

Neuroprotection in Age-related Macular Degeneration

Preventing apoptosis may not be enough to prevent cell death.

BY DEMETRIOS G. VAVVAS, MD, PhD

Degeneration and death of photoreceptor cells is the cause of vision loss in many retinal disorders, including retinitis pigmentosa, retinal detachment (RD), geographic atrophy in dry age-related macular degeneration (AMD), and neovascular AMD. An effective method of neuroprotection would potentially be a valuable therapy for these disorders, as well as for glaucoma, stroke, and other diseases. Yet, despite more than a decade of attempts to develop neuroprotective drug therapies for retinal diseases, these efforts have not been successful.

The principal cause of photoreceptor death in retinal and other diseases has been assumed to be apoptosis, a process of programmed cell death. However, increasing evidence suggests that there is another pathway involved, called programmed necrosis or necroptosis.^{1,2} This may help to explain why attempts to use neuroprotective strategies in clinical trials for stroke and glaucoma have failed: These studies addressed only 1 of the pathways involved, the apoptotic pathway.

If this is the case, then for future efforts at neuroprotection in retinal diseases to be effective, therapeutic strategies must address these redundant pathways of cell death with a therapeutic combination approach. This article summarizes some of what we have learned in recent years about photoreceptor cell death in retinal diseases and what these findings may mean for the development of effective neuroprotective strategies in the future.

CELL DEATH IN RETINAL DISEASE

Photoreceptor death occurs when photoreceptor cells are physically separated from the underlying retinal pigment epithelium (RPE). This separation is seen in a vari-

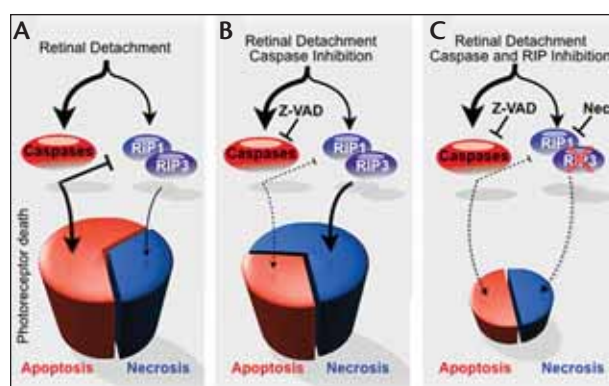


Figure 1. Proposed mechanism of photoreceptor loss after retinal detachment. (A) After retinal detachment, photoreceptor death is caused mainly by apoptosis. (B) Caspase inhibition by Z-VAD decreases apoptosis but promotes RIP-mediated programmed necrosis. (C) Blockade of both caspases and RIP kinases is essential for effective prevention of photoreceptor loss. (Reproduced from Trichonas G et al. *Proc Natl Acad Sci U S A*. 2010;107(50):21695-21700.)

ety of disorders, including AMD, diabetic retinopathy, and rhegmatogenous RD.

Apoptosis and necrosis are 2 major modalities of cell death. Apoptosis is a regulated process involving cysteine proteases called caspases. In the characteristic morphology of apoptosis, cells become rounded and shrunken, followed by chromatin condensation and engulfment by phagocytes. Necrosis, by contrast, has been considered a passive, unregulated process of cell death, marked by swelling of the cell and rupture of the plasma membrane, with subsequent release of intracellular contents resulting in inflammation.

It has recently been recognized that some necrosis,

induced by regulated signal transduction pathways such as those mediated by receptor interacting protein (RIP) kinases, is responsible for photoreceptor cell death. This recently characterized mechanism of cell death is termed programmed necrosis or necroptosis. Necroptosis is induced by apoptotic stimulation of the Fas/TNFR family of death domain receptors, particularly in conditions when caspases are inhibited. The cell morphology in necroptosis is similar to that of necrotic cell death, with activation of autophagy. The process is regulated through a specific set of genes, 7 of which are common to both apoptosis and necroptosis.

Photoreceptor cell death after RD has been thought to be caused mainly by apoptosis, which as mentioned is regulated by caspases. However, although the caspase pathway is indeed activated after RD,^{3,4} inhibition of caspase does not prevent photoreceptor death.⁵

In a rodent model of RD, we showed that expression of tumor necrosis factor- α (TNF- α) increases substantially and caspases are activated after RD. However, the caspase inhibitor Z-VAD does not prevent photoreceptor cell death induced by RD.⁵

We therefore investigated other pathways that lead to photoreceptor death. In a series of investigations, we demonstrated that necrotic photoreceptor death occurs after RD with about half the frequency of apoptosis.¹ We also showed that treatment with the caspase inhibitor Z-VAD after RD causes a decrease in apoptosis but an increase in necrosis. Further, when necrostatin-1 (Nec-1) is added to induce blockade of RIP kinases, this cotreatment effectively reduces both types of cell death (Figure 1).

This work demonstrated that necroptosis is an essential, complementary and redundant mechanism of programmed cell death after RD. And in fact, in the presence of caspase inhibition, this programmed necrosis becomes the predominant means of photoreceptor death. This suggests that simultaneous inhibition of RIP kinase and caspase pathways are needed for prevention of photoreceptor cell death.

RIP1 and RIP3 are members of the RIP family of serine/threonine protein kinases. An unexpected finding of our study was that deficiency in RIP3, such as by knockout of the RIP3 gene, attenuates both necrotic and apoptotic photoreceptor cell death after RD. By contrast, Nec-1 prevents necrotic photoreceptor death but does not affect apoptosis.¹ Further study is needed to characterize the roles of the RIP signaling pathways in cell death.

LOOKING AHEAD

Photoreceptor loss occurs acutely after RD, and successful anatomic reattachment does not always restore patients' visual acuity. In several common and potentially

blinding diseases, vision loss occurs after the detachment of photoreceptor cells from the RPE. Neuroprotection therefore represents a promising avenue for potential therapeutic approaches to these vision-threatening conditions.

To date, however, investigations of neuroprotective agents have not been fruitful. The work described above suggests that these failures are due to a concentration on monotherapy, when a double-pronged approach is needed. Our investigations suggest that both RIP-mediated necroptosis and caspase-mediated apoptosis are at work in photoreceptor cell death, and that inhibition of both pathways is necessary for success. In our animal work, inhibition of these pathways with small-molecule compounds protected photoreceptors after detachment, and this may offer a model for potential therapies to prevent vision loss in retinal diseases associated with photoreceptor death.

We have conducted further work, yet unpublished, exploring the potential for neuroprotection in AMD through chemical and animal models of the disease. Without going into detail prior to formal publication, this work has shown that the necroptosis pathways are active in a chemical model of AMD, and that genetic deletion of these pathways is protective. Further, in two genetic models of AMD, necroptosis pathways are active, and genetic ablation is protective.

This new evidence, demonstrating that these death pathways are activated by multiple upstream degenerative signals in multiple models of AMD, gives us hope that interfering with these pathways will yield effective ways to therapeutically address this polygenetic, multifactorial disease. ■

Demetrios G. Vavvas, MD, PhD, is an Assistant Professor of Ophthalmology in Disease of the Vitreous and Retina; the J. W. Miller Scholar in Retina Research; and Principal Investigator in the Angiogenesis Laboratory at Harvard Medical School, Massachusetts Eye and Ear Infirmary (MEEI), and Massachusetts General Hospital, Boston. Dr. Vavvas discloses that MEEI has patent applications on the subject matter and he is listed as inventor. He can be reached at +1 617 573 6874; vavvas@meei.harvard.edu.



1. Trichonas G, Murakami Y, Thanos A, et al. Receptor interacting protein kinases mediate retinal detachment-induced photoreceptor necrosis and compensate for inhibition of apoptosis. *Proc Natl Acad Sci U S A*. 2010;107(50):21695-21700. Epub 2010 Nov 22.
2. Murakami Y, Miller JW, Vavvas DG. RIP kinase-mediated necrosis as an alternative mechanisms of photoreceptor death. *Oncotarget*. 2011;2(6):497-509.
3. Zacks DN, Hanninen V, Pantcheva M, Ezra E, Grosskreutz C, Miller JW. Caspase activation in an experimental model of retinal detachment. *Invest Ophthalmol Vis Sci*. 2003;44:1262-1267.
4. Zacks DN, Zheng QD, Han Y, Bakhru R, Miller JW. FAS-mediated apoptosis and its relation to intrinsic pathway activation in an experimental model of retinal detachment. *Invest Ophthalmol Vis Sci*. 2004;45:4563-4569.
5. Hisatomi T, Sakamoto T, Murata T, et al. Relocalization of apoptosis-inducing factor in photoreceptor apoptosis induced by retinal detachment in vivo. *Am J Pathol*. 2001;158:1271-1278.