FALL 2008

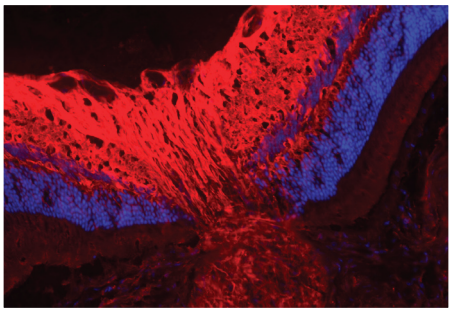
Point of View
THE EYE RESEARCH INSTITUTE UNIVERSITY OF WISCONSIN–MADISON

Under Pressure: Preventing Cell Death

It is often said in research that for every question answered, ten more are created. This is true for molecular and developmental biologist Robert W. Nickells, PhD, professor in the Department of Ophthalmology and Visual Sciences and a member of the UW Eye Research Institute, who has devoted the past 17 years to researching the multivariate aspects of glaucoma—each discovery opening new pathways to explore.

Glaucoma is a complex disease described as a *"sneak thief of sight"* because it steals vision almost imperceptibly over time. According to the World Health Organization, it is the second leading cause of blindness in the world. A person with glaucoma may not notice vision loss until the disease is already quite advanced, as one's field of vision shrinks at the periphery first. Once vision is lost, it cannot be regained.

While glaucoma can be treated with medication and/or surgery to slow vision loss, it remains a chronic and currently incurable neurodegenerative condition. Basic science researchers like Nickells are studying the mechanisms underlying glaucoma damage and susceptibility to help determine which genetic, molecular and cellular events are directly responsible for the death of one type of nerve cell in our eyes—the retinal ganglion cells. This progressive cell death, a defining feature of glaucoma, may be linked to the high intraocular pressure long identified as the primary risk factor for the disease. Also true of any complex disease, family history is perhaps the second most important risk factor.



A microscopic image of the retina and optic nerve of a mouse eye. The axons of the ganglion cells (bright red) are the cables that connect the eye to the brain. Other cells are stained blue. As the axons leave the eye and enter the optic nerve (image top toward bottom), they form the optic nerve head. This is likely the first region of damage in glaucoma.

photo by Greg Anderson

Rob Nickells, PhD, with a glaucomatous mouse valuable to his research

Screening for glaucoma is usually performed as part of a standard eye examination, which includes a measurement of intraocular pressure. A certain minimum pressure is required to maintain the shape and size of the eyeball so that it can work efficiently as an optical instrument. Pressure above the norm, but without a corresponding loss of visual field, makes one a "glaucoma suspect" or "ocular hypertensive." Chronic pressure elevation causing visual field loss due to optic nerve damage, which can be detected during an eye exam, mandates intervention.

Yet lowering eye pressure does not address the fundamental causes of retinal ganglion cell death. It is these essential nerve cells, transmitting electrical impulses via the optic nerve from the retina to the brain, which trigger our experience of sight. When retinal ganglion cells die, vision is impaired. It would therefore be desirable for the next generation of anti-glaucoma agents to function in a way that prevents or delays the loss of retinal ganglion cells.

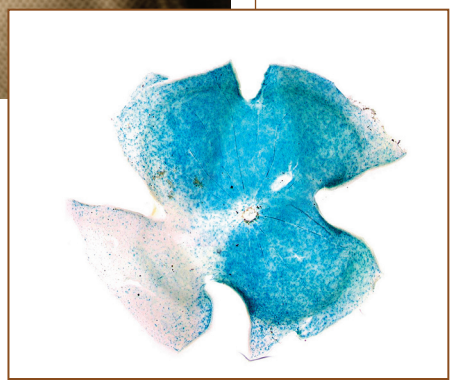
"Ganglion cell death occurs by a process of 'cell suicide'—known as apoptosis—whereby key biochemical pathways conspire in a programmed chain of events leading to the cell's death," Nickells explains. "We think that new and effective treatments can be developed specifically to interfere with ganglion cell death, providing important avenues of therapy for many neurodegenerative disorders, including glaucoma."

Understanding the biochemical processes underlying cell death can give access to the suicide program and allow researchers to target a point that could alter the outcome. Pursuing this goal, Nickells' lab identified the importance of the *Bax* gene and detected that ganglion cells expressing *Bax* at a higher level are more susceptible to activation of cell

death. Nickells found that manipulating cells in the laboratory to produce lower levels of *Bax* made them completely resistant to death-related signals present in glaucoma. While the biochemical process of cell death involves many pathways, *Bax* represents the critical intersection in cell death. Nickells notes, "We have learned that *Bax* is essential to the cell suicide process. When the *Bax* gene is 'active,' ganglion cells die in glaucoma. How do we shut it off? If we can target *Bax*, then we can keep the ganglion cells alive."

Mouse models have provided the genetic tools required for Nickells' work. Certain strains of inbred mice spontaneously develop progressive eye abnormalities that closely mimic human glaucoma. From those inbred mouse populations, Nickells studies mice that have been engineered to carry mutant *Bax* genes and compares them to the normal *Bax*-containing mice by simulating the cell damage caused by glaucoma in both.

photo by Greg Anderson



Graduate student Heather Pelzel mounts mouse retinas on slides for study (shown right). Using a special stain for ganglion cells (blue), scientists can quickly identify regions where these cells have been lost in glaucoma.

> ***"There are things known and there are things unknown, and in between are the doors of perception."***
>
> ***Aldous Huxley***

"We learned that loss of function of the *Bax* gene blocks cell death in experiments involving both acute trauma to the optic nerve and in the mice that have the naturally-occurring form of glaucoma. But in the mice with glaucoma, the axons—the part of the nerve cell that acts as the cable connecting the ganglion cell to the brain—died even though the ganglion cell survived." Nickells believes that this information marks a major advance in understanding that ganglion cell death and axonal loss are distinct problems, indicating that treatment of the cell death process may require two stages: blocking ganglion cell death, and then stimulating the axon to regenerate.

Hypothesizing that the process of retinal ganglion cell death may be affected by genetic background, Nickells turned to mouse genetics to identify genes tied to cell death. His lab screened 15 different lines of mice, finding that genetic background did indeed influence retinal ganglion cell loss. Building on this finding, the Nickells group screened hundreds of mice that were progeny of the most resistant strain and the most susceptible strain. Using DNA markers that were unique to each parental strain, they identified a single stretch of DNA associated with cell death and named it Rgcs1 (Retinal ganglion cell susceptible 1). Computer analysis of this stretch of DNA has led to the identification of seven genes that may potentially affect ganglion cell death.

Further study is needed to understand the role of Rgcs1 and to determine whether these other genes factor into the complexity of glaucoma. Future work will pinpoint the respective roles of each of the genes and then compare and apply this data to the human genome. The task is simplified since the genes of mice and humans have between 70% and 90% similarity, and there are only a few cases in which no mouse counterpart can be found for a particular human gene.

"In the scheme of things, we are still in the A's of the research dictionary—we have so much yet to learn," Nickells concludes. "I hope that in ten years we will be able to identify a series of genes that contribute to a patient's glaucoma susceptibility. Eventually we would like to be able to do genetic testing to provide an early alert for patients predisposed to glaucoma. I also think we will finally understand how elevated pressure causes the death of the ganglion cells. Our ultimate goal is to preserve, and perhaps even restore some of the sight of those with glaucoma. And typical of any kind of medical research, we keep putting together the small pieces until the larger puzzle has been solved."

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