other devastating conditions.²

Compartmentalized Neurodegeneration in Glaucoma

Early studies of animal models of glaucoma naturally focused on outcome measures after induced injury. From these, we understand that the critical end-stages of RGC degeneration in glaucoma involve caspase-dependent apoptotic cascades.³ Unfortunately, such knowledge has not led to a clear path for neuroprotective interventions. Apoptosis is a complex process defined by a multitude of interlacing and redundant components. Focusing on arresting apoptosis pharmacologically, as one would for the human disease, is a highly daunting proposition. When glaucomatous vision loss is detected in the clinic, our hope is that the underlying neurologic processes that are at work early in pathogenesis can be abated. This window of opportunity has not been adequately defined, mostly because our understanding of the earliest degenerative events in glaucoma is still developing.

In recent years, evidence has emerged that supports the hypothesis that distinct programs in the retina and in the retinal projection to the brain underlie the progression of RGC degeneration in glaucoma. Like other neurons, RGCs are compartmentalized into dendritic, somatic, and axonal units defined by a unique set of functional and morphologic characteristics. As is becoming true of glaucoma, it is often convenient to approach neurodegeneration from this vantage point and to break down separate events in progression by compartment. However, one must bear in mind that, like all other neurons, each RGC forms a functional unit for collecting information (dendritic arbor), integrating it (cell body), and transmitting the result to postsynaptic targets (axons). Although each compartment may present distinct elements as parts of pathogenesis, the compartments are likely to interact in complex ways that render true *independence* among compartments an empirical construct defined only by a narrow set of outcome measures.

Notwithstanding this caveat, it is useful to categorize outcomes into specific compartments and examine the extent to which contributing mechanisms are specific as well. In terms of the neurodegeneration in glaucoma, the concept of compartmentalization has been applied most thoroughly in the context of RGC axonopathy.² The question to consider in what follows is whether evidence supports moving from considering *axonopathy in glaucoma* to considering *glaucoma an axonopathy*.

Axonopathy Occurs Early and Can Be Independent of Cell Body Loss

Whatever the early events are that precipitate RGC degeneration in human glaucoma, as in animal studies, they eventually diminish the population of RGC somas in the retina through apoptosis.⁴ Since glaucoma is a progressive disease, this final outcome is not surprising, as opposed to an acute injury resulting in necrotic loss of tissue. However, even in animal models in which progression is compressed into weeks and months rather than years and decades, apoptosis is no longer viewed necessarily as the most meaningful outcome, because it occurs relatively late.

One of the most common techniques used to measure RGC survival is to inject a neuronal tracer into one or more retinal projection sites in the brain (for example, the lateral geniculate nucleus or superior colliculus) and quantify labeled cell bodies in the retina at some point farther down the road. However, these assays can confound cell loss with diminished retrograde axonal transport and/or axon survival. If axons are challenged early, and this is increasingly thought to be the case, then lack of retrograde-labeled RGCs does not necessarily indicate that they are absent. We have shown in the DBA/2J mouse model of pigmentary glaucoma that RGC somas labeled by neuronal markers persist long after retrograde transport is depleted and axon degeneration in the nerve has set in.⁵ These data suggest that axons can be damaged without loss of cell bodies in the retina. Similarly, deletion of the proapoptotic gene *Bax* in the DBA/2J has a protective effect on the cell body, but does not prevent RGC axon loss in the nerve.⁶

How early are axons damaged in glaucoma? By injecting tracers into the eye that require active uptake and anterograde transport to visualize the RGC projections to the brain, we have been able to quantify the progression of physiological deficits in axon function. In the DBA/2J, anterograde transport is challenged very early compared with retrograde transport from the brain to the eye—even without significant exposure to elevated IOP.⁷ Indeed, age seems to be the most relevant stressor to axonal transport. With increasing age, less and less elevation is necessary to challenge axons. The same is true in an acute model (microbead occlusion).⁷

Measurements of the progression of loss of transport tell us something else about glaucoma as well. In rodents, the primary target in the brain for RGC axons leaving the optic nerve is the superior colliculus of the thalamus. In primates (such as human beings), this role is played by the lateral geniculate nucleus. It is important to note that the spatial organization of the retina (which part of the retina encodes each part of the visual world) is conserved in the mapping of RGC axons to the colliculus. That is to say there is a perfect retinotopic representation of the visual world in the colliculus. In glaucoma, vision loss typically (but not always) progresses in a particular retinotopic manner, depleting one sector completely before moving on to the next. Furthermore, deficits within a sector generally progress from the peripheral edge toward the central retina where the number of RGCs is the greatest. Similarly, in animal models, deficits in anterograde transport to the colliculus progress sectorially and from the edge of the visual field toward the central region of highest RGC density.⁷ This finding lends credence to the idea that among the earliest events in pathogenesis is a distal loss of axonal function that contributes to actual vision loss (Fig. 1).

Persistence of the Neural Substrate

Results from the DBA/2J mouse indicate that RGC somas persist in the retina well after significant axonal degeneration in the optic nerve.⁵ Similarly, in an acute rat model of laser-induced ocular hypertension, we found a similar progression of degenerative outcomes: deficits axon transport deficits, followed by optic nerve damage, followed by RGC soma loss.⁹ This distal (brain) to proximal (retina) progression applies to both anterograde transport loss in the brain and axon degeneration in the optic nerve.^{7,10} Just as the retinal substrate (cell body) persists after axonopathy has progressed significantly, we found that

RGC axon terminals and synapses persist for a time at distal targets in the brain, even after axonal transport is completely depleted.⁷

The upshot of quantitative assessment of progression is that, between loss of functional axonal transport and actual degeneration of the neural substrate, there appears to be a window of opportunity. We know that, eventually, RGC projection sites degenerate with loss of postsynaptic neurons in thalamic relay nuclei.¹¹ Recent animal studies have shown that this transneuronal degeneration occurs relatively late in progression, at least after failure of the RGC optic projection.⁷ The question becomes whether early intervention can prevent progression. A recent study demonstrated that systemic delivery of the α_2 -adrenergic receptor agonist brimonidine (a common hypotensive agent), although it does not reduce elevated IOP, prevents RGC soma loss in the retina and axonal loss in the nerve and reduces deficits in anterograde transport.⁹ These findings make sense, since loss of anterograde transport began earliest and was the most severely affected outcome measure described in the study. It is possible that transport could have been rescued completely had brimonidine been delivered either at a higher dose, prophylactically, or more directly to the retina and nerve. If so, promise for neuroprotective therapies in the human disease may rest on identifying individuals not only most susceptible to the disease, but also at greatest risk for nonresponse to hypotensive regimens.

Understanding Axonopathy: Key Needs and Opportunities

Should we consider glaucoma an axonopathy? At the very least, axonopathy is an early event that shows signs of promise as a therapeutic target.^{6,7,9} Confounding the question of whether axonopathy is truly an independent compartment in pathogenesis is the nature of the initiating events. That the optic nerve head is an important site of early injury is established, but that does not preclude mechanisms in the retina (dendritic or somatic) or even in the brain from participating early in progression. Focusing exclusively on the nerve head in attempting to understand how disease-related stressors are transduced pathogenically carries the risk of missing possible neuroprotective targets along the retinal axonal–glial–vascular axis. We have discussed elsewhere the evidence of contributions from calcium-dependent intracellular and extracellular cascades.⁸ These cascades and others like them are likely to form a complex network of feedforward and feedback response pathways that, when stressed beyond a certain point, are likely to facilitate degeneration within all compartments, including the unmyelinated axon in the retina. A key need is the application of emerging tools, such as proteomics and metabolomics, that are sufficiently sensitive to identify the molecular signature of the transition from ocular etiology (e.g., IOP and scleral mechanics) to the beginnings of axonopathy and vision loss.

Age is a critical risk factor for glaucoma and certainly influences the susceptibility of the RGC projection to IOP-related stress.⁷ Clearly, IOP-dependent mechanisms associated with glaucoma take place against a backdrop of age-dependent changes in the structure and function of the RGC projection.¹² These changes are likely to involve not only the RGC, but ultimately the vasculature and glial cell intermediaries, both in the retina and throughout the projection. Given the increasing prevalence of age-related sensory loss and degenerative disorders, the lack of systematic investigations of how the visual system ages at the

molecular level is difficult to understand. Through animal models, stressors associated with age can be teased from those associated with IOP and other risk factors and presents an important opportunity for probing how genetic, environmental, and metabolic factors tip the balance between normal aging and glaucomatous neurodegeneration.

To translate mechanistic studies of early axonopathy into meaningful clinical applications, we need new and more sensitive means of detecting loss of function before the neural substrate becomes irreversibly damaged. Noninvasive imaging of the retina and nerve head through modalities such as optical coherence tomography has demonstrated great utility in establishing structure–function relationships in glaucoma and other optic neuropathies. However, to image distal sites in the optic projection necessitates a shift to new tools. Although magnetic resonance imaging (MRI) has been used with some success in imaging the optic projection,¹³ clearly a functional MRI protocol is needed to detect loss of axon function early. The use of manganese as a contrast agent in MRI is promising, since it is an analog of Ca^{2+} and has been used as a measure of changes in neuronal activity in models of glaucoma.^{14,15} It is our hope that by leveraging cross-disciplinary consortia of investigators, such modalities can be brought to practical fruition in the clinic.

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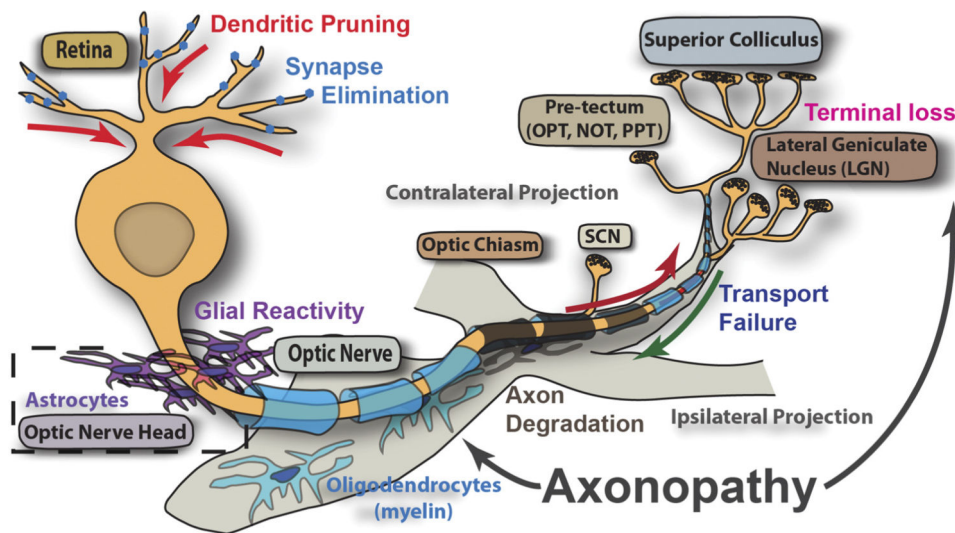


Figure 1.

Compartmentalized RGC degeneration in glaucoma. The axon of the RGC passes unmyelinated through the astrocyte-rich optic nerve head, an established site of vulnerability exhibiting glaucoma-related stressors such as glial reactivity. A contralateral projection is shown depicting the axon passing through the optic chiasm with terminals targeting the superior colliculus and other more anterior sites. These include the suprachiasmatic nucleus (SCN), lateral geniculate nucleus (LGN), and the pretectal nuclei: olivary pretectal (OPT), the nucleus of the optic tract (NOT), and the posterior pretectal (PPT). Degenerative events in glaucomatous axonopathy include failure of axonal transport, both anterograde (red arrow) and later retrograde (green arrow).

RGC axon terminals and their synapses degrade later. Later, degeneration in the retina includes loss of excitatory synapses, dendritic pruning, and finally cell body loss (modified from Crish and Calkins⁸).