Progression Under the Microscope – Can Basic Science Provide the Link Between Structural and Functional Deterioration?

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Abstract

The mechanisms of progression in glaucoma remain incompletely understood, but basic science research is gradually elucidating the processes involved. Axonal injury is a major event in glaucoma and the cellular mechanisms involved in axonal degeneration are different from those that lead to retinal ganglion cell body death. Thus, preventing cell body death is necessary but not sufficient to prevent progressive loss of function in glaucoma, and axonal protection is an important therapeutic goal. Basic science approaches are also suggesting new ways to detect and monitor progression. Techniques to image individual retinal ganglion cells and their axons in the living eye are improving rapidly. Cells undergoing apoptosis can be labelled and identified, and it may be possible in the future to use retinal ganglion cell apoptosis rates as a biomarker of glaucoma progression. However, slow progression may be difficult to detect using such techniques; therefore, biomarkers that identify ganglion cells that are sick but not yet committed to die are an important research goal.

Keywords

Glaucoma, neuroprotection, apoptosis, high-resolution imaging, Wallerian degeneration, axoprotection

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Mechanisms of Progression in Glaucoma

Glaucoma is a neurodegenerative disease characterised by progressive loss of retinal ganglion cells (RGCs) over time with associated progressive visual field loss. Elevated intraocular pressure is the strongest risk factor for glaucoma,[1] but the mechanisms by which damage occurs in the disease remain incompletely understood; however, a number of important principles have been established. First, there is strong evidence that the optic nerve head is an important site of injury. Characteristic optic nerve changes are observed in glaucoma and correlate with the pattern of visual field loss. Glaucomatous visual field defects often pertain to the horizontal mid-line and such a pattern would be difficult to explain if the site of injury were predominantly to the RGC bodies in the retina. The arcuate nature of many field defects observed in glaucoma also suggests the optic nerve head as the site of injury. In addition to such clinical evidence, recent basic science studies have pointed to the lamina cribrosa region of the optic nerve as a major site of injury in the disease.

Groundbreaking work by Howell et al. using mice with hereditary chronic glaucoma (the DBA/2J strain) provided experimental evidence for a focal insult to optic nerve axons at the glial lamina, the rodent analogue of the lamina cribrosa.[2] Damaged axons at the glial lamina appeared dystrophic compared with other portions of the optic nerve in the early stages of DBA/2J glaucoma. The exact nature of the insult experienced by RGC axons at the optic nerve head is unclear, but oxidative stress,[3] mitochondrial dysfunction[4,5] and failure of glutamate homeostasis[6] have all been implicated; other mechanisms are also likely to be involved.

However, although there is strong evidence that the optic nerve head is an important site of injury in glaucoma, this does not preclude the possibility that glaucomatous neurodegeneration may be compounded by factors affecting other RGC compartments. Numerous other mechanisms can potentially affect RGC survival, including toxic and ischaemic injuries at the level of the retina and secondary degeneration, where RGCs not directly injured degenerate later as a consequence of neighbouring cell death.[7] Thus, progression in glaucoma is likely to involve a complex interaction between RGC injury at multiple locations, with the optic nerve head probably most significant; the individual's susceptibility and compensatory responses to glaucomatous stress are also important.

Protecting All Compartments of Retinal Ganglion Cells Is Essential to Prevent Progression

In order to preserve function in glaucoma, it is necessary – but not sufficient – to keep RGC bodies alive in the retina. Maintenance of visual function requires preservation of all compartments of the RGC including the axon, dendrites and distal synapses.[8] Only if all components of the cell survive and continue to function can vision be preserved. However, it is becoming clear that the therapeutic strategies required to protect different compartments may differ. As an example, RGC bodies in the retina are known to die by apoptosis,[9]

which is an active cell death programme that involves a cascade of events that lead to the orderly demise of a cell. In transgenic mice with hereditary glaucoma that also lack *Bax*, a major component of the apoptotic pathway, RGC loss from the retina is greatly reduced, but axonal degeneration continues.[10] Thus, blocking apoptosis is insufficient to prevent RGC axonal degeneration in glaucoma, and therapeutic strategies to protect axons are potentially worth exploring for their ability to alleviate glaucoma progression.

Axoprotection

In order to protect axons it is necessary to understand the mechanisms by which they degenerate. Axonal degeneration can occur by several mechanisms, but Wallerian degeneration frequently occurs, especially following major axonal injury. Wallerian degeneration is an active process that leads to the orderly breakdown of axons in response to injury, and mutations that block this process can enhance axonal survival. The best characterised example mutation that reduces Wallerian degeneration is the slow Wallerian degeneration mutation (*Wld^S*). *Wld^S* arose spontaneously in mice, where it delayed injury-induced degeneration of distal axon stumps by several weeks in both the peripheral and central nervous system.[11] No other mutation is known to protect axons so robustly from Wallerian degeneration, and several studies have demonstrated that WldS protein expression delays axonal degeneration in experimental glaucoma.[2,12] We found that WldS delayed axonal degeneration in experimental rat glaucoma for at least two weeks. The duration of axonal protection was similar to that after optic nerve transection and crush, providing further evidence that axonal degeneration in glaucoma follows a Wallerian-like mechanism.[12] Axonal degeneration must be prevented to maintain RGC function; therefore, pharmacologically mimicking and enhancing the protective mechanism of WldS could offer an important route towards therapy. However, WldS did not protect RGC bodies in glaucoma or after other types of optic nerve injury, suggesting that combined treatments to protect both axons and cell bodies may offer the best therapeutic prospects.

Although Wallerian degeneration is the end result of severe axonal injury, lesser insults can also have an effect on axonal function. As an example, axonal transport blockade occurs soon after elevation of intraocular pressure in glaucoma models.[13,14] Axonal transport is essential for normal RGC function and is mediated by motor proteins, such as dynein and kinesin, which convey cargo along the microtubules of the axonal cytoskeleton. RGC retrograde transport of brain-derived neurotrophic factor (BDNF) is interrupted by elevated eye pressure, leading to the accumulation of BDNF and its receptor, trkB, at the optic nerve head.[14] Impaired delivery of BDNF to the RGC bodies is likely to adversely affect RGC survival, and supplementation of BDNF to the retina, for example by gene therapy, has been shown to mitigate the effect of glaucomatous transport blockade in animal models.[15] Other approaches to reduce the effects of axonal transport dysfunction in glaucoma are worth further exploration.

Problems with Detecting Progression in Glaucoma

In addition to helping us understand the mechanisms underlying degeneration of RGC in glaucoma, basic science research is playing an important role in the search for new ways to measure progression. Accurate determination of the presence and rate of progression is essential to the management of glaucoma patients, and is also crucial in the quest to develop new therapies for the disease. However, existing methods to measure progression have significant limitations. Visual field analysis is widely used and is arguably the most clinically relevant measure of glaucoma change as visual function is measured directly. However, most visual field analysis relies on psychophysical testing and as a result variability is a major difficulty. Variability in visual field testing means that multiple tests are often required to confirm progression; therefore, it can take a considerable period of time to confirm whether a patient is deteriorating. This has implications not just for the treatment of individual patients, but also for clinical studies of new potentially neuroprotective treatments where the 'noisiness' of visual field end-points contributes to the need for long, and therefore expensive, clinical trials with large numbers of patients.

In addition, visual field changes tend to lag behind RGC loss, with defects developing only after 25–35% of RGCs have been lost at a given retinal location.[16] Testing strategies can be modified to improve progression detection using visual field testing, for example by optimising the inter-test interval[17] and establishing a rate of progression.[18] However, visual fields remain a relatively insensitive measure of glaucoma progression, at least in early disease.

Other approaches to the measurement of progression in current clinical use include assessment of the nerve fibre layer and the 3D structure of the optic nerve head using scanning laser ophthalmoscopy, ocular coherence tomography and polarimetry.[19] These techniques can provide evidence of structural changes associated with glaucoma progression, but are relatively insensitive to small changes. Thus, new techniques with the ability to rapidly determine with precision the exact rate of glaucomatous progression would be a major advance.

Using Basic Science to Improve Detection of Progression

Detailed understanding of the mechanisms of RGC loss in glaucoma has the potential to suggest new approaches to the assessment of progression. As an example, the observation that RGC death in glaucoma occurs by apoptosis means that existing techniques to visualise apoptosing cells may be applied to the glaucomatous eye. Technology to image individual RGCs in the living eye and to trace the fate of individual labelled retinal cells over time has advanced rapidly over the last few years.[20–22] Cordeiro et al. used annexin V labelling and carefully refined imaging techniques to identify apoptosing retinal cells in the living eye.[23] Annexin V specifically labels cells with surface membrane changes characteristic of apoptosis. This technology has also been applied to glaucoma models,[24] and a clinical trial in glaucoma patients is also planned. Such techniques represent a potentially important breakthrough in the assessment of glaucomatous progression by allowing individual dying RGCs to be visualised. However, the ability of apoptosis imaging to separate normal RGC attrition due to ageing from glaucomatous progression remains to be established. Given that apoptosing cells can only be detected by annexin V for four to six hours, it is likely that the eye of a slowly progressing glaucoma patient will have a relatively small number of apoptosing cells detectable at a given point in time.

Additional challenges will be introduced if the apoptosis rate in glaucoma subjects and controls is not uniform, with cell death

occurring in bursts distributed in time and retinal location. High variability in apoptotic rate between individuals will require a greater number of images to determine the average rate of deterioration. It is also conceivable that other tests, such as visual field and nerve fibre layer analysis, could be used to target areas of the retina deemed 'at risk' of progression for apoptosis imaging. Such questions can only be answered fully in human glaucoma and thus the results of human trials are awaited with great interest, assuming the technique is found to be safe and effective.

One limitation of apoptosis imaging is the relatively small number of cells likely to be undergoing apoptosis at any given time when glaucoma progression is slow. An alternative approach would be to identify sick RGCs that are not yet committed to die. The rationale for this approach is that there may be a larger population of sick cells evident for much longer than the end stage of apoptosis. The challenge here is to identify detectable markers of sick RGCs. To this end, there are several possible approaches that are showing promise. As discussed earlier, axonal transport dysfunction is a major early event in glaucoma. Techniques now exist to visualise axonal transport in living axons, for example by imaging the movement of mitochondria in transgenic animals where these organelles are fluorescently labelled.[25] The combination of such techniques with high-resolution *in vivo* imaging of the eye could theoretically allow axonal transport to be assessed as a marker of RGC health.

Other possible approaches include searching for characteristic optical 'signatures' of sick RGCs using techniques such as ocular coherence tomography (James Morgan, unpublished data). In transgenic animal models that possess small numbers of fluorescent retinal neurons, it is also possible to track the effect of injuries such as glaucoma on the dendritic tree of individual RGCs.[22] Although some of these techniques are not likely to be useful in humans, the principle of detecting sick cells may be as useful as detecting those committed to die, and is worthy of further exploration.

Summary

Basic science approaches are the key to understanding the links between RGC injury in glaucoma and the resultant structural and functional deterioration that occurs as the disease progresses. Understanding the basic mechanisms of RGC degeneration can not only facilitate the identification of new therapeutic targets, but also suggest new ways in which progression can be detected and quantified. Moreover, basic science is essential for the ultimate goal of rapidly identifying progressing patients for their inclusion in shorter, more affordable clinical trials of novel potential neuroprotective therapies. ■

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