Before retinal cells die, they regenerate, vet blindness study finds

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Until relatively recently, the dogma in neuroscience was that neurons, including the eye's photoreceptor cells, rods and cones, do not regenerate. This is the reason that nerve damage is thought to be so grave. More recent studies have poked holes in this belief by showing that, in some vertebrate species, neurons can be stimulated to divide.

Yet the dogma continued to hold firm with regard to retinal neurons - until a surprising finding in 2011 by University of Pennsylvania researchers. They showed that in a form of canine blindness retinal cells continue to differentiate for a period of time early in a dog's life before overwhelming cell death caused the retina to degenerate.

In a new study, the Penn researchers have expanded this line of inquiry to consider two other forms of blindness. They found that these diseases, too, possess this unexpected feature of temporarily rejuvenating retinal cells. The findings suggest this feature may be common across many forms of inherited blindness. Further investigation into the reasons for this period of retinal neuron proliferation could lead to molecular targets for intervening in cell death and maintaining functional photoreceptor cells and a working retina.

The work is reported in the journal *BMC Genomics* and was led by Penn School of Veterinary Medicine researchers Kristin L. Gardiner, a resident in the Department of Laboratory Animal Medicine; Gustavo D. Aguirre, professor of medical genetics and ophthalmology; and Sem Genini, a senior research investigator.

The study picked up where the 2011 work left off. The Penn Vet team had found in early retinal degeneration, or erd, an inherited canine retinal disease which leaves dogs sightless within a year of birth, that photoreceptor cells in the retina continued to divide when the animals were between 7 and 14 weeks of age.

"After 14 weeks, the balance between death and division tips, and the retina degenerates," Aguirre said. "We weren't sure whether this was unique to erd, so we thought we'd look at other diseases that have a similar time course."

They chose to examine two other early-onset blinding diseases. One is X-linked progressive retinal atrophy, or xlpra2, a disease very similar to the human disease X-linked retinitis pigmentosa, one of the most common forms of retinal degeneration. The other is rod cone dysplasia 1, or rcd1.

Gardiner took on the project while still a veterinary student and continued during summers and into her current residency.

The team wanted to know whether retinal cells were proliferating and, if they were, what specific types of cells were doing so.

Using chemical markers that label cells going through division, along with markers that only tag rod cells, the primary photoreceptor retina, Gardiner says they saw "beautiful labeling."

"To our great surprise, in these other two diseases we also saw a period of cell proliferation," Aguirre said.

Under a confocal microscope, they could see individual cells labeled with both markers surrounded by other cells that were labeled with the rod marker but not the cell division label. They also specifically looked to see if other types of neurons in the retina, such as microglia or Müller cells, were dividing and found that they were not.

The timing of this cell proliferation differed between the diseases. While erd cells had entered a proliferation stage at seven weeks, the researchers observed similar increases in cell division at two weeks in rcd1 and at eight weeks in xlrpa2, all time

points that precede or coincide with the known peaks of cell death in the three diseases.

A further experiment ruled out the possibility that the same cells that were proliferating were also then undergoing cell death.

"We wanted to make sure that these weren't some aberrant cells that were expressing all these different markers," Gardiner said. "We showed that there appears to be a distinct population of rod cells that is proliferating and another that is dying."

Finally, the team performed genetic and protein analyses to look for variations at these critical time periods between diseased and normal eyes. Of note, they found that changes in expression of cell cycle-related genes and proteins coincided with specific diseases stages, including expression patterns that were shared across erd, xlpra2 and rcd1.

"These commonalities were unexpected," Genini said, "but made sense in light of some of our previous studies, which found that, in all three diseases, the expression levels of key genes were statistically different compared to a normal retina.

"Furthermore, the expression dysregulation occurred in different subsets of retinal cells that might play a primary or bystander role in the degenerative process," he added. "This has to be investigated in more detail."

Additional research will also narrow in on the genes involved in turning cells on to divide, with a hope of developing a therapy that could interfere with the eventual cell death and retinal degeneration that characterizes the three diseases studied as well as many other forms of inherited blindness.

"If you have a cell that is functional but sick, perhaps we could provide it with some agent that will allow it to keep replenishing itself and maintain a functional retina for a longer period of time," Aguirre said.

Penn Vet's section of ophthalmology members Louise Downs, Evelyn Santana and Agnes I. Berta-Antalics, now at Germany's Augenklinik Uniklinik Erlangen, were coauthors on the paper.

More information: Kristin L. Gardiner et al. Photoreceptor proliferation and dysregulation of cell cycle genes in early onset inherited retinal degenerations, *BMC Genomics* (2016). DOI: 10.1186/s12864-016-2477-9

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