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Gene Therapy and Animal Models for Retinal Disease

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Abstract

Those plagued by retinal diseases are often robbed of their vision, as often, effective treatments do not exist. Knowledge of the pathophysiology of retinal diseases stems from research on available animal models. Gene therapy may be useful for both genetic and acquired retinal diseases. This review will focus on retinal diseases for which gene therapy has demonstrated promise. The diseases are presented in order of the age at which they are generally first symptomatic and include retinopathy of prematurity, Leber congenital amaurosis, mucopolysaccharidoses, retinoblastoma, retinitis pigmentosa, diabetic retinopathy, glaucoma and age-related macular degeneration. We will describe the animal models used to study these disorders and emphasize the progress that has been made in using gene therapy for the treatment of retinal disease.

Introduction

The retina is a highly organized, complex, neural structure that is responsible for visual processing. Diseases affecting this tissue can be particularly devastating, as vision loss is irreversible and frequently severe. These diseases are often multifaceted, and treatments are limited by our incomplete understanding of the mechanisms that lead to blindness.

In order to further our understanding of acquired and inherited retinal diseases we depend heavily on animal models. Many models have arisen from spontaneous mutations, while others have been induced by environmental factors. Technological advances in genetic engineering have also enabled

researchers to develop genetically engineered models that either overexpress genes known to lead to retinal disease or disrupt normal retinal gene function. These animals continue to expand our understanding of basic retinal biology and help to unravel the mysteries surrounding mechanisms of retinal disease.

Gene therapy may prove to be a feasible treatment option for patients with retinal diseases. Viral vectors can be used to transport therapeutic material to cells of the retina. Adenovirus (Ad), adeno-associated virus (AAV) and lentivirus are most commonly used for retinal transgene delivery. These vectors differ in their tropism for specific cell types; consequently, it is necessary to select a viral delivery system based on the ocular target [1]. Genes may themselves cause disease if they are targeted to the wrong cells. As different viruses vary in their onset and duration, these factors must be considered in selecting an appropriate delivery vehicle [1].

Here we review nine different disorders affecting the retina. They include retinopathy of prematurity, Leber congenital amaurosis, mucopolysaccharidosis, retinoblastoma, autosomal recessive retinitis pigmentosa, diabetic retinopathy, autosomal dominant retinitis pigmentosa, glaucoma and age-related neovascularization. These diseases are listed chronologically; according to the age symptoms first appear. We provide updates on the pathophysiology of each disease and provide an overview on results of gene therapy studies.

Retinopathy of Prematurity (ROP)

ROP is responsible for most cases of childhood blindness in the developed world, and specifically affects premature neonates [2]. Infants delivered at full term normally possess a completely vascularized retina. In contrast, premature neonates possess an incompletely vascularized retina. The incomplete vascularization coupled with the changes in oxygenation after birth often leads to a relative hypoxia in the avascularized retina. This stimulates the growth of new abnormal vessels at the juncture of the vascularized and avascularized retina. The vessels extend from the retina into the vitreous, causing hemorrhage and retinal detachment [2].

Current treatments for ROP are limited to cryosurgery and laser ablation of avascular retina [2]. The treatments are far from perfect as they themselves destroy functioning retina. Fortunately, a reproducible and quantifiable murine oxygen-induced retinopathy model of ROP exists, which may be used to test potential candidate treatments [3]. In this model, 1-week-old C57BL/6J mice are exposed to 75% oxygen for 5 days and then returned to room air. Retinal neovascularization results with a maximal response seen between postnatal days 17 and 21. Similar models have also been developed in rats [4, 5].

Antiangiogenic therapy may prove to be an effective treatment for ROP. Angiostatin, a proteolytic fragment of plasminogen, is a potent angiogenesis inhibitor and may contribute to the positive effects of photocoagulation therapy in neovascular retinal diseases [6]. An AAV vector carrying a shortened recombinant angiostatin derivative was used to produce angiostatin in vitro [7]. Following subsequent purification of the recombinant protein, subcutaneous delivery to the murine oxygen-induced retinopathy model resulted in effectively reducing blood vessel formation [7].

More recently, subretinal injection of an AAV carrying any of a variety of antiangiogenic genes including endostatin, pigment epithelium-derived factor (PEDF) and tissue inhibitor of metalloproteinases 3 (TIMP3) was found to significantly inhibit neovascular growth in the ROP model [8]. Further studies must be carried out in order to characterize the stability of the effect and to assure that the procedure is safe to the eye and to other organ systems.

Leber Congenital Amaurosis (LCA)

LCA is a childhood-onset retinal degeneration resulting in complete night blindness from birth or early childhood. Mutations in at least 6 genes have independently been shown to result in LCA [9]. One of these genes, RPE65 is, as its name suggests, localized to the retinal pigment epithelium (RPE), where recent evidence indicates it plays an essential role in the recycling of vitamin A [10].

Currently, two animal models of LCA exist. Both, as in a significant percentage of humans with LCA, have an RPE65 deficiency. The first model was identified as a spontaneous mutation in the Swedish Briard dog [11, 12]. Affected Briard dogs have a homozygous 4-bp deletion (485delAAGA) in putative exon 5 of the canine RPE65 gene, and suffer from the same severe visual impairments seen in human LCA [13, 14]. The second model, a knockout of RPE65, was developed in mice. These animals, like the Briards, suffer progressive retinal degeneration, have severely depressed visual function, lack the rhodopsin photopigment, and accumulate all-*trans* retinyl esters and droplets within the RPE [10, 15].

Recently, gene therapy has successfully recovered vision in the canine model of LCA [16]. The researchers designed an AAV carrying the wild-type canine *RPE65* cDNA and injected it into the subretinal space of dog eyes. They demonstrated functional recovery in the treated dogs by performing visual function and behavioral tests. Assuming that the therapeutic findings persist and there are no toxic consequences, a gene therapy trial may be developed to treat humans with the same genetic disease.

Mucopolysaccharidoses (MPSs)

MPSs are a group of inherited metabolic diseases characterized by the abnormal accumulation of glycosaminoglycans (heparan sulfate, dermatan sulfate, keratan sulfate) in the lysosomes of various tissues, as a result of defects in carbohydrate metabolism [17]. Patients have a short life expectancy and generally suffer disease in multiple organ systems. Ocular abnormalities range from corneal clouding to retinal degeneration, optic atrophy and glaucoma.

Multiple animal models of MPSs exist. These include a feline model of MPSVI (deficiency of arylsulfatase B), and murine, feline and canine models of MPSVII (deficiency of β-glucuronidase) [18, 19]. These models share multiple pathological abnormalities with humans including those seen in the eye.

To date, there have not been any successful treatment options for the ocular manifestations associated with MPS, although keratoplasty has had limited success in treating corneal opacification in an animal model of MPSIV [20]. In a promising gene therapy study, Li and Davidson [17] delivered a recombinant Ad carrying the human β-glucuronidase gene to the eyes of MPSVII mice. These mice develop late-onset photoreceptor degeneration secondary to defects in the RPE, and they possess lysosomal storage vacuoles in keratocytes in the corneal stroma, corneal endothelial cells, RPE cells, and cells in the choroid and sclera [17]. Intravitreal injection resulted in complete clearance of the storage defect in RPE cells, with partial phenotypic correction in corneal endothelial cells. Intravenous administration of a recombinant AAV encoding the human β-glucuronidase cDNA also resulted in nearly complete elimination of lysosomal storage vacuoles in the RPE of these animals [21]. In order to address the corneal clouding in the MPSVII mouse, Kamata et al. [22] delivered an Ad expressing human β-glucuronidase into the intrastromal region of the cornea following lamellar keratotomy. Histology revealed a rapid and nearly complete elimination of vacuoles in the areas of the cornea examined. Recently, a larger animal model has been used to explore the efficacy of treating MPSVI ocular disorders via gene therapy. Ho et al. [23] used an AAV to deliver arylsulfatase B to the subretinal space of the MPSVI cat. This model is characterized by the presence of vacuolated inclusions in the RPE, cornea, conjunctiva, sclera, choroid and the stroma of the iris and ciliary body [14]. AAV treatment appeared to reverse the diseased phenotype in the RPE [33].

Retinoblastoma

Retinoblastoma is an ocular tumor of childhood and is fatal if left untreated. Two thirds of all cases are diagnosed by 3 years of age, and tumors

may present unilaterally or bilaterally. Tumor formation results from mutations in the retinoblastoma gene (*pRb*) [24]. This gene normally encodes a nuclear phosphoprotein that is important in regulating the cell cycle. In the heritable form of the disease, patients possess a single altered allele. When a spontaneous mutation disrupts the second retinoblastoma allele in retinal cells the tumor develops. Nonheritable disease occurs when both alleles are inactivated by spontaneous mutations.

Gene therapy studies have focused on developing an effective eye-sparing treatment for retinoblastoma. Current protocols use enucleation to treat large unilateral tumors in patients. Chemotherapy and radiation therapy may also be used, but are not always effective, and can cause further damage. An ideal therapy would potentially eliminate the need for such drastic treatments and preserve the integrity of the eye. Researchers have recently taken advantage of an Ad vector that encodes the herpes simplex virus thymidine kinase gene (*HStk*). Virus was directly administered to experimental tumors in mice, and animals were subsequently treated with ganciclovir [25]. Transduced cells were susceptible to ganciclovir cytotoxicity and tumor growth was inhibited. These results allowed for approval of a phase I clinical trial for the treatment of retinoblastoma. In this trial, ganciclovir is used to ablate the tumors that have been treated with AdV-thymidine kinase. This trial is currently underway, and early results appear promising [Hurwitz, pers. commun.].

Retinitis pigmentosa (Autosomal Recessive Disease)

Retinitis pigmentosa (RP) represents a group of inherited retinal disorders that affect approximately 1 in 3,000 individuals worldwide. RP is characterized by early-onset night blindness, which is followed by central vision loss. Patients with autosomal recessive (AR) disease often begin having symptoms in childhood or early adolescence. These symptoms become progressively worse with time, leading to complete visual impairment (age 30–60) [26].

AR RP can result as a consequence of lack-of-function mutations found in genes that play critical roles in the visual transduction cascade and outer segment maintenance [see 9]. An AR retinal degeneration occurs spontaneously in a number of animal models for RP, including the *rd* mouse, Irish setter dog, Royal College of Surgeons (RCS) rat, and the *rds* mouse. In order to rescue the lack of function phenotype it is necessary to introduce a correct version of the inactivated gene to its target cell. Gene therapy has served well for this purpose. βPDE was delivered to the *rd* mouse using Ad, AAV, gutted Ad and lentivirus [27–30]. These mice are homozygous for a mutation in βPDE and experience a rapid initial loss of rod photoreceptor cells that is

followed by cone photoreceptor loss. Each of the four different virus treatments proved to successfully delay the rate of photoreceptor degeneration in the *rd* model. Similar approaches were used to treat the RCS rat. This animal has a mutation in the gene for the receptor tyrosine kinase Mertk [31]. These animals develop an abnormal build-up of outer segment debris in the subretinal space, due to the RPE's inability to phagocytize photoreceptor outer segments. This leads to a progressive loss of rod and cone photoreceptor cells [31]. Vollrath et al. [32] used subretinal injection to deliver an Ad encoding the rat *Mertk* cDNA to RCS rats. Treatment restored the RPE phagocytosis defect and preserved outer segment structure in areas surrounding the site of injection. The *rds* mouse possesses a lack of function mutation in the Prph2 (rds/peripherin) gene. This animal is a model for AR RP, although interestingly, *Prph2* mutations in humans with RP or macular dystrophy are inherited in an autosomal dominant (AD) fashion. Delivery of the wild-type *Prph2* gene to photoreceptors of the *rds* mouse has extended the lives and function of these cells [33].

One promising area of gene therapy for retinal degenerative diseases involves use of antiapoptotic genes, as photoreceptors ultimately die via the apoptotic cascade [34–36]. Ad-bcl-2 was subretinally delivered to *rd* mice [37]. Treatment resulted in a rescue effect that was maintained for 6 weeks. Such treatment may also be applicable to AD RP.

Diabetic Retinopathy

Diabetic retinopathy is the leading cause of blindness in individuals under the age of 65. Proliferative diabetic retinopathy (PDR) is characterized by the development of retinal capillary occlusions and small vessel damage. The capillaries develop microaneurysms and the retinal veins become tortuous and dilated, resulting in hemorrhage and retinal detachment causing sudden visual loss. The pathogenesis and symptoms of diabetic retinopathy appear very similar to those of ROP. Therefore, gene therapy strategies aimed at ROP are likely to apply to PDR, as well. Indeed, the ROP mouse is used as a model for studies involving treatment for both ROP and PDR.

Retinitis pigmentosa (Autosomal Dominant Disease)

AD RP may be caused by any of a large number of mutations in a number of photoreceptor-specific genes [9]. Multiple gene therapy approaches have been applied to rodent models of AD RP. Dominant disease results in unwanted

gain of function, thus mutant protein must be eliminated in order for recovery to occur. Ribozymes are very useful for this purpose. These RNA enzymes are designed to exclusively target and cleave mutant mRNA. A ribozyme specific for the P23H mutant mRNA was delivered to the transgenic rhodopsin P23H rat via an AAV [38, 39]. Photoreceptor degeneration was successfully delayed for 3 months.

A second strategy for treating RP relies on the delivery of growth factors to promote photoreceptor survival or to limit apoptotic cell death. AAV delivery of basic fibroblast growth factor delayed photoreceptor death in the S344ter rhodopsin transgenic rat [40]. Delivery of ciliary neurotrophic factor resulted in long-term protection of retinal structures in the both rhodopsin S344ter and P23H animals [41]. AAV-mediated delivery of glial-derived neurotrophic factor also delayed cell death in S334ter transgenic rats [42].

Glaucoma

Glaucoma is the second leading cause of blindness in the world. It is an ocular dystrophy that is characterized by retinal ganglion cell degeneration, altered nitric oxide synthase levels, and elevated glutamate in the vitreous [for review, see 43]. The most common cause of glaucoma is impaired aqueous outflow from the anterior chamber, resulting in increased intraocular pressure (IOP). Interestingly, conventional treatment aimed at lowering IOP does not necessarily prevent ganglion cell death, suggesting other factors are involved.

A number of animal models for glaucoma have been developed in rodents. Two models that rely on mechanical interventions to increase IOP include episcleral vein cauterization and laser treatment of the trabeculum. Alternatively, optic nerve crush, optic nerve transection, or intravitreal injection of NMDA are performed as a means of inducing ganglion cell death.

Gene therapy for glaucoma has been limited to the delivery of growth factors and antiapoptotic proteins. Ad vectors were used to deliver brain-derived neurotrophic factor (BDNF) to a rat model of glaucoma [44]. Increased expression of BDNF in the Müller cells resulted in delayed retinal degeneration. AAVs encoding GFP were also used to target ganglion cells. The authors demonstrated that the ganglion cells are efficiently transduced and expression persists for up to 6 months [45]. Surprisingly, Dreyer et al. [46] illustrated that AAV-GFP alone was able to protect the ganglion cells from NMDA toxicity. However, intravitreal injection of AAV-bcl-2 in models of axonal injury and NMDA toxicity exacerbated retinal ganglion cell death [47], while AAV-bFGF appears to be protective [48].

Age-Related Macular Degeneration (AMD)

AMD is the leading cause of blindness in people over the age of 65. The 'wet' form of AMD results in the most damage – often leading to blindness overnight from hemorrhage under the retina originating from blood vessels which have aberrantly entered this area (after traversing Bruch's membrane and the RPE). Treatment is currently limited to laser photocoagulation of abnormal choroidal vessels. Unfortunately, this therapy is only suitable for a small subset of patients (~10%) and generally stabilizes vision loss for only a limited time.

It has been difficult developing an animal model of wet AMD. This is because, as yet, no genes have been conclusively identified as causing this disease and also because the only animal with a macula is the primate. Mechanical rupture of Bruch's membrane with laser photocoagulation will stimulate CNV in primates, rabbits and rodents; however, lesions generated in this way generally resolve spontaneously, unlike CNV found in humans with AMD. Attempts have also been made to generate transgenic rodents that over-express VEGF in photoreceptors. These animals develop neovascularization originating from the vitreal side of the retina – not the choroid [49, 50]. Despite the imperfect animal models for the wet form of AMD, they have been useful in testing potential treatments for this disorder. Mori et al. [51] recently delivered PEDF through Ad and found that this inhibits neovascularization in the transgenic VEGF mouse and the ROP model. This has led to the proposal of a human clinical trial testing this treatment in neovascular AMD.

Because of the general lack of animal models for wet AMD, gene therapy reagents have also been put to use to create an animal model so that medical treatments can be tested. Recently, an Ad vector encoding human VEGF was used to induce CNV in the Long-Evans rat [52]. This viral vector was delivered subretinally, and animals developed reproducible CNV two weeks post-injection, suggesting it may be a useful model to study neovascularization associated with AMD.

Conclusions

Retinal diseases are found at all stages of life and often cause significant morbidity. This review summarizes valuable animal models for a variety of retinal diseases and their roles in development of gene therapy-based treatments. Currently, rodents are the primary animals used to mimic human disease partly because of the abundance of spontaneous mutants that are available and also partly because of our ability to create genetically engineered mice. With

ongoing advances in vector biology and the identification of novel genes it is possible that gene therapy will become an acceptable form of treatment for patients with at least certain forms of retinal disease.

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