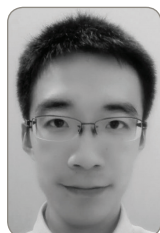


GENE THERAPY IN A DROP

The lowdown on an investigational, noninvasive gene delivery system for the treatment of retinal disease.

BY KUAN JIANG AND GANG WEI, PhD



Age-related macular degeneration (AMD) and proliferative diabetic retinopathy (PDR) are sight-threatening diseases with limited treatment options. VEGF has been identified in the pathogenesis of both disease states

and is consequently regarded as an important therapeutic target. Angiogenesis is a principal component in the pathogenesis of these diseases, and VEGF is recognized as a major mediator of this process.¹⁻³ In the past decade, treatment of patients with AMD and PDR has increasingly been performed using monthly intravitreal anti-VEGF injections.

Research into gene therapy for the treatment of patients with AMD and PDR emerged as a way to potentially decrease the frequency of these injections. In one potential approach to gene therapy for AMD, a viral or nonviral vector is employed to carry desired genetic information to target cells, and a gene vector then constantly expresses therapeutic factors antagonizing VEGF.⁴⁻⁶ The goal of gene therapy is to provide a sustained therapeutic benefit by way of continual expression of the protein or proteins that modulate the pathogenesis of the relevant disease. However, as encouraging as the concept of gene therapy may seem, there are barriers to its use.

In the clinical trials that have been conducted to date, ocular gene therapy is typically administered to the vitreous and the retina subretinally or by intravitreal or suprachoroidal injection. Although there is minimal risk of immune reaction and systemic spread when a gene vector is injected into the eye,⁷ these procedures do carry the risk of endophthalmitis, retinal detachment, and increased intraocular pressure.⁸⁻¹⁰

A NONINVASIVE GENE DELIVERY OPTION?

An ideal method of ocular gene delivery that would not involve the risks mentioned above would be noninvasive and easy to prepare and apply, as in the case of a topical eyedrop. However, most therapeutic gene therapy administered in this manner would be eliminated because of tear rinsing. Furthermore, the corneal epithelium is almost impermeable to hydrophilic macromolecules, so topical application would result in unacceptable waste of expensive gene medicine.

The same predicament exists for gene therapy delivered

systemically: Few macromolecules could penetrate the blood-retina barrier and distribute themselves into the eye, and their presence in the bloodstream could induce adverse effects on the body as a whole.^{11,12}

THE CONCEPT

Penetratin is a cell-penetrating peptide (CPP) derived from antennapedia homeoprotein. Cellular internalization of CPPs often involves the crossing of a biological membrane, and these molecules are capable of carrying hydrophilic cargoes into cells. With good biocompatibility to ocular tissues, penetratin efficiently delivered conjugated fluorescence probes to the retina after topical instillation.¹³

Poly(amidoamine), or PAMAM, is a class of dendrimer that possesses strong gene condensation and protection ability. In a 2014 study, low molecular weight PAMAM (G3 PAMAM) did not show cytotoxicity to ocular tissues.¹⁴

To study the potential for use of these molecules for topical gene therapy delivery, we constructed and evaluated in vitro and in vivo a simple, noninvasive gene delivery system composed of penetratin and G3 PAMAM.¹⁵

The physical mixture of G3 PAMAM and penetratin in an appropriate ratio formed a compact nanoscale complex with 150 nm diameter and a zeta potential of approximately



AT A GLANCE

- Gene therapy aims to provide a sustained therapeutic benefit by continually expressing a protein or proteins that modulate the pathogenesis of a particular disease.
- Typically, gene therapy targeting a retinal disease is administered to the vitreous and retina subretinally or by intravitreal or suprachoroidal injection, procedures that involve some risk.
- In a study conducted by the authors, a nanoscale complex formed by the combination of penetratin and poly(amidoamine) showed impressive gene expression in ocular cells when administered topically.



30 mV. The complex, when instilled in the conjunctival sac of rats, demonstrated impressive gene expression in ocular cells. Besides facilitating powerful cellular internalization, after topical instillation the complex penetrated rapidly from the ocular surface to the fundus and resided in the retina

for more than 8 hours, resulting in efficient expression of red fluorescent protein plasmid in the posterior segment.¹⁵ This finding suggests that the delivery system provided protection for the gene against degradation by enzymes during its journey from the ocular surface to the back of the eye. Additionally, the intraocular distribution of the complex illustrated that the plasmids were absorbed into the eyes through a noncorneal pathway, perhaps through the sclera, which is permeable to hydrophilic micromolecules.¹⁶ Fluorescence images of the retinæ subjected to the double fluorescence-labeled complex revealed that penetratin and plasmid reached the fundus oculi together after topical

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OCULAR GENE THERAPY: CLINICAL TRIUMPHS AND REMAINING CHALLENGES

An editorial commentary by Szilárd Kiss, MD

Gene therapy involves the delivery of genetic material that encodes a therapeutic molecule via a nonpathogenic virus capable of infecting a target cell. Alternatively, the genetic material can be delivered via a nonviral vector, as in the work of Jiang and Wei. In theory, once this genetic material is delivered, the target cell will produce the therapeutic molecule indefinitely. In many ways, the eye is an ideal site for gene therapy—it is a relatively small, self-contained, immune-privileged organ, and many of the well-characterized disorders that affect it require long-term treatment strategies.

The successful management of RPE65-associated Leber congenital amaurosis has taken the concept of gene therapy from science fiction to clinical reality. In a phase 3 clinical trial, subretinal delivery of SPK-RPE65 (Spark Therapeutics) demonstrated the ability to safely and durably restore functional vision in patients who otherwise would have gone blind.¹

A surgical approach to treating patients with RPE65-associated retinal degenerations may be ideal and acceptable, but, if ocular gene therapy becomes a viable treatment strategy, then a less invasive method may be more desirable.

The human eye contains numerous barriers (eg, the internal limiting membrane) that prevent infection by naturally occurring viruses. Because many of the viral vectors used in gene therapy are variants of naturally occurring viruses—disease-causing viruses, such as adeno-associated virus type 2—many people already have antibodies to these viruses that may also prevent any gene therapy product from delivering the desired therapeutic molecule to its target cell.

In the accompanying article, Jiang and Wei report an interesting approach to overcoming some of these barriers using penetratin in combination with a nonviral gene vector. They report that, using this method in vivo, they have been able to deliver

genetic material to the posterior segment with a topical drop administered to the ocular surface. Although their preliminary preclinical data using reporter constructs look promising, experiments in larger animals (eg, nonhuman primates) and proof of delivery of therapeutic molecules in sufficient concentrations are needed before moving their work into humans. Moreover, a drop form of gene therapy may not be the most desirable delivery method, as it could allow systemic exposure of the therapeutic molecule, which may lead to systemic adverse events (eg, Anti-Platelet Trialists' Collaboration events) due to high systemic expression of anti-VEGF molecules.

Regardless of which gene delivery methods (subretinal, intravitreal, suprachoroidal, or topical) become mainstream, there is no denying that we stand at the cusp of a gene therapy revolution in the treatment of ocular disorders. There are perhaps a dozen gene therapy clinical trials under way or in planning stages for the treatment of a variety of inherited and acquired ocular disorders. Most exhilarating of all is the possibility in the next 1 to 2 years of a gene therapy product becoming available for the treatment of patients with RPE65-associated retinal degenerations. It is nice to see the field moving forward both in the laboratory, as with Jiang and Wei's research, and on the clinical front.

1. Maguire AM. Phase 3 trial of AAV2-hRPE65v2 (SPK-RPE65) to treat RPE65 mutation-associated inherited retinal dystrophies. Paper presented at: American Academy of Ophthalmology Annual Meeting; November 14-17, 2015; Las Vegas, NV.

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instillation, implying that penetratin played a crucial role in transporting plasmid into the eye by virtue of the powerful capability of membrane translocation and cellular uptake.

To further demonstrate that the nanoscale complex could be a promising system for posterior delivery of ocular gene therapies, experiments assessing the therapeutic effects of a complex containing small interfering RNA targeting VEGF are in progress in murine models.

CONCLUSION

With ongoing clinical trials studying the use of gene therapy in various retinal diseases, including PDR and AMD, this is an exciting area of drug development, and further advances could change the way clinicians treat these and other sight-threatening conditions. An effective topical mode of gene therapy delivery to the back of the eye would remove significant barriers to further development in this field. ■

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