Podcast Interview: Karina Guziewicz and Artur Cideciyan

PNAS: Welcome to Science Sessions. I'm Paul Gabrielsen.

Macular degeneration is a retinal disease and a leading cause of vision loss in the United States. There are age-related forms of macular degeneration as well as monogenic forms where just one gene mutation is responsible for the development of the disease. Monogenic retinal diseases are a promising target for gene therapy treatments. Karina Guziewicz and Artur Cideciyan, both of the University of Pennsylvania, and their colleagues recently published a paper in PNAS that reported a gene therapy treatment for macular degeneration in dogs. The dogs carry a mutation in a gene called *BEST1* that codes for the retinal protein bestrophin, so the disease arising from that mutation is called a bestrophinopathy. Humans are also affected by bestrophinopathies.

Vision occurs as light passes through the eye and hits photoreceptor cells called rods and cones. These cells are nourished by the retinal pigment epithelium, or RPE. In a bestrophinopathy, the RPE cells degrade, causing them to detach from the retina and form large lesions. These large detachments are common to both human and canine disease. Guziewicz explains how gene therapy can correct this condition.

Guziewicz: The gene therapy strategy is designed to introduce genetic material into the cells of interest to compensate for either an absent or abnormal gene and to make protein. So if the mutated gene does not produce a protein or produces a faulty one, the gene therapy introduces a normal copy of the gene to restore the function of the protein. In our application, we use adeno-associated virus 2 as a vehicle, delivering the cargo - in our case either a canine or human version of the bestrophin gene by subretinal injection and delivery is specific to the RPE cells which express the mutant copy of bestrophin.

PNAS: The researchers found that in addition to the large detachments, canine bestrophinopathies also have widespread microdetachments which expand with exposure to moderate light and contract in darkness. Cideciyan explains what this finding means.

Cideciyan: Light interacts with the retina, and that is how we see. Human vision works so exquisitely well that we can even perceive individual photons of light and the reason that it works so well is that there is this cycle that is predicated on the tight physical and molecular interaction between the photoreceptor light-sensing antenna called outer segments, and the retinal pigment epithelium extensions called microvili. These sets of protrusions originate from two neighboring cells and interlock almost like a 2D-zipper or a patch of Velcro across the whole retina. Only with the advance of in vivo imaging methods it has been possible to detect structural changes occurring between these outer segments and microvilli. In the dogs, these light-dependent changes can be much much larger separating the two cell layers up to 20 microns upon light exposure.

PNAS: The researchers say the treatment was effective and long-lasting.

Guziewicz: In the dog model the treatment is pretty much permanent. We observe a stable disease reversal in all three models tested, and after a single subretinal injection we observe reversal of disease exactly up to six years post-injection; so far we have treated more than 30 eyes.

Cideciyan: Normally subretinal delivery of the adeno-associated virus causes a small bleb or a blister that surgically splits the photoreceptors from the RPE to either directly transfect the photoreceptors or the RPE or both cell populations. It is well-known that the area transfected is usually a little bigger than area of the initial surgical bleb. What was absolutely fascinating in the *BEST1* mutant dogs is that this penumbral region of transfection could be so large as to sometimes cover nearly the whole retina for a very small volume of sub retinal injection. We think this was because of the existence of the microdetachment allowing the subretinal injection to distribute to much further areas than we are accustomed to. If proven to be relevant for human studies, one can envision use of much smaller volumes of vector and thus much smaller total dose delivered to cover much larger areas of retina that would otherwise be not covered with such small volumes.

PNAS: Despite the loss of normal chromophore transport between the rod and cone cells and the RPE cells in bestrophinopathy, some people with substantial retinal lesions have been found to retain good visual acuity.

Cideciyan: We had the opportunity to evaluate cone based day vision and rod based night vision in patients with autosomal recessive bestrophinopathies and the results show that the rod function is affected to a much greater degree compared to cone function. Rods drive night vision and cones drive day vision. There appears to be two sources of chromophore in the retina. One source involves the RPE and another source is thought to be for the private use of cones and does not involve the RPE. We think the private one for the cones may provide enough chromophore for vision in cones despite their physical separation from the RPE in this condition. This obviously needs further evaluation in different patients.

PNAS: Several steps remain on the road to bringing gene therapy to human bestrophinopathies.

Cideciyan: For the autosomal recessive bestrophinopathy, there is now a clear proof of concept. There were no obvious toxicity signals associated with the canine subretinal AAV2 injections, but a formal toxicity analysis remains to be performed. Also important is a formal dose response function to determine the minimal effective titer and very relatedly the minimal effective volume to cover a certain area considering the tendency we saw for a very wide distribution of the treatment beyond the initial injection site. The expansion of the treatment from recessive disease to dominant disease will require further evidence, and such studies are currently ongoing.

PNAS: William W. Hauswirth, a co-author on Guziewicz and Cideciyan's work and a researcher at the University of Florida, owns equity in a company that hopes to commercialize some aspects of this work in the future.

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