Gene Transfer Vectors: Applications to the Treatment of Retinal Degenerations

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Introduction

The first two genes implicated in blindness were identified and cloned in 1990: the choroideremia gene (*CHM*) and the rhodopsin (*RHO*) gene (Cremers et al., 1990; Dryja et al., 1990; Farrar et al., 1990). Two decades later, 172 different genes have been identified which, when mutated, can cause retinal degeneration (http://www.sph.uth.tmc.edu/RetNet). This number is likely 80% of the total number of retinal disease-causing genes because loci have been identified for at least 32 additional blinding conditions. The progress in genetics has led to the identification of disease-causing genes in spontaneous mutant animal models and, in turn, to the development of additional animal models of blindness and an improved understanding of disease pathogenesis. With the simultaneous development of reagents and approaches with which to carry out retinal gene transfer, it has become possible to harness the pathogenetic data to develop rational gene-based treatments.

The mammalian eye, because of its ease of access, benign immunologic response to gene transfer, and ability to perform noninvasive functional and structural studies, has been at the forefront of therapeutic trials based on gene transfer. Gene transfer strategies have been used in both small and large animal models to demonstrate proof-of-concept. These preclinical studies have allowed the field to reach the point where gene therapy to treat a form of inherited blindness has been tested in clinical trials.

Background

Vectors and retinal gene transfer: nonviral gene delivery

A number of physicochemical methods have been evaluated for their ability to deliver nucleic acids to retinal cells. These methods include the use of physicochemical agents to compact the DNA and/or transport it across the membrane lipid bilayer (Table 1). Retinal gene transfer has also been achieved through electroporation or iontophoresis. Nonviral gene transfer is attractive because it can be used to deliver DNA of unlimited size and is less likely than viral gene transfer to incur a detrimental immune response. Several studies have demonstrated proof-of-concept for retinal gene therapy using nonviral DNA delivery, and additional studies are expected to reveal the long-term safety, stability, and efficacy of this approach.

Vectors and retinal gene transfer: viral vector–mediated gene delivery

A large number of recombinant viruses have been tested for their ability to target the retina. Different viruses have different attributes and challenges, including cargo capacity, ease of purification, cellular specificity, and immune response (Table 1). Many of these have been used to demonstrate efficacy in animal models of retinal degeneration.

Adenovirus type 5 (Ad5) vectors, deleted of the adenoviral *E1, E3* genes, were the first to be evaluated for retinal gene transfer in the differentiated retina (Bennett et al., 1994; Li et al., 1994). Adenovirus vectors result in high levels of gene expression within 24 to 48 h. When injected subretinally, they target retinal pigment epithelium (RPE) cells efficiently in the adult eye and also Müller cells. When injected intravitreally, they target Müller cells and many cells in the anterior segment (including cells in the cornea, lens, iris, and outflow tract). Because the early generations of vectors carry viral open-reading frames, these vectors can elicit an immune response that limits the duration of transgene expression. Efforts have been made to generate adenovirus vectors lacking any viral open-reading frames, the so-called gutted or helper-dependent vectors, thereby reducing immune clearance and allowing stable transgene expression (Kumar-Singh and Chamberlain, 1996). Such vectors result in more stable transgene expression than did the first-generation vectors and, further, have a much greater cargo capacity than the original adenoviral vectors (Table 1). They are more difficult to manufacture, however.

Lentivirus vectors, unlike recombinant adenovirus (rAd) and recombinant adeno-associated virus (rAAV) vectors, have RNA genomes that are reverse-transcribed by virally encoded reverse transcriptase. These vectors integrate into the host genome and thereby can result in stable gene transfer. Vectors developed from a variety of different wild-type viruses, including human, simian, and feline immunodeficiency virus and equine infectious anemia virus, have been generated. Lentiviral vectors target RPE cells efficiently after subretinal injection and, in undifferentiated retina, target neural progenitor cells. Lentiviral vectors can carry a cargo of ~7.5 kb (Table 1).

Recombinant AAV vectors do not carry any viral open-reading frames and therfore are generally more favorable from an immunologic standpoint than adenovirus vectors (Table 1). Also, an abundant amount of safety data is available on AAV

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Table 1. Vectors tested *in vivo* in retinal gene therapy proof-of-concept studies[a].

Vector	Cargo limits	Integration	Stability (in large animal models)	Easy to purify for animal studies	Retinal cell targets	Risk of toxic immune response	Used in human ocular studies
Electroporation	Unlimited	No	Unknown (unlikely)	Yes	RPE, PRs; BPs	Low	No
Compact nanoparticles; POD	Unlimited	No	Unknown	Yes	PRs, RPE; GCs, IRs	Low	No
Adenovirus	7.5 kb	No	No	Yes/No	RPE, Müller	High	Yes
Helper independent ("gutted") adenovirus	34 kb	No	Unknown	No	RPE, PRs	Unknown	No
Adeno-associated virus	4.8 kb	No	Stable	Yes	RPE, Müller, PRs, GCs	Low	Yes
Lentivirus	7.5 kb	Yes	Stable	Yes	RPE, PRs	Low	Yes

[a]Although a number of retinal cell targets are listed, the exact targets depend on the route of administration, dose, species, and modifications to the vector. BP, bipolar cell; GC, ganglion cell; IR, inner retinal cell; Müller, Müller cell; POD, peptide for ocular delivery; PR, photoreceptor cell; RPE, retinal pigment epithelium.

administration in animals and in humans, both systemically and intraocularly. Recombinant AAV vectors have the added benefit that they target a more diverse set of cell types than do adenoviral (or other) vectors. AAV vectors do not integrate, or do so only rarely (Table 1). However, AAV-mediated transgene expression in the retina is stable since the transgene persists in episomal fashion in postmitotic differentiated cells. Expression persists for the life of small animals (e.g., mice and rats) and at least for many years in large animals and humans. rAAV vectors are useful for delivering genes efficiently to many types of retinal cells. A disadvantage of these vectors is their relatively limited cargo capacity (a maximum of 4.8 kb) (Table 1).

The retinal gene delivery properties (e.g., cellular specificity, onset of expression) of rAAV vectors can be modified by swapping the capsid from one AAV serotype with another (i.e., creating cross-packaged AAVs) or by altering amino acids in the capsid. This iformation is important for selecting vectors for particular applications. For example, an AAV that targets photoreceptor cells efficiently (AAV8) will be more useful in treating a photoreceptor disease than an AAV that predominantly targets retinal pigment epithelium cells (AAV2) (Vandenberghe et al., 2011).

Preclinical studies: proof-of-concept

Gene augmentation strategies, whereby a wild-type copy of a gene is delivered, have been tested successfully in animal models of approximately 12 different diseases. The animals model conditions such as autosomal recessive retinitis pigmentosa (ARRP), autosomal dominant (AD) RP, Leber congenital amaurosis (LCA), cone rod dystrophy, macular dystrophy, oculocutaneous albinism, achromatopsia, mucopolysaccharidosis VI, AR Stargardt disease, and RP found in disorders such as Bardet-Beidl syndrome and Usher syndrome. Keys to the success of retinal gene augmentation studies include selecting the appropriate vector (see above) and deciding when and where to deliver the vectors. The outcome measures used in the various studies include physiological assays such as electroretinograms (ERGs), evaluations of pupillary light reflexes and optokinetic responses, and tests of visual behavior (e.g., ability to swim through a water maze or to select light or dark areas).

Some toxic gain-of-function mutations have also been treated successfully using gene transfer techniques. Such strategies are necessarily more complex than gene augmentation strategies. The best studied examples of intervention in gain-of-function gene defects include rhodopsin mutations found in ADRP. Such defects result in abnormal cellular trafficking as well as altered functional properties. Deleterious effects of the endogenous mutant genes can be minimized by either a knock-down or a combined knock-down and gene augmentation strategy. The mutant mRNA can be specifically targeted, leaving the wild-type mRNA (either endogenous or delivered via gene augmentation) intact. Knock-down has been achieved successfully by using ribozymes, RNA interference (RNAi), delivery of micro RNAs, and zinc finger nucleases.

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"Generic" gene therapy strategies have been devised that are not specific to a particular disease-causing gene and potentially could be applied to a diverse set of conditions. One approach is to use genes encoding growth or neurotrophic factors or hormones to maintain the health of the diseased photoreceptors. Another approach is to deliver light-sensitive channels, originally isolated from single-cell organisms, to either inner retinal ganglia or remnant cone photoreceptors. This so-called optogenetic therapy has been used to deliver retinal/visual behavior to animals that were previously insensitive to light (Bi et al., 2006; Tomita et al., 2007; Lagali et al., 2008; Busskamp et al., 2010; Caporale et al., 2011; Doroudchi et al., 2011).

Current Status of Gene Therapy Trials for Retinal Degeneration

LCA is a severe, congenital blindness that can be caused by mutations in any one of at least 15 different genes. LCA2, the form resulting from mutations in the *RPE65* gene, which is involved in the retinoid cycle, has been the target of three different gene augmentation therapy clinical trials, all initiated in 2008. Each of the studies used an AAV serotype 2 (AAV2) vector delivering the wild-type human *RPE65* cDNA subretinally to the RPE in one eye. However, the studies differed in terms of dose, inclusion criteria, type of promoter, location of injection, and outcome measures. Each group reported a high degree of safety, and the various groups demonstrated efficacy in the first set of subjects through increases in light sensitivity, improved visual acuity and visual fields, improved pupillary light reflex, and/or improved mobility (Bainbridge et al., 2008; Hauswirth et al., 2008; Maguire et al., 2008). A fourth clinical study, in which the investigational AAV gene therapy was provided under compassionate use only, reported encouraging results for one patient (Banin et al., 2010).

The entire set of results of the Phase 1/2 study were reported by the group at The Children's Hospital of Philadelphia. They indicated that not only was the AAV delivery safe, but each of the 12 clinical trial subjects, aged 8–45 years, showed evidence of improved retinal and visual function, as judged by both subjective and objective testing (Maguire et al., 2009). The children in the study showed particularly large improvements, now being able to read books and play sports, although the older individuals also showed evidence of gain in function.

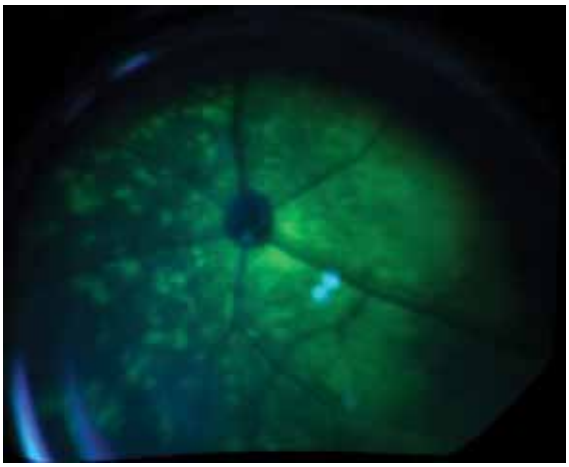

Figure 1. GFP is visible through illumination with blue light with an ophthalmoscope in the injected control eye of this mouse. The mouse had received subretinal injection of 1E11 vector genomes (vg) AAV2/8.CMV.EGFP. CMV, cytomegalovirus promoter; EGFP, enhanced green fluorescent protein.

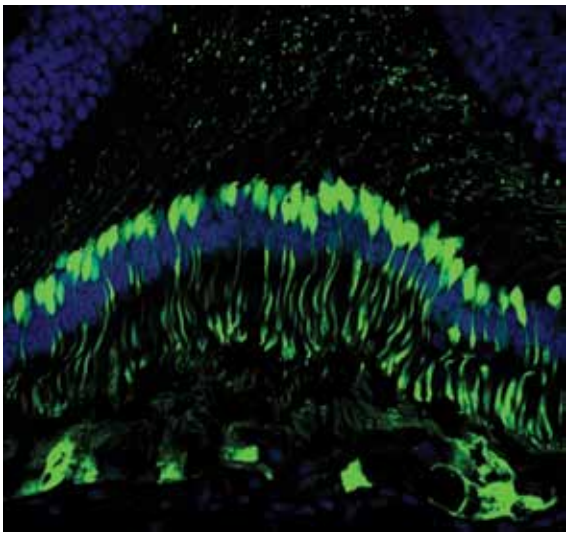

Figure 2. Histologic section from a retina of a monkey injected subretinally in the macula with 1E11 vg AAV8.CMV.EGFP. CMV, cytomegalovirus promoter; EGFP, enhanced green fluorescent protein.

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Challenges of Bringing Retinal Gene Transfer from Bench to Bedside

The successes of the first human gene augmentation therapy studies involving retinal degeneration, the LCA-RPE65 studies (Bainbridge et al., 2008; Hauswirth et al., 2008; Maguire et al., 2008, 2009; Banin et al., 2010; Simonelli et al., 2010), provide the foundation for gene therapy approaches to the treatment of other forms of inherited retinal degenerative diseases. There will be many challenges in extrapolating these approaches to treat other retinal degenerative diseases, as follows:

- Treatment of some retinal diseases will require use of a large transgene cassette — one that does not fit into the current AAV capsid or even the larger confines of lentiviral vectors;
- It will be important to continue to expand the vector toolkit in order to generate reagents that are efficient at targeting photoreceptors and other inner retinal cells;
- Although many animal models of retinal diseases have been described, many are not accessible or are imperfect; thus, additional models are needed;
- It will be important to continue to evaluate the safety of retinal gene transfer, both with respect to responses to the vector and the transgenic protein and with respect to repeat administration (in the contralateral eye); and
- Systematic genetic screening programs of wide breadth are needed to identify subjects who could participate in retinal gene therapy clinical trials.

Many physicians in the United States still tell their patients, "There is nothing that we can do." There are very few guidelines on what is an acceptable level of improvement in retinal/visual function. Additional studies will be needed to develop and adapt outcome measures in order to assess the efficacy of retinal gene therapy. The initial results from functional magnetic resonance imaging (fMRI) studies have shown that the visual cortex can become responsive to visual input after retinal gene therapy, even after prolonged (up to 35 years in the oldest patient) visual deprivation. Additional studies should evaluate the limits to restoration of retinal-cortical pathways.

Conclusions

A huge amount of progress has been made toward developing proof-of-concept of gene therapy for retinal degeneration. In addition, the results of the first few human clinical trials have shown both safety and efficacy. It will not be long before clinical trials are developed for additional gene targets. With continued improvements in vector design and progress toward understanding the genetic and pathologic bases of retinal degenerative diseases, it is likely that gene therapy successes will be reported for other blinding diseases that are currently untreatable.

Acknowledgments

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