Retinal Neuroprotection

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Separation of the neurosensory retina from the underlying retinal pigment epithelium (RPE) is a common form of injury that can occur alone (a retinal detachment) or as a result of other disease processes such as ocular trauma, inflammation, traction secondary to diabetic retinopathy, or exudation from neovascular age-related macular degeneration (AMD). Despite significant advances in the medical and surgical management of retina-RPE separation from these various causes, patients often have significant vision loss, primarily due to the death of the photoreceptors.

APOPTOSIS AND PHOTORECEPTOR LOSS

Research over the past decade has made it increasingly clear that apoptosis, or programmed cell death, is the primary mechanism of separation-induced photoreceptor death. To study this phenomenon, we developed a rodent model of experimental retina-RPE separation.[1] In this model, we inject a solution of 1% hyaluronic acid into the subretinal space to elevate the retina off the RPE (Figure 1). Our model is derived from a well established and accepted feline model of experimental retina-RPE separation.[2] This is a highly reproducible technique for creating detachments that can persist for extended periods of time. The changes that occur to photoreceptors in our model mimic the changes seen in the human condition, with the early histologic appearance of apoptotic markers and the progressive loss of photoreceptors with chronic separation (Figure 2). This model provides a convenient, easily controlled system for perturbing photoreceptor homeostasis and studying the molecular biology of photoreceptor apoptosis.

Using the rodent model of retinal detachment, we have shown that the FAS-apoptosis pathway is the crucial activator of photoreceptor apoptosis after separation from the RPE.[3] Retina-RPE separation induces a rapid activation of the FAS-receptor and downstream components of the FAS-pathway including caspase 8, BID, caspase 3, caspase 7, and caspase 9. This is accompanied by a marked increase in the transcription of FAS-pathway intermediates within the area of the detached retina. Interventions that prevent FAS-receptor activation or transcription of new FAS-receptor provide significant protection against the separation-induced death of the photoreceptors.

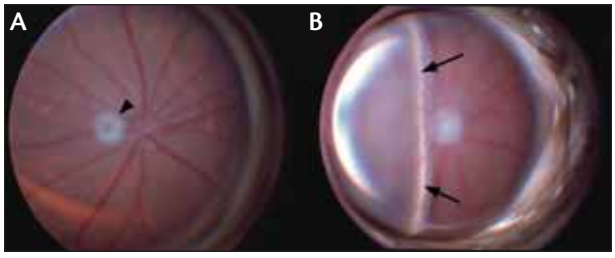

Figure 1. A normal rat retina; arrowhead points to a photographic artifact (A). The arrows show the detached retina (B).

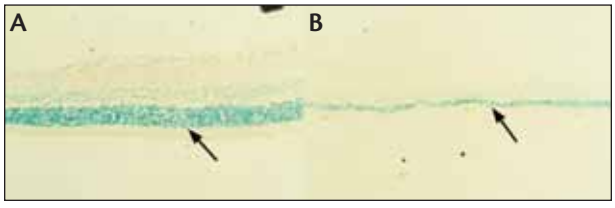

Figure 2. Arrows point to the outer nuclear layer in attached retina (A) and in a retina detached for 2 months (B).

FAS-receptor activation occurs within hours after retina-RPE separation and controls the downstream activation of the intrinsic (mitochondrial) apoptosis pathway. Several independent methods for inhibiting FAS-receptor activation resulted in increased survival of photoreceptors after retinal detachment. Injection of a FAS-neutralizing antibody (FAS-NAb) or injection of a small inhibitory ribonucleic acid (siRNA) against the FAS-receptor transcript prevents photoreceptor apoptosis (Figure 3). In addition, death of photoreceptors after retina-RPE separation is attenuated in the lpr mouse, a strain that contains a defective FAS-receptor. Similarly, we recently described photoreceptor protection by a small molecule inhibitor of the FAS-receptor called Met12.[4]

Photoreceptor apoptosis can also be prevented by further downstream inhibition of the apoptotic pathway. The X-linked inhibitor of apoptosis (XIAP) is a key member of the inhibitors of apoptosis (IAP) family of proteins and suppresses the activity of caspases 3, 7, and 9.

Figures 1 and 2 are were originally published in: Zacks DN, Hänninen V, Pantcheva M, Ezra E, Grosskreutz C, Miller JW. *Invest Ophthalmol Vis Sci.* 2003;44:1262–1267 Copyright © Association for Research in Vision and Ophthalmology.

Previous studies showed that XIAP is neuroprotective in various models of neuronal injury, including forebrain ischemia, methyl-phenyl-tetrahydropyridine (MPTP)-induced Parkinson disease, and cisplatin-induced ototoxicity. Exogenous administration of XIAP has been shown to protect retinal ganglion cells in animal models of optic nerve axotomy, increased intraocular pressure, and retinal ischemia. Not surprisingly, XIAP can also protect photoreceptors from N-methyl-N-nitrosourea (MNU)-induced retinal injury and in two rodent models of retinitis pigmentosa. In conjunction with the laboratories of Catherine Tsilfidis, PhD (University of Ottawa Eye Institute), and Dr. William Hauswirth, PhD (University of Florida, Gainesville), we have shown that XIAP delivered subretinally through an adenoassociated virus (AAV) vector inhibits apoptosis and significantly prevents retinal detachment-induced photoreceptor death.[5] The photoreceptors expressing exogenous XIAP were protected for at least 2 months of continual detachment and were positive for rhodopsin staining, indicating that these photoreceptors remained functionally viable.

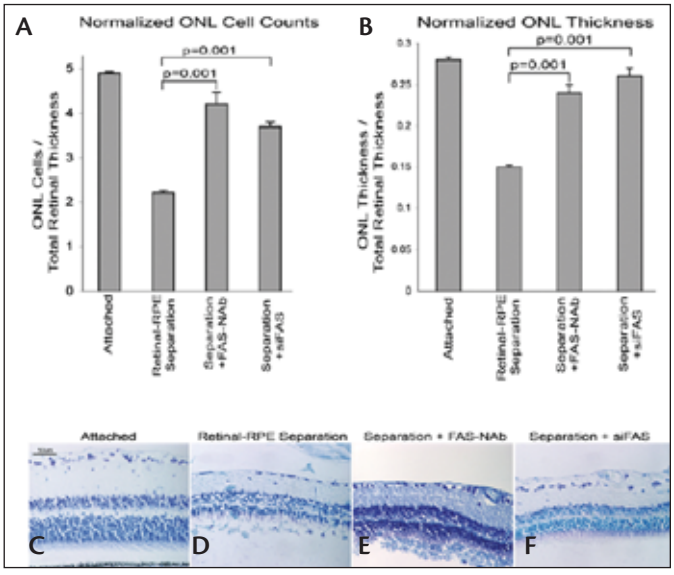

Figure 3. Outer nuclear layer (ONL) cell counts (A) and thickness (B) before and after separation of the retina from the retinal pigment epithelium (RPE). Histologic analysis of an attached section (C), a section in which the retina and RPE are separated (D), a separated section treated with a FAS-neutralizing antibody (FAS-NAb) (E), and a separated section treated with small inhibitory RNA against the FAS transcript (siFas) (F) demonstrating the ability of reduced FAS-receptor activity to preserve ONL cell counts and thickness after retina/RPE separation (toluidine blue, original magnification x 40).

INTRINSIC PROTECTIVE PATHWAYS IN RETINA-RPE SEPARATION

A paradox seemingly exists when the retina separates from the RPE, in that photoreceptors can actually survive for prolonged periods of time, despite the early activation of pro-apoptotic pathways. To better assess the multitude of potential other pathways activated after retina-RPE separation, we performed a gene microarray analysis looking at the transcriptional activity in detached versus attached retinas.[6] Our results showed that beside apoptotic pathways, numerous pro-survival signaling pathways are also activated within the retina. One such pathway is the interleukin-6 (IL-6) pathway. Using both loss-of-function and gain-of-function experiments, we demonstrated that the intraretinal activation of the IL-6 receptor is crucial for preventing photoreceptor cell death after retina-RPE separation.[7]

NEUROPROTECTIVE AGENTS

Despite IL-6 pathway activation, however, photoreceptors will die if the retina remains separated from the RPE, suggesting that the endogenous production of IL-6 primarily serves to slow the rate of this death. Loss of the endogenous IL-6 signaling results in a more rapid rate of cell death, whereas addition of exogenous IL-6 further slows the rate of death.

The neuroprotective effect of IL-6 is similar to the effect of ciliary neurotrophic factor (CNTF, a member of the IL-6 family of cytokines), which has been shown to reduce photoreceptor death in animal models of hereditary retinal degenerations. Several growth factors and neurotrophic agents including FGF, BDNF and CNTF have survival-promoting activity in the central and peripheral nervous systems including the retina and the optic nerve. Beside retinal degeneration models, CNTF has been shown to prolong photoreceptor survival in light-induced photoreceptor damage. Exogenous CNTF is also effective in promoting retinal ganglion cell survival and axonal growth in several experimental models of glaucoma and other optic neuropathies. A phase 1 trial showed that CNTF delivered by cells transfected with the human CNTF gene and sequestered within capsules implanted into the vitreous cavity is safe in patients.[8] Phase 2 and 3 trials of the CNTF implant are ongoing for patients with AMD and early or late stage retinitis pigmentosa (RP; clinical trials.gov identifiers: NCT00447954, NCT00447980, and NCT00447993).

Another neuroprotective agent that has received great attention in recent years is brimonidine, a selective alpha 2-adrenergic agonist. Brimonidine is currently used for reducing intraocular pressure in ocular hypertension and glaucoma. The neuroprotective effect of brimonidine has

been studied extensively in retinal ganglion cells after optic nerve injury. Based on the success of animal studies, human trials examined the neuroprotective effect of brimonidine in nonarteritic anterior ischemic optic neuropathy (NAION) and Leber hereditary optic neuropathy. Unlike in experimental animal models, however, none of the human studies demonstrated any neuroprotective efficacy of brimonidine. The neuroprotective effect of brimonidine was also studied for photoreceptor survival. A recent prospective placebo-controlled, double-masked, randomized clinical trial of 17 patients with RP and cone-rod dystrophy found no statistically significant protective effect of topical brimonidine treatment; however, a non-significant trend showed slower progression of visual field loss in treated eyes.[9] In another small study, topical brimonidine demonstrated a small effect on reducing collateral damage caused by laser photocoagulation for choroidal neovascularization.[10] An ongoing study is examining the neuroprotective effect of brimonidine in AMD (clinical trials.gov identifier NCT00658619), but as yet the results of this trial are not available.

NUTRITIONAL SUPPLEMENTS

Protective effects of nutritional supplements have been studied extensively in patients with degenerative retinal diseases. The neuroprotective effect of vitamin A was studied in a randomized, controlled, double-masked trial enrolling patients with RP.[11] This study showed that patients receiving 15,000 IU/d of vitamin A palmitate had a slower rate of decline of retinal function compared with patients receiving placebo, based on cone electroretinogram amplitude. Another randomized trial analyzed whether a therapeutic dose of docosahexaenoic acid (DHA), an omega-3 fatty acid, would slow the course of retinal degeneration in patients with RP who are also receiving vitamin A.[12] Although the effect of DHA was not as robust as that of vitamin A, this study showed that the addition of 1200 mg/d DHA slowed the course of RP for 2 years in patients beginning vitamin A therapy.

GENE THERAPY

Another strategy for cell rescue is to insert a fully functional allele into a photoreceptor carrying a mutated gene. Animal studies have successfully demonstrated that insertion of a normal gene into mice prevents degeneration of the photoreceptors. In particular, in RPE65 genetic subtypes of Leber congenital amaurosis (LCA), successful photoreceptor and visual rescue have been achieved in mice and canine models. A similar gene therapy approach was recently studied in a subset of patients with LCA. RPE65 replacement was evaluated in multiple phase 1 trials conducted by several groups to assess the effect of gene therapy on retinal and visual function in children and adults with LCA. Remarkably, subretinal injection of an AAV containing RPE65 was well tolerated, and patients showed improvement in subjective and objective measurements of vision.[13] Perhaps more important, the greatest visual improvement was seen in children, who all gained ambulatory vision. Results of these trials suggest that favorable response to retinal gene therapy will depend on the patient's age and extent of retinal degeneration.

Future research for identifying novel neuroprotective agents will require improved methods of measuring photoreceptor death and function in vivo.

FUTURE RESEARCH

Future research for identifying novel neuroprotective agents will require improved methods of measuring photoreceptor death and function in vivo. A promising new tool that was recently described employs autofluorescence of flavoproteins.[14] Preapoptotic cells exhibit mitochondrial stress and develop impaired electron transport by the energy-generating enzymes in the respiratory chain. This in turn causes increased percentages of flavoprotein that are capable of absorbing blue light and emitting green autofluorescence. A novel method for the clinical detection of early metabolic dysfunction in the human ocular fundus involves the measurement of retinal flavoprotein autofluorescence. Unlike current diagnostic tools, this new method permits detection of cellular metabolic dysfunction in the retina in the pre-death stage. Validity of this instrument was demonstrated in patients with diabetic retinopathy, pseudotumor cerebri, AMD, RP, and central serous retinopathy.

Similar tools will also be integral to testing neuroprotective agents in animal models. Studies using animals currently require the sacrifice of animals for histologic examinations of the retina. This prevents monitoring therapeutic effects of neuroprotective agents over time in individual animals. Several groups have described custom-built or adapted commercially available optical coherence tomography (OCT) systems for in vivo imaging of the retinal structures in normal and diseased rodent eyes.[15] These studies showed that rodent OCT images have excellent correlation with histology, and OCT may become an important new tool for the in vivo analysis of rodent eyes.

SUMMARY

Research in animal models and human subjects has produced promising agents for retinal neuroprotection and will continue to improve our understanding of basic mechanisms of cell death in the retina. Neuronal cell death is a complex process with several molecular checkpoints regulating the fine balance between the pro-death and pro-survival signals. Successful neuroprotection will likely require combination treatments, with rapid application of anti-apoptotics to arrest cell death and exogenous pro-survival agents to prevent cells from reentering the apoptotic pathway. These techniques hold much promise for providing significant structural and functional rescue in the retina and preventing vision loss. ■

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