Gene Therapy for Treatment of Retinal Disease

BY LAUREN S. TANEY, MD, AND ELIAS REICHEL, MD

In the 1990s, gene therapy emerged as a novel strategy for treatment of human diseases. Early attempts at gene therapy in the United States centered on treatment of severe combined immunodeficiency due to adenosine deaminase deficiency, ornithine transcarbamylase deficiency, and hemophilia. Early setbacks gave way to later successes, resulting in increased acceptance of the concept of genetic therapy for treatment of human disease.

In ophthalmology, early efforts in gene therapy focused on treatment of inherited monogenic diseases such as Leber congenital amaurosis (LCA). Subsequent studies investigated the use of gene therapy for, among others, Usher syndrome 1B, choroideremia, and Leber hereditary optic neuropathy (LHON). Recently, attention has expanded to encompass the use of genetic therapy for more common and multifactorial eye diseases such as age-related macular degeneration (AMD) and diabetic retinopathy. Many preclinical and clinical studies that employ genetic therapeutic techniques are under way, with promising results.

New treatment approaches for retinal disease by way of genetic therapy have important implications. Ultimately, these techniques may complement or supplant existing approaches to treating retinal disease.

CONCEPTS IN GENE THERAPY

Gene therapy can be approached in two primary ways: (1) delivery of genetic material into cells, or (2) direct introduction of proteins into cells. Either can be accomplished at a cellular level by use of a variety of vectors, viral or nonviral, that target specified cells for delivery. In the case of retinal diseases, target cells may include Müller, photoreceptor, and retinal pigment epithelial cells.

The term *transduction* refers to the delivery of somatic genetic material, specifically nucleic acids, into cells. Transduction can be used to achieve gene addition, gene correction, or gene knockdown.

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Gene addition, the most commonly employed strategy, involves the introduction of genetic material into cells that would otherwise lack such material. For inherited retinal diseases, gene addition techniques can be used to target loss-of-function genetic mutations in photoreceptors or the retinal pigment epithelium (RPE).

Gene addition targeting the photoreceptors has been accomplished in preclinical studies in X-linked retinoschisis, Stargardt disease, and achromatopsia.[1-4] Gene addition targeting the RPE has been applied in clinical studies on LCA and preclinical studies on choroideremia, ocular albinism, and Usher syndrome 1B. Treatment of LCA2 currently centers upon gene addition in RPE cells that lack the genetic material responsible for the production of the protein RPE65. Complementary DNA (cDNA) for RPE65 has successfully transduced RPE cells in patients with LCA2, enabling production of RPE65 protein that functions in isomerization of 11-cis-retinal from all-trans-retinyl esters.[5] Another example of gene addition is that of delivery of choroideremia cDNA to cells lacking the CHM/REP1 gene that encodes for production of Rab escort protein 1.[6,7]

Gene correction is the least commonly performed of the three techniques. It depends upon the delivery of genetic material that produces nucleases which edit preexisting cellular genetic material and thereby modify expression of target proteins.

Gene knockdown involves gene silencing through introduction of noncoding genetic material that inhibits production of target proteins either by repressing translation or by altering cleavage patterns such that undesired target proteins are not produced. This strategy may work best for conditions attributable to gain-of-abnormal-function mutations, many of which are inherited in an autosomal dominant pattern. Gene knockdown can be accomplished by introduction of different subtypes of RNA, such as RNA interference (RNAi), microRNA (miRNA), short hairpin RNA (shRNA), or double-stranded RNA.[8] The concept has been demonstrated in vitro and in vivo in preliminary studies on suppression of RHO-linked retinitis pigmentosa using adeno-associated virus (AAV)–mediated delivery of RNAi molecules.[9]

The alternative to introducing genetic material into cells is to directly introduce proteins into cells in order to effect a desired response. Typically, proteins are introduced into cells via viral or nonviral vectors, just as in the case of transduction. Several classes of proteins may be utilized, and the choice of protein naturally depends on the basis of the disease being treated. Structural protein replacement may be a goal of treatment. Insertion of necessary enzymes into deficient cells may likewise be a target, as is the case in neuronal ceroid lipofuscinosis.[10]

Alternatively, introduction of protein factors may be a modality for disease treatment. Neurotrophic and antiapoptotic factors such as fibroblast growth factor (FGF), ciliary neurotrophic factor (CNTF), and glial-derived neurotrophic factor (GDNF) may play a role in promotion of photoreceptor cell survival in retinal degenerative diseases such as retinitis pigmentosa. For diseases characterized by neovascularization, antiangiogenic factors such as pigment epithelium-derived factor (PEDF), angiostatin, and endostatin are more appropriate choices for therapy.

A limitation of protein-based genetic therapy is that continued replacement or replenishment of proteins may be necessary, as the framework for continued production of such proteins does not exist without the introduction of coding nucleic acids into cells. In the case of transduction, however, the continued presence of genetic material that encodes for desired proteins makes cells theoretically capable of producing such proteins long-term through ongoing transcription/translation.

DELIVERING GENE THERAPY IN THE EYE

In general, some of the challenges posed by gene therapy center on safety, immunogenicity, mutagenesis, and feasible vector manufacturing and delivery. The eye, however, has intrinsic features that make it an ideal target for gene therapy. Courtesy of the blood-retina barrier, the eye is an immunologically privileged space where classical immune responses are limited. Thus, vectors introduced into the eye are much less likely to incite a systemic immunologic response capable of damaging the eye itself or destroying the vector and its intended therapy. Additionally, the eye is a relatively isolated compartment with a small volume, so direct introduction of a finite amount of genetic material with minimal systemic exposure is possible. By avoiding intravascular infusions or intramuscular injections, intravitreal or subretinal introduction of genetic material minimizes attendant systemic safety risks. Finally, responses to treatment can be directly assessed with relative ease by ophthalmic examination or monitoring with use of routine ancillary testing.

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Viral and nonviral vectors have been studied for delivering genes and proteins to ocular tissues.

Viral Vectors

Viral vectors include adenovirus (Ad), AAV, retrovirus (RV), and lentivirus (LV). When used as vectors, these viruses are disabled genetically so that they are unable to cause disease once a target cell is infected. The selection of a viral vector depends on the intended target cell and duration of effect.[11] Recognition of viral capsids by specified receptors on certain cells influences cell tropism; therefore, much research currently revolves around engineering viral capsids in order to enhance the transductive properties of a viral vector.[12]

The two viral vectors most commonly used are AAV and LV. AAV, a member of the parvovirus family, is a nonenveloped, replication-defective virus 18 to 26 nm in size.[13] LV belongs to the Retroviridae family, which tend to be larger viruses (80-120 nm) that are capable of infecting nondividing cells.[14] Because AAV is smaller in size than LV, less genetic material can be introduced into AAV. AAV can accommodate up to 4.7 kb of genetic material, while LV can incorporate up to 10 kb.8 However, the smaller size of AAV makes it a more ver-

satile choice in terms of delivery to the outer retina and photoreceptors.

Another difference between AAV and LV is that AAV does not integrate its genome into the host cell genome. Transgenic material exists as an episome in the case of AAV, while LV vectors integrate genetic material into host chromosomes, resulting in a higher likelihood of causing mutagenesis.

Viral vectors targeting the outer retina and/or the RPE can be introduced in multiple ways, but the main routes of delivery include intravitreal, subretinal, and suprachoroidal delivery. Intravitreal delivery is less invasive than subretinal delivery as it can be easily performed in the office. However, intravitreal delivery relies on diffusion through the retina in order to target deficiencies in the photoreceptors or RPE. AAV is small enough to diffuse through the retina, but diffusion may nonetheless be hampered by anatomic barriers such as the internal limiting membrane (ILM) and the inner retina. In some models, laser or intravitreal enzymes (proteases capable of ILM digestion) may be used as adjuncts in order to facilitate diffusion. LV is too large to diffuse through the retina and thus must be delivered subretinally. Subretinal delivery traditionally must be combined with vitrectomy; therefore, it is more invasive, costly, and prone to complications.

Nonviral Vectors

Nonviral vectors include liposomes, lipoplexes, polyplexes, nanoparticles, microparticles, or a combination of these. A major benefit of nonviral vectors is their unlimited carrying capacity. However, while nonviral vectors may represent a safer choice than viral vectors as vehicles for transfection, they tend to transfect less effectively and have shorter lives than viral vectors. Techniques that rely upon electricity (iontophoresis, electrotransfer), hydrostatic pressure, or ultrasound (sonophoresis) can be used to enhance the efficacy of transfection.[15]

FROM MONOGENIC ORPHAN DISEASES TO MULTIFACTORIAL DEGENERATIVE DISEASES

Early efforts in genetic therapy naturally focused on monogenic retinal diseases with an identified culprit such as absent RPE65, CHM/REP1, and MYO7A proteins. However, the relative rarity of orphan diseases such as LCA2, choroideremia, and Usher syndrome contrasts with commonplace clinical entities such as AMD and diabetic retinopathy. At a time when research is shedding light on the genetic basis for complex multifactorial conditions such as AMD and diabetic retinopathy, attention has shifted in the direction of researching genetic therapeutics for such diseases.

Several clinical trials have been completed or are in progress for treatment of exudative AMD with genetic therapy. A phase 1 clinical trial was conducted on intravitreal delivery of the antiangiogenic factor PEDF via an Ad vector (Ad-CMV-PEDF.11) for treatment of eyes with choroidal neovascularization due to AMD. The study results were promising, suggesting that delivery of PEDF may limit progression of angiogenesis.[16]

A more recent phase 1 study (NCT01024998) has centered on intravitreal injection of genetic material encoding a soluble VEGF receptor decoy, sFLT01, via AAV2 vector. sFLT01 genetic material results in the production of a modified soluble Flt1 receptor with antiangiogenic properties, due to the protein's ability to bind to and neutralize circulating VEGF-A and placental growth factor (PlGF).[17,18] Thus, intravitreal injections of AAV2-sFLT01 have the potential to inhibit choroidal neovascularization due to exudative AMD. An ongoing phase 1 study (NCT01301443) is looking at subretinal injections of LV–vector-based genetic material capable of expressing the angiostatic proteins angiostatin and endostatin. Introduction of RNAi via viral vectors has also been studied in phase 1 and phase 2 studies on the following: intravitreal bevasiranib (Cand5), intravitreal Sirna-027 (AGN211745), and intravitreal PF-04523655 (REDD14NP).

Multiple preclinical studies of treatments for nonexudative AMD are also under way. Many of the treatment strategies focus on interfering with complement-mediated cellular destruction, thought to be responsible for a significant portion of AMD-mediated damage to RPE and choroidal cells.[19] Hemera Biosciences has developed the molecule HMR59. When injected intravitreally, AAV2-HMR59 results in increased production of a naturally occurring membrane-bound protein known as soluble CD59 (sCD59) in ocular cells.[20] sCD59 inhibits cellular destruction by blocking membrane attack complex (MAC), which represents the final step of the classic complement cascade. Increased production of sCD59 thus protects cells from MAC-activated destruction while not disrupting upstream portions of the complement cascade that are necessary for maintenance of ocular homeostasis.[21] Protection from MAC-mediated cellular lysis may ameliorate damage associated with dry AMD.

Wellstat Ophthalmics has developed AVT-101, a protein that downregulates the alternative complement pathway and can theoretically inhibit attendant changes associated with dry AMD. The company Retrosense has developed AAV-delivered RST-001, a gene that codes for channelrhodopsin-2 proteins that may increase the photosensitivity of retinal cells damaged by dry AMD.[22,23]

In diabetic retinopathy, early studies have focused on the AAV-mediated delivery of angiotensin converting enzyme 2 (ACE2) and angiotensin 1-7 (Ang 1-7) genes capable of local retinal production of these vasodilatory proteins.[24] Another example of ongoing work is use of LV vectors such as HIV to deliver genes capable of producing antiangiogenic proteins such as angiostatin.[25]

The list of preclinical and clinical studies above is not intended to be a comprehensive survey of work in the field. Rather, these investigations are representative of the many directions gene therapy is taking as initial research has bred increased interest and successes in treatment of retinal diseases.

CONCLUSION

Although many advances have been made in the use of gene therapy for treatment of retinal diseases, challenges remain. One significant obstacle concerns the timely delivery of gene products so that components of the visual system can be salvaged by introduction of therapeutic proteins; this may pose a significant challenge, independent of compounding factors such as amblyopia and developmental abnormalities due to visual dysfunction. Another poorly understood issue is the duration of effect of transgenes.

It remains to be seen whether long-term gene expression can truly be achieved after a single delivery of transgenetic material to target cells, or whether continued administration may be necessary. Development of adequate animal models on which to conduct preclinical testing may be fraught with difficulty. Additionally, complex societal factors such as funding appropriation and disease prioritization must be taken into account.

Despite these challenges, gene therapy has clearly emerged as a viable technique for managing disease and promises to revolutionize our ability to treat retinal disorders in the future. ■

Elias Reichel, MD, is vice chair for research and education, department of ophthalmology, at New England Eye Center, and a professor of ophthalmology at Tufts University School of Medicine in Boston. He is a Retina Today *Editorial Board Member. He has a financial interest in Hemera Biosciences. Dr. Reichel may be reached at ereichel@tuftsmedicalcenter.org.*

Lauren S. Taney, MD, completed a medical retina fellowship at New England Eye Center, Tufts Medical Center in Boston, MA. She currently practices at Washington Eye Physicians and Surgeons, with offices in Chevy Chase, MD and Washington, DC. She has no financial interests in any of the material presented in this article. Dr. Taney may be reached at laurentaneymd@gmail.com

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