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COMMENTARY

Gene therapy to treat inherited and complex retinal degenerative diseases

Tonia S Rex[1]

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These are exciting times in the field of retinal gene therapy, as impressive successes have been achieved by recombinant adeno-associated viral (rAAV) gene delivery of Rpe65 or Rep1 to the retinal pigment epithelium of patients with Lebers congential amaurosis or choroideremia, respectively.[1–5] Instead of being left with incurable blindness, patients are reporting restoration of vision. An advantage of these disease targets is that the affected cell type, the retinal pigment epithelium, is amenable to gene therapy since it is naturally phagocytic, resulting in efficient transduction, and is not adversely affected by overexpression of exogenous transgenes. In contrast, the majority of inherited retinal degenerations are due to mutations in photoreceptor-specific transcripts causing primary death of these cells and permanent vision loss.[6] Novel studies published in *Molecular Therapy–Methods & Clinical Development* address such challenges for photoreceptor gene therapy, including rapid onset of cell death (resulting in pathological changes to the retinal environment) and inefficient transduction of photoreceptors by gene therapy vectors.

Palfi *et al*.[7] address the latter challenge by demonstrating that rAAV2/rh10 transduces photoreceptors as efficiently as the current gold standard, rAAV2/8.[8,9] The number of cells transduced and level of transgene expression achieved by Palfi *et al*., with rAAV2/rh10 is sufficient to provide histological, electrophysiological, and behavioral improvements in a mouse model of autosomal recessive retinitis pigmentosa due to mutations in rhodopsin. Their studies are among the first to show success in the most common form of retinitis pigmentosa.[10–12] While very exciting and full of promise, the effect is not long-lasting, consistent with results in other studies using rAAV-mediated gene addition to rescue photoreceptors.[12–19] A decreased rate of degeneration is certainly clinically relevant as it could translate to the addition of years of precious sight in patients. At the same time we need to understand why the efficacy of gene therapy in photoreceptors decreases over time despite addressing the intrinsic deficit (lack of functional protein). This article will discuss two potential explanations: (i) insufficient gene expression within the cells causing intrinsic cell death pathways to still activate; and (ii) changes in the retinal milieu that initiate cell death by extrinsic signaling pathways.

Palfi *et al*.[11], both in the recent article in this journal[7] and in their 2010 paper,[12] achieved a fantastic feat—improvement of structure and function by gene delivery of rhodopsin into the rhodopsin knock-out mouse. Their studies also highlight the challenges of gene augmentation therapy for retinal degeneration. Despite a significant effort by Palfi *et al*. to increase expression of rhodopsin, the disorganization of the outer segment discs and lower visual function (spatial acuity threshold and electroretinogram) in the treated mice as compared to wild-type controls suggest that normal levels of rhodopsin were still not achieved. Further, the mice still exhibited progressive retinal degeneration. Studies by other groups suggest that increasing gene expression alone may not be sufficient to completely block progression of inherited retinal degenerations. First, increasing gene expression either through repeated injections of rAAV or by use of a more efficient rAAV vector does not overcome this progressive decline in photoreceptors.[16,17] Second, studies in both dogs and mice show that the therapeutic effects of augmentation gene therapy are more sustained when it is given early, prior to onset of photoreceptor degeneration.[13,15]

Many inherited retinal degenerations due to deficits in photoreceptor-specific proteins have a very early onset. Therefore, the retinal environment is likely altered prior to intervention by gene therapy. For example, glial reactivity can be detected prior to significant retinal degeneration in multiple models of retinal degeneration.[20–24] Glial cell reactivity can be beneficial acutely, but can cause oxidative stress and neuroinflammation, leading to neuronal death if the glia remain reactive long-term (for review, see ref. 25). In fact, Palfi *et al*., detected infiltrates in the rhodopsin knock-out mouse, although they did not assess the level of reactivity of the endogenous glial cells.

However, robust and long-term benefit may be achieved by a combined approach targeting both the intrinsic gene defect and extrinsic signaling (*i.e.*, neuroprotection). This approach has shown success in a mouse model of inherited retinal degeneration.[26] Gene delivery of several neuroprotective agents has shown promise in models of inherited retinal degeneration.[27–34] It is important to note that not all neuroprotective factors are created equal, and all inherited retinal degenerations are not the same. It is critical to have a good understanding of the molecular pathways both activated by the inherited retinal degeneration of interest and inhibited by each potential neuroprotective factor in order to properly pair them. For example, if the photoreceptor degeneration is driven entirely by intrinsic pathways that activate apoptotic cell death, then only those factors that block apoptosis will be effective. In contrast, if autophagy, pyroptosis, or necroptosis are critical, then one needs a factor that will target those pathways. In addition, late stage inherited retinal degenerations, as well as complex retinal degenerations such as glaucoma, diabetic retinopathy, and age-related macular degeneration, are affected by extrinsic factors such as glial reactivity (as mentioned above), oxidative stress, and/or neovascularization, thus requiring factors that will target these processes. Fortunately,

[1]Vanderbilt Eye Institute, Vanderbilt Brain Institute, Vanderbilt University, Nashville, Tennessee, USA. Correspondence: TS Rex (tonia.rex@vanderbilt.edu)
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most neuroprotective factors are pluripotent such that they can address both intrinsic and extrinsic pathways.

Shanab *et al*.[35] present a promising neuroprotective gene therapy approach that targets neovascularization in diabetic retinopathy. In the retina, neovascularization most commonly occurs during retinopathy of prematurity and as a common secondary complication to diabetic retinopathy. It can also occur in advanced stages of inherited retinal degenerative diseases, in which the lack of photoreceptors creates a more oxidative environment leading to neovascularization in the retina.[36] This group previously demonstrated that an oxidative environment leads to decreased MMP-7 activity resulting in decreased processing of pro-nerve growth factor (NGF) into NGF in both experimental and clinical diabetes.[37–39] While it is well known that NGF is neuroprotective through activation of TrkA (for review, see ref. 40), they showed that proNGF promoted neuroinflammation and cell death by activation of $p75^{NTR}$.[41–43] In their current paper, the authors used virus-mediated (lentivirus) gene delivery of a cleavage resistant proNGF to induce endothelial cell death and counteracted the damage by treatment with a lentivirus carrying shRNA targeting the $p75^{NTR}$. This approach successfully protected the endothelial cells both *in vivo* and *in vitro*. These results are encouraging and further studies should be performed to test the general applicability of this approach for retinal neovascularization as a result of retinopathy of prematurity, and choroidal neovascularization as a result of age-related macular degeneration or end stage inherited retinal degenerations. There is a need for a new approach for blocking neovascularization since recent studies show that anti–vascular endothelial growth factor therapies are ineffective at blocking neovascularization in a subset of patients.[44]

In summary, two studies now published in *Molecular Therapy–Methods & Clinical Development* highlight the great strides being made in retinal gene therapy and the future of the field. Palfi *et al*. demonstrate great progress in the treatment of retinal degenerations due to lack of functional rhodopsin using a new rAAV serotype. Shanab *et al*. illustrate the utility of virus-mediated gene delivery for understanding the molecular events that underlie disease, in this case neovascularization, and demonstrate a new approach for blocking pathological angiogenesis. This and other neuroprotective approaches will be necessary for treating complex retinal degenerations and may improve outcomes in inherited retinal degenerations. The lack of long-term preservation of vision after gene addition into photoreceptors underscores the need for both gene augmentation and neuroprotection approaches in order to achieve great clinical success. The field is also propelled forward by the development of new serotypes of rAAV that have greater transduction efficiency, faster onset of transgene expression, and target different cell types (*i.e.*, photoreceptors, glia, or ganglion cells). In conclusion, for the field of retinal gene therapy to reach the ultimate goal of treating all forms of blinding disease, multiple groups will need to work together, collaboratively, bringing their expertise on the biology of disease, vector development, and both gene targeted and neuroprotective strategies together to develop synergistic, multipronged gene therapies.

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