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Please cite this article as:


DOI: doi: 10.1097/ICU.0b013e3283541eb6

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Gene therapy for corneal dystrophies and disease, where are we?

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Number of words excluding references = 2251 (2500 max excluding refs)

Keywords

viral vectors, nanoparticle gene delivery, corneal dystrophies, neovascularization, scarring and haze, corneal transplantation
Abstract

Purpose of review
We assess studies on vector systems for delivery of transgenes to the cornea that have been published over the last year, and summarise new work on the identification of specific transgenes for corneal diseases.

Recent findings
Adeno-associated viral vectors (AAV) are increasingly being successfully applied to the cornea, although transgene expression requires corneal epithelial debridement, or intrastromal injection of the vector. Gene delivery platforms based on nanoparticles of chitosan or gold also show promise. Over-expression of vasoinhibin-1 or decorin, or siRNA-mediated blockade of the cannabinoid receptor CB1, can all reduce corneal neovascularization. Over-expression of decorin or matrix metalloproteinase 14 can reduce corneal fibrosis and haze, while over-expression of c-Met accelerates epithelial wound healing. Induction of corneal endothelial cell replication by over-expression of E2F2, p16 or p21 can maintain or even increase corneal endothelial cell density in eye bank corneas. Over-expression of the anti-apoptotic transgenes Bcl-xL or p35 significantly enhances corneal endothelial cell survival and reduces apoptosis in stored human corneas.

Summary
Despite a wealth of information on methods for the delivery of nucleic acids to the human cornea, and ever-increasing information transgenes with substantial therapeutic potential, gene therapy for corneal disorders has yet to reach the clinic.
Key Points

- Adeno-associated viral vectors (AAV), and delivery platforms based on nanoparticles, are being successfully applied to the cornea.

- Corneal neovascularization can be reduced by over-expression of inhibin-1 or decorin. Blockade of the CB1 cannabinoid receptor shows particular promise because of its specificity for actively-proliferating endothelial cells.

- Use of gene therapy to prevent stromal scarring and haze after refractive surgery, or to accelerate corneal epithelial healing, now appears within reach.

- Gene transfer approaches designed to increase or maintain corneal endothelial cell density in human donor corneas retrieved for corneal transplantation include the reduction of endothelial cell apoptosis, or the induction of endothelial cell replication by modulation of the cell cycle.

- Despite ever-increasing availability of suitable viral and non-viral vector delivery systems, and a plethora of suitable transgenes, gene therapy for the human cornea has yet to reach clinical practice.
Introduction

Gene therapy for the correction of diseases affecting the cornea has been mooted for twenty years, and has been applied in a wide variety of experimental models. In this review, we assess recent work on vector systems for delivery of transgenes to the cornea, and also examine the outcomes of delivery of specific transgenes for specific conditions. Virtually all the reported work on corneal gene therapy over the past year has been in animal models, or on human cells in vitro or on human corneas ex vivo.

Viral vectors for use in the cornea

Viral gene therapy vectors are incomparably efficient, producing rapid, high-level expression of the requisite transgene. The major classes of viral vectors used hitherto to transfect the cornea successfully have been adenoviral vectors and lentiviral vectors. However, over the past year, the use of adeno-associated (AAV) viral vectors has been further explored [1-3]. Serotypes AAV6, AAV8 and AAV9 were shown to transduce both murine and human corneas following removal of the epithelium, with AAV9 being the most efficient [1]. AAV5 was shown by the same investigators to be able to transduce equine corneal fibroblasts in vitro [2] and the rabbit cornea [3]. In corneas, expression of the transgene was maximal after about 7-14 days. An alternative strategy was delivery by injection into the corneal stroma, which also yielded good expression for serotype AAV2 in murine corneas [4]. However, these approaches may be limited in their clinical utility, since extensive corneal epithelial debridement and stromal injection both carry some risks of morbidity. However, the application of AAV vectors to the cornea is interesting in the light of the successful use of AAV2 in clinical trials for Leber's congenital amaurosis [e.g. 5], because many of the issues in producing clinical-grade vector stocks and in
handling the ethical and regulatory frameworks have already been overcome.

Further work has also been reported with lentiviral vectors. An HIV-1-based lentiviral vector carrying the transgene ovine interleukin 10 was used to transfect ovine corneal endothelium \textit{ex vivo} prior to orthotopic corneal transplantation in outbred sheep [6]. Corneal allograft survival, the endpoint of interest, was prolonged to a modest but statistically significant extent, compared with the control group. In another study, a commercially-available lentiviral vector was shown to transduce rabbit primary epithelial cells cultured \textit{ex vivo}. After a short delay in expression, a reporter gene was stably expressed over time in a dose-dependence fashion, and was transferred not only to mature corneal epithelial cells, but also to putative epithelial stem cells [7], paving the way for future gene transfer approaches to improve outcomes for limbal stem cell dysfunction.

Relatively little new work has been performed with adenoviral (AV) vectors, but of note was the successful delivery of the c-Met gene to human corneal epithelium \textit{in vitro}, using a combination of long transduction time (48 hours) and sildenafil (Viagra) at 75 µg/ml as a transduction enhancer [8]. In another study, subconjunctival injection of an AV vector expressing human vasohibin-1 resulted in expression in the central corneal stroma in an \textit{in vivo} mouse model [9]. It is clear that despite the many problems associated with adenoviral gene therapy vectors, they are still favoured by a number of investigators for use in the cornea.

**Gene delivery platforms involving nanotechnology**

A number of groups are investigating the use of nanoparticles and nanomaterials as
platforms for drug, cell and nucleotide transfer to the eye. One such interesting and well-developed system is based on the hydrophilic, cationic polysaccharide chitosan, which has been adapted for the delivery of both hydrophilic and lipophilic drugs such as indomethacin and cyclosporin A, and (of more importance in the current context) polynucleotides, to the ocular surface [10]. Chitosan therefore has some potential for gene therapy directed to the cornea and conjunctiva. Commercially-available ultrapure (bacterial endotoxin-low) chitosan oligomers complexed with cDNA encoding a reporter gene have been shown to transfect rat keratocytes following intrastromal injection, but the formulation of chitosan-containing nanoparticles was not more effective than was the direct injection of naked DNA [11]. Topical delivery of the chitosan nanoparticles to the eye, which would have been of great interest, was not examined in this study.

Another platform for gene delivery is based on gold nanoparticles. Sharma et al [12] have explored the potential of polyethylenimine-conjugated gold nanoparticles (GNPs) for gene transfer to the corneal stroma. Successful transduction of human corneal fibroblast cultures in vitro, and rabbit corneas in vivo, was observed. Of particular interest was the relatively slow release of the nucleic acid cargo and the slow clearance from the target tissue. However, in vivo clearance rates may be affected by the degree of neovascularization. The GNPs exhibited good biocompatibility, although toxicity was dependent on the nitrogen to phosphorus ratio used to prepare the particles. Despite gold particles being demonstrable in the cornea, there was no apparent visual disturbance. The fact that gold nanoparticles were found both within corneal cell nuclei and the extracellular matrix suggests that such particles may also be suitable for slow-release drug delivery (rather than gene
delivery) to the cornea.

**Gene therapy for corneal dystrophies**

Whilst mutations in the genes causing some of the corneal dystrophies are now well characterised, little practical progress has been made in gene therapy in humans [13]. However for Meesmann epithelial corneal dystrophy, which is caused by a dominant-negative mutation in either keratin K3 or K12 in the anterior corneal stroma, *in vitro* data on cultured cells suggested that an siRNA against a mutant allele of the keratin gene might provide some therapeutic benefit [14].

**Approaches to reduce corneal neovascularization**

The significant role of vascular endothelial growth factor-A (VEGF) in corneal neovascularization has long been recognised, and interference with the VEGF signalling pathway has been approached by several groups in different ways. Subconjunctival injection of an AV vector encoding human vasohibin-1 significantly reduced corneal neovascularization in mouse corneas subjected to alkaline burns, and the effect appeared to be related to down-regulation of VEGF-R2-expression [9]. Only the effect on inflammation-driven neovascularization was examined, and potential effects on other types of ocular neovascularization would be of interest.

Blockade of the cannabinoid receptors CB1 and CB2 has been previously reported to inhibit VEGF signalling, and CB1-specific pharmacological blockade or siRNA-mediated knockdown of CB1 has been shown to inhibit basic fibroblast growth factor (bFGF)-driven vascular endothelial proliferation, migration and tube formation [15]. A strong synergy between VEGF and bFGF in the induction of neovascularization is
well recognised. These effects were replicated in an *in vivo* model of rabbit corneal neovascularization (an example of an inflammatory process), and also in a mouse model of oxygen-induced retinopathy, indicating the importance of this pathway for neovascularization independent of the initiating factor. Importantly, only actively proliferating vascular endothelial cells were affected, suggesting that blockade of CB1 might be an interesting target for new anti-angiogenic strategies, with little risk of a non-specific toxic effect in the pre-existing mature vasculature.

In an *in vivo* rabbit model of VEGF-induced corneal neovascularization, AAV-mediated expression of decorin, a small leucine-rich proteoglycan, also resulted in significant inhibition of neovascularization [16]. A marked decrease in VEGF expression at the mRNA level in corneas treated with decorin suggests that the anti-angiogenic effects observed were at least in part due to attenuation of VEGF expression by inflammatory cells recruited to the tissue. However, decorin, which is expressed by normal cornea, also interacts with growth factors including transforming growth factor-beta (TGFβ) to regulate collagen fibrillogenesis and extracellular matrix deposition, both of which are crucial in corneal wound healing. Decorin is thus an attractive target for gene therapy approaches to reducing corneal scarring and haze [17].

**Gene therapy for corneal scarring**

Expression of TGFβ in the healthy cornea is restricted to the corneal epithelium, but this growth factor is secreted into the stroma during wound healing where it contributes to myofibroblast formation, haze and scarring [18]. AAV-mediated over-expression of decorin by human corneal fibroblast cultures did not alter cell
morphology or viability, but prevented TGFβ-driven transformation of keratocytes and corneal fibroblasts to myofibroblasts [19]. Subsequent in vivo studies in a rabbit model of laser-induced corneal fibrosis confirmed the inhibition of myofibroblast formation and reduction of haze, and demonstrated no immunogenic or toxic effects [20].

Other approaches have included over-expression of matrix metalloproteiinase 14 by corneal stroma in vivo in a murine model of corneal scarring. In this model, fibrotic repair also occurs via differentiation of keratocytes into myofibroblasts, which was significantly inhibited, along with collagen type III deposition [21]. However, some severe toxic effects were evident at higher doses when virus was injected into the corneal stroma.

One of the difficulties with these and similar models is the need to either debride the corneal epithelium prior to gene delivery, or to inject the viral vector directly into the corneal stroma. For improvement of wound healing in human corneas of diabetes-affected donors, who suffer from down-regulation of migratory growth factors such as hepatocyte growth factor (HGF), over-expression of the HGF receptor c-Met led to normalisation of HGF signalling and accelerated epithelial wound healing in vitro [8]. This approach was especially attractive because an improved delivery technique did not involve corneal epithelial ablation to facilitate gene delivery. Adenoviral-mediated gene expression is only short term, and diabetic patients may benefit most from this type of therapy in the event of an acute injury. In any case, short-term gene expression is probably preferable, given that long-term expression of the proto-oncogene c-Met might yield unexpected side-effects, including malignancy.
Approaches to protection of corneal endothelium and epithelium

Gene transfer approaches designed to increase or maintain corneal endothelial cell density in human donor corneas retrieved for corneal transplantation have recently been elegantly reviewed by Kampik, Ali and Larkin [22]. The options include the reduction of endothelial cell apoptosis, or the induction of endothelial cell replication by modulation of the cell cycle. Anti-apoptotic transgenes under investigation include Bcl-xL and p35 [23,24]. Transgenes designed to induce endothelial cell replication include E2F2, encoding a transcription factor that induces cell cycle progression, and p16 and p21, which encode cyclin-dependent kinase negative regulators of the cell cycle [22]. Over-expression of either mammalian Bcl-xL or baculoviral p35 in human Eye Bank corneas, following transduction with a lentiviral vector, significantly enhanced corneal endothelial cell survival and reduced apoptosis in corneal storage [23,24].

Reviews

A number of reviews of gene therapy for the cornea have been published over the past year, each with a somewhat different emphasis [13,22,25].

Conclusion

Where are we with respect to translating gene therapy into benefits for individuals with cornea disease? Sadly, not very far. Despite a wealth of information on methods for the delivery of nucleic acids to the cornea, including the human cornea, and increasing information on the mutations that are disease-causative in humans, especially in corneal dystrophies, clinical trial data on gene therapy for corneal disorders have yet to be published.
Acknowledgements

KAW is supported by the National Health & Medical Research Council of Australia.

Conflicts of interest

There are no other conflicts of interest.
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1 Sharma A, Tovey JCK, Ghosh A, Mohan RR. AAV serotype influences gene transfer in corneal stroma in vivo. Exp Eye Res 2010; 91:440-448.


* A comprehensive review on the potential of some chitosan-based nanomaterials.


* Describes a gene therapy approach for the eventual treatment of Meesmann epithelial corneal dystrophy.


* Interesting because the target appears to have specificity for actively-proliferating vascular endothelial cells.


** An excellent review that includes a useful discussion of gene therapy approaches to maintain corneal endothelial cell density in human donor corneas.


** A description of the value of anti-apoptosis strategies to protect the corneal endothelium in corneal storage in the eye bank.