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Gene therapy in age related macular degeneration and hereditary macular disorders

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1. ABSTRACT

In ophthalmology, administration of the therapeutic agent can be difficult due to the tight barriers in the eye. Multiple injections may be needed to allow the therapeutic agent to reach adequate levels in retina and choroidea which may increase the risk of complications including endophthalmitis, cataract and haemorrhages. Optimal methods for the delivery of therapeutic agents to the posterior segments of the eye have not yet been developed. Gene therapy offers an alternative where the therapeutic protein or proteins can be induced in the target tissue for a prolonged period of time after a single injection. The eye is a promising target for gene therapy due to its small size and tissue boundaries preventing leakage of the therapeutic material to other tissues or systemic circulation. However, most of the work in ocular gene therapy is still at the preclinical phase; only three vectors have reached phase 1/2 clinical trials. This review summarizes basic principles and current status of gene therapy in age related macular degeneration and hereditary macular disorders.

2. INTRODUCTION

Gene therapy is a promising strategy for the treatment of several inherited and acquired diseases of the eye. In gene therapy, the genes are delivered into the target cells in order to treat some disease. The therapeutic gene may be designed to induce the expression of a useful gene, to block that of a harmful gene, or to replace a defective mutant gene with a functional version. However effective gene therapy is dependent on being able to deliver therapeutic genes to specific cells at high efficiency, express the gene for a prolonged period of time and ensure that the introduction of the therapeutic gene is not harmful to the target tissue. There are several methods and vectors being used to deliver therapeutic nucleic acids into cells. These methods can be classified as viral and non-viral technologies, and a number of different vector systems for ocular gene transfer have been developed (Table 1). Viral vectors are very efficient in transducing genes into cells. However, their use has been restricted due to immunological problems and the risk of insertional mutagenesis. Non-viral vectors are easier to engineer and

Table 1. Gene therapy strategies and different vectors in preclinical trials

Vector	Advantages	Disadvantages	Gene expression after (ref.)	
			Intravitreal injection	Subretinal injection
AAV	Broad host spectrum Long expression No association with human diseases	Difficult production Limited DNA capacity	TM GC MC (15, 108)	RPE PRC (15, 108)
Adenovirus	Easy production High transduction efficiency Broad host spectrum	Immunogenic Transient expression	COE TM CIE GC (21, 109)	Occasional MC RPE (22)
Lentivirus	Broad host spectrum Long expression Random integration of DNA	Difficult production Low transduction efficiency Limited DNA capacity	COE CIE GC RPE (30, 31)	RPE PRC (32)
Baculovirus	Easy to produce No association with human diseases High DNA capacity	Inactivation by complement fractions Moderate transduction efficacy Transient expression	COE Lens PRC RPE (39)	RPE (39)
Plasmid DNA, complexes and physical methods	Easy production Safe	Low transduction efficiency Transient expression	GC (110)	PRC MC (111)
RNA interference	High transduction efficiency High specificity to target RNA	Transient expression	With appropriate delivery systems gene silencing can be achieved in any ocular cell type	

Abbreviations: GC=Ganglion cells, COE=Corneal endothelium, CIE=Ciliary epithelium, RPE= retinal pigment epithelium, PRC=Photoreceptor cells, TM=trabecular meshwork, MC= Müller cells

manufacture, but significantly less efficient in gene delivery compared with their virus-based counterparts. In addition, their lack of chromosomal integration precludes long-term therapeutic effects (1).

The eye is a promising organ for *in vivo* gene transfer. It is easily accessible by microsurgical techniques under direct visual control. Localized targeting of vector within the eye minimizes dissemination to other tissues and the risk of systemic side effects. Furthermore, the optical transparency of the eye enables transgene expression and effects of treatments to be monitored by noninvasive examinations. The small size of the eye means that small vector suspension volumes can transduce an adequate proportion of cells within the ocular tissue. Immune responses following intraocular vector administration are typically attenuated compared to those following systemic administration; a relatively immune priviledged eye protects from immune responses directed against vector antigens that might otherwise evoke inflammation and limit transgene expression (2, 3). When only one eye is treated, the untreated eye can be used as a control for the evaluation of the efficacy of the treatment. The therapeutic gene can be delivered into the target tissues within the eye by several routes. Intracameral injections are mainly used in treating the anterior segments of the eye. Intravitreal and subretinal injections are better if one wishes to place the therapeutic gene to the posterior segments (Figure 1). The major barriers and determining factors in ocular drug delivery are physiochemical properties of the drug, molecular weight and lipophilicity which can influence the penetration of drug into the eye (4). This review summarizes basic principles and current status of gene therapy in age related macular degeneration and hereditary macular disorders.

3. VIRAL VECTORS

Viruses are intra-cellular parasites with specialized molecular mechanisms to transport their genomes to cells. Viruses carry their own DNA or RNA into the host cells, which are hijacked to produce new viral particles. By replacing genes that are needed for the replication phase of the viral life cycle with therapeutic genes, the recombinant viral vectors can transduce the cell type it would normally infect (Figure 2). The most extensively studied viruses in ocular gene therapy are adenoviruses (Ad), adeno-associated viruses (AAV) and lentiviruses. Adenoviral vectors can efficiently target cells of the outer retina but the duration of gene expression is limited to a few weeks by immune responses generated against the vector. AAV vectors have been used for sustained transduction of photoreceptor cells (5). The major limitations associated with AAV are difficulties in producing the virus, rather low maximal insert size of 9 kb and potential induction of insertional mutagenesis. Lentiviral vectors stably transduce RPE cells but are less efficient than AAV in transducing photoreceptors (6). Furthermore, lentiviral vectors are often based on human immunodeficiency virus (HIV) and the safety of these vectors needs to be established carefully before clinical applications.

3.1. AAV

AAVs are nonenveloped parvoviruses with many properties that make them advantageous for use as viral vectors (7). In humans, AAV vectors appear to evoke minimal immune responses and therefore have low toxicity and they also achieve prolonged transgene expression (8). However, AAV administration has been associated with the induction of a cellular immune response leading to transient and acute hepatotoxicity in patients (9, 10). Randomly

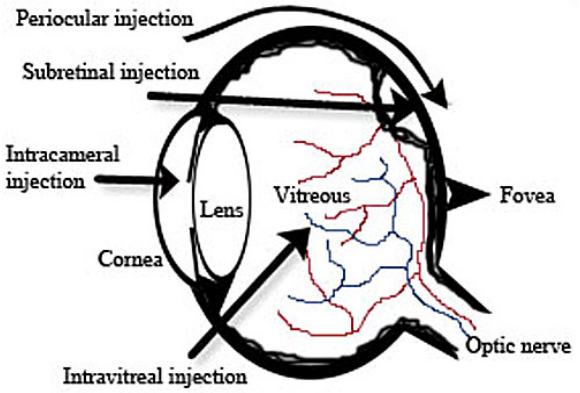

Figure 1. Delivery routes in ocular gene therapy. Intraocular injections are usually performed through pars plana: intravitreal vectors are injected to the vitreous humour whereas subretinal vectors pass the vitreous humour and the retina and are injected to subretinal space. Periocular injections are performed beneath the conjunctiva along the outer border of the sclera. Intracameral injections into the anterior chamber are mainly used in treating the anterior segments of the eye.

integrating recombinant AAVs have been developed that are able to evoke stable transduction of the retina for more than 1 year (11). There are currently eleven known serotypes of AAV, of which type 2 has been most extensively studied as a potential vector (12). Different AAV serotypes vary in their abilities to bind to and transfect different host cell types (13). Recombinant vectors can be generated using both capsid proteins and genomes from the same serotype or the vector genome can be derived from one serotype and included in the capsid from an alternative AAV serotype (14). Intravitreous injections of AAV2/2 vectors result in transduction of ganglion cells, trabecular meshwork cells and various cells of the inner nuclear layer, including Müller cells. Subretinal administration of AAV5/5 or AAV2/5 results in more efficient RPE transduction than that achieved with AAV2/2 (15).

3.2. Adenovirus

Adenovirus is a non-enveloped virus with maximal carrying capacity of 30kb of foreign DNA (16). It is a widely used vector in gene therapy since it has many advantageous properties. Ad vectors are relatively easy to produce, possess good capacity, and with an appropriate promoter, can achieve high expression levels in multiple cell types in the eye. The transduction efficiency for a certain cell type varies depending on the serotype of the Ad vector. The most widely studied serotypes are first or second generation types 2 and 5 (17). Adenoviruses enter the cell via CAR receptor mediated endocytosis and remain in the nucleus as episomes (18). Therefore, they lack the ability to integrate the transferred gene into chromosomal DNA and their presence in cells is short-lived, typically limited to a few weeks (19). Furthermore, in the first-generation adenoviruses, the expression of wild-type adenoviral genes stimulate the immune system, trigger a cytotoxic T lymphocyte immune response towards infected cells leading to the elimination of transduced cells and, therefore, to the lost of therapeutic gene expression. To avoid this problem, gutless adenoviruses were generated showing long-term stability and less immunogenicity in many tissues (20). Intravitreous injections of Ad vectors have resulted in transduction of corneal endothelium, trabecular meshwork, iris pigmented epithelium, ciliary epithelium, and ganglion cell layer of the inner retina in mice (21). Furthermore, subretinal injections result in transduction of RPE cells and occasional Müller cells, but little or no transduction of retinal neurons in mice (22). In clinical trials, Ad vectors were well tolerated and there were no severe inflammation or dose-limiting toxicity observed (23-25).

3.3. Lentivirus

Lentivirus-based vectors are attractive candidates for ocular gene transfer because they efficiently transduce a variety of nondividing cells while evoking little or no host response, resulting in long-term transgene expression (26). Vectors based on nonprimate lentiviruses that are not known to cause human disease, such as feline immunodeficiency virus (FIV) and bovine immunodeficiency virus (BIV), have transduction efficiencies and durations of expression in ocular tissues that are comparable to human immunodeficiency viruses -1 and -2 (HIV-1 and -2) based vectors and may represent alternatives with several safety advantages (27, 28). HIV-1 based lentiviral vectors efficiently transduced the corneal endothelium and trabecular meshwork after their administration into the anterior chamber (29). Intravitreal administration of HIV-2 viral vectors transduced the ganglion cells, RPE, corneal endothelium and ciliary epithelium (30, 31). Subretinal injection of FIV vector led to stable transgene expression in the RPE cells in rodents for at least 2 years (32). HIV-1 based lentiviral vector has been shown to mediate therapeutic effects in cases of retinal degeneration (33).

3.4. Baculovirus

Baculovirus (Bv) is a large, approximately 130 kb virus which can accomodate a transgene with up to 100 kb of foreign DNA. Bv cannot replicate in vertebrate hosts and it is capable of transducing differentiated, nondividing cells (34). Bvs have a low cytotoxicity in mammalian cells even at a very high virus load and they can be easily produced at high titers (35). Recombinant Bvs have the capability of transducing a variety of mammalian cells *in vitro*(36). Despite the ability to transduce mammalian cells *in vitro*, only limited success has been reported *in vivo*. This is most likely due to viral inactivation by the complement system (37). In the eye, the anterior chamber, the subretinal space, and to a lesser extent, the vitreous cavity, are relatively immune-privileged sites (38). Antigens in these areas are not subjected to attack by the complement pathway and therefore Bvs represent potential vectors for ocular gene therapy. Intravitreal injections of Bv resulted in GFP expression in the corneal endothelium, lens, RPE, and photoreceptor cells. GFP expression was observed for up to two weeks after injection. Subretinal injection of BvGFP has resulted in transduction of RPE cells. No alteration in

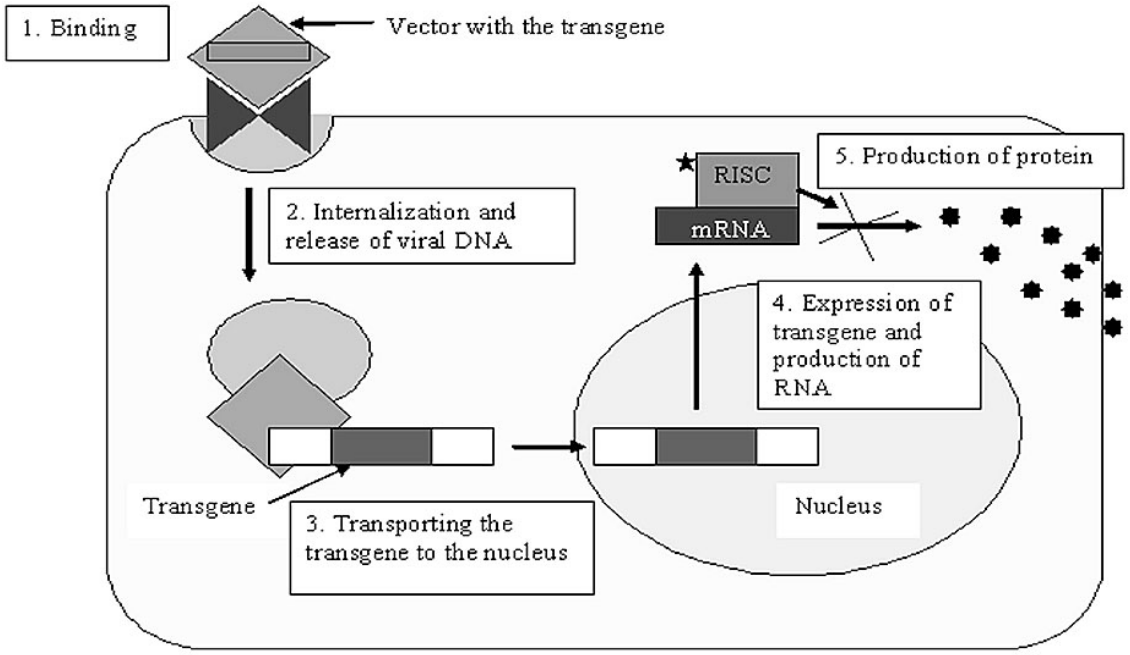

Figure 2. Basic principals of gene transfer with viral and non-viral vectors and siRNAs. A vector is used to introduce the therapeutic gene into the target cells. The vector binds to the cell membrane, is internalized and the viral DNA is released. Further, the therapeutic gene is transported to the nucleus, where it is expressed and RNA is produced. Then, RNA is released to the cytoplasm and the therapeutic protein is produced. * In the cell, siRNA is processed to a RNA-induced silencing complex (RISC), which binds specifically to messenger RNA (mRNA) leading to cleavage and digestion of the mRNA, resulting in an efficient inhibition of the production of the targeted protein.

electroretinogram responses was observed after injection of BvGFP (39).

4. NON-VIRAL VECTORS

Problems associated with virus vectors have led to the development of non-viral methods. The advantages of non-viral systems include their reduced immunogenicity, large size of therapeutic expression cassette and improved safety profiles. In addition, non-viral vectors are easier and less expensive to manufacture than viral vectors (40). However, these approaches tend to suffer from inefficient delivery, resulting in transient transgene expression (41).

Non-viral techniques can be subdivided into two general groups. Naked DNA can be delivered to the cell by a physical method, including electroporation, gene gun and ultrasound, where a physical force that permeates the cell membrane is employed to facilitate intracellular gene transfer (42-44). Simple injection of plasmid DNA directly into tissue without additional help from either a chemical agent or a physical force is theoretically able to transfect cells (40). However, because of the rapid degradation by nucleases in the serum and clearance by phagocytosis, the expression levels after injection of naked DNA are generally low (45). Furthermore, vitreous humour has been shown to substantially limit nonviral gene delivery to RPE cells. As a polyanionic gel it might affect the gene transfer by binding the positively charged complexes, reorganizing their structure, releasing DNA from the complex too early, or it could act as a diffusional barrier that slows down the distribution of the complexes after the injection (46, 47). Alternatively, chemical approaches use synthetic or naturally occurring compounds such as cationic polymers and lipids as carriers to deliver transgenes into cells. Following the internalization of the DNA-polycation complex by endocytosis, a large fraction of the complex is targeted to the lysosomal degradation, and only a small fraction of internalized plasmid DNA penetrates the cytoplasm (48). In the eye, because of the micro-environment of the eye, the degradation and clearance is more limited compared to systemic administration. Furthermore, it is possible to add ligands to the complexes, modify their DNA binding properties, or change the surface of the complexes with coating molecules to improve transgene expression in the target cells (47).

The transduction efficiency of non-viral vectors in the retina can be substantially improved by adjunctive electroporation. Electroporation is the use of an electric field to facilitate the penetration of macromolecules into cells based upon the observation that electric fields can alter the structure and permeability of the cell membrane (49). Following intravitreal injection of plasmid DNA, electroporation results in short-lived but efficient transduction of retinal ganglion cells (50). The gene gun technique consists of “bombarding” a tissue with gold or tungsten bullets covered with DNA. It is potentially applicable for the treatment of ocular surface diseases, particularly for corneal diseases. Ultrasound can alter the

permeability of plasma membrane transiently and thereby facilitate DNA uptake (51).

Lipofection reagents are molecules composed of phospholipids that contain both hydrophobic and hydrophilic domains. These reagents form complexes with DNA and the lipid/ DNA complexes can then be used to deliver foreign DNA to cells *in vitro* and *in vivo* (52). After entering the cell, the majority of the DNA is degraded by lysosomes. However, some of the DNA reaches the nucleus probably in a concentration dependent manner (53). Disadvantages of lipofection include reduced efficiency compared to viral vectors and short duration of expression. Avoiding these problems, compacted DNA nanoparticles have proved to be a very useful vehicle for gene therapy. These nanoparticles typically contain a segment of DNA or RNA compacted with a polycationic polymer and are taken up at the cell surface and trafficked to the nucleus. Delivery of compacted DNA nanoparticles to the target is very efficient; in many cases expression levels are several folds greater than those observed after treatment with naked plasmid DNA. The results are dependent on specifics of the nanoparticle formulation, size, or electric charge (54).

One special form of gene therapy is RNA interference. It is a mechanism for inhibiting the intracellular production of a specific protein by silencing gene coding. Small interfering RNAs (siRNA) are double-stranded RNAs consisting of 21-22 nucleotides. In the cell, it is processed to a RNA-induced silencing complex (RISC), which binds specifically to messenger RNA (mRNA) leading to cleavage and digestion of the mRNA. Subsequently, RISC can bind to other mRNA molecules, resulting in an efficient inhibition of the production of the targeted protein (55). The challenges in achieving optimal *in vivo* RNA interference include effective siRNA delivery and serum stability. The naked siRNA molecule has negative charges, and it is therefore susceptible to serum nucleases and nontargeted biodistribution having many limitations, such as poor stability, short half-life, and low efficiency (56). siRNA injected into the vitreous cavity is shown to diffuse throughout the eye and is detectable for at least five days(57). Various types of transfecting agents are used to improve the stability and delivery of the siRNAs *in vivo*. Polymer particles and cationic liposomes have been shown to be suitable for the delivery of siRNA (58). Cationic liposomes bearing siRNAs have been intravenously injected into mice and electroporation has been used to deliver siRNAs to post-implantation embryos and post-natal retinas (59, 60). Depending on the vector, siRNA can be delivered and gene silencing achieved in any ocular cell type. Several siRNA-based therapeutic agents are already in clinical trials (61, 62).

5. GENE THERAPY IN AGE RELATED MACULAR DEGENERATION

Age related macular degeneration (AMD) is the leading cause for visual impairment in the developed countries. The disease has two forms, the dry form with RPE and photoreceptor atrophy, and the wet form with choroidal neovascularization (CNV). The wet AMD is responsible for 90% of cases of severe visual loss in AMD patients (63). In the wet AMD, CNV causes sub- and intraretinal accumulation of hemorrhages leading to structural and metabolic damages and eventually vision loss due to the secondary cell death and reactive gliosis. Several new molecules are tested in preclinical trials targeting either CNV and angiogenesis or other molecular pathways providing new therapeutic strategies in treating AMD (25, 64, 65). Current gene therapy strategies for acquired ocular neovascular diseases focus mainly on this condition and the most extensively studied molecules are discussed here.

5.1. PEDF

Pigment epithelium derived factor (PEDF) is one of the most extensively studied proteins that has been shown to inhibit ocular neovascularization seen in AMD. PEDF is a 50 kDa glycoprotein isolated from cultured RPE cells as a neurotrophic factor (66). *In vitro* it has also been shown to inhibit the migration of endothelial cells in a dose-dependent manner (67). Furthermore, it promotes neuron survival and protects photoreceptors from the effects of excessive light exposure (68, 69). In the eye, PEDF is produced by the RPE, cornea and ciliary epithelium (70). PEDF is normally present at high concentrations in the vitreous humour, the lens and the cornea. The vitreous concentration of PEDF declines in elderly people and especially in patients with AMD (71). Systemic delivery of recombinant PEDF inhibited ischemia-induced retinopathy in mouse models (72, 73). Intravitreal or subretinal AAV-mediated gene transfer of PEDF has also been shown to inhibit retinal and choroidal neovascularization in mouse models (74, 75). Furthermore, intravitreous or subretinal injection of adenoviral vector expressing human PEDF (AdPEDF) suppressed the development of retinal or choroidal neovascularization and also caused regression of established neovascularization in mice (76). Periocular injection of AdPEDF resulted in transduction of episcleral cells producing PEDF outside the eye and causing regression of CNV in mice (77). One phase I trial in wet AMD patients with AdPEDF has been conducted. The aim was to investigate the safety of intravitreous AdPEDF in subjects with advanced neovascular AMD. Eight dose levels of AdPEDF were investigated, each subject was monitored for safety and tolerability for 12 months. There were no serious adverse events and a significant proportion of the patients displayed an improvement in lesion size from the baseline (24, 25). However, Apte *et al.* demonstrated that PEDF has opposing effects on CNV and endothelial cell function. Whereas low doses were inhibitory, high doses augmented the development of CNV. These results suggest that the effects of PEDF on neovascularization are more complex than originally believed (78).

5.2. sFlt-1

Soluble (s)Flt-1 is a naturally occurring protein antagonist of VEGF formed by alternative splicing of the pre-mRNA for the full length VEGF receptor-1 (VEGFR-1, a.k.a. Flt-1). The angiostatic activity of sFlt-1 results from inhibition of VEGF by two mechanisms: 1) sequestration of VEGF and 2) forming inactive heterodimers with VEGFR-

1 and VEGF receptor-2 (VEGFR-2) (79). Several studies have investigated the effect of overexpression of sFlt-1 in ocular neovascularization models. Inhibition of VEGF by repeated intravitreal injections of recombinant sFlt-1 has been shown to reduce retinal neovascularization in the oxygen induced retinopathy (OIR) mouse model (80). Intravitreal or subretinal injection of adenoviral sFlt-1 (AdsFlt-1) suppressed retinal or choroidal neovascularization in mice and rats (81, 82). Periocular injection of AdsFlt-1 resulted in transduction of episcleral cells, penetration of the sclera and high levels of AdsFlt-1 in the choroid, suppressing CNV significantly in mice (83). In addition, long-term suppression of CNV was achieved with subretinal injection of AAVsFlt-1 in mice and monkeys (65, 84). A Phase 1 clinical research study is underway to examine the safety and tolerability of AAV2-sFLT01 in 34 patients with wet AMD (ClinicalTrials.gov; ID NCT01024998). Initial results of the trial should be available in early 2012.

5.3. RNA interference

Bevasiranib, an siRNA targeting VEGF, was able to inhibit retinal neovascularization in a mouse model (85). In non-human primates, intravitreal injection of bevasiranib significantly decreased the area of laser induced CNV (86). In the phase II CARE (Cand5 Anti-VEGF RNAi evaluation) study, 129 patients with AMD induced CNV were randomized to receive three different intravitreal doses of bevasiranib at baseline level and at 6 weeks. No local or systemic serious adverse events were found. Intravitreous or periocular injection of AGN211745 (siRNA-027), an siRNA targeting VEGFR-1, significantly decreased the area of neovascularization in mouse models of retinal and choroidal neovascularization (87). One phase I study with AGN211745 in 26 patients with AMD revealed that a single intravitreal injection of the siRNA-027 was safe and well tolerated. Visual acuity had stabilized in 92% of patients at 3 months and decreased foveal thickness was seen in some patients (88). RTP801i-14 (PF-4523655) is an siRNA designed to inhibit the expression of the hypoxia-inducible gene RTP801. The RTP801 gene has been demonstrated to be upregulated in response to ischemia, hypoxia and oxidative stress both *in vitro* and *in vivo* (89). In both RTP801-knockout and therapeutic mouse and primate models of laser-induced CNV, inhibition of RTP801 expression has been shown to inhibit or reduce CNV and vessel leakage (90, 91). In addition, knock-out of RTP801 could ameliorate diabetes-induced retinal vascular permeability and ERG abnormalities in diabetic mice (92). Due to its anti-inflammatory and anti-apoptotic properties it may also be useful for the treatment of dry AMD. The results from a Phase I/II trial demonstrated that RTP801i-14 was safe and well tolerated in patients with wet AMD who had failed to respond to the currently approved therapies (93).

However, the mechanism of action with siRNAs is not completely clear. The recent study of Kleinman *et al* revealed that untargeted 21-nucleotide or longer siRNAs were as effective as VEGFA-targeted siRNA at suppressing CNV. According to that study, the anti-angiogenesis property was not due to target knockdown but due to activation of toll-like receptor 3 and to immunity pathways (94). More studies need to be performed to elaborate the mechanism of action of siRNAs in ocular diseases.

6. GENE THERAPY IN HEREDITARY MACULAR DISORDERS

Compared to AMD, in hereditary diseases affecting the posterior segment of the eye the major problem is not neovascularization. The inherited macular dystrophies comprise a heterogeneous group of disorders characterized by bilateral central visual loss and atrophy of the macula. Degeneration of the photoreceptors in the macular area and underlying retinal pigment epithelium are responsible for the symptoms and visual loss in most cases (95).

The first clinical trials in ocular gene transfer strategy have been focused on gene replacement in inherited retinal degeneration, Leber's congenital amaurosis (LCA), caused by mutations in the RPE65 gene (96-98). This type of LCA is an ideal target for the development of therapies because it accounts for ~20% of the disease in the human population. RPE65 is expressed in the retinal pigment epithelium and encodes a 65-kD protein that is involved in the conversion of all-trans-retinoids to 11-cis-retinoids, a biochemical pathway that regenerates the visual pigment after exposure to light. A lack of functional RPE65 results in a deficiency of 11-cis retinal so that rod photoreceptor cells are unable to respond to light, leading to blindness in early childhood (99). In animal studies with the Briard dog, a canine strain that has a spontaneously occurring RPE65 mutation similar to that in humans, as well as with the genetically engineered RPE65-/- knock-out mouse, gene therapy with AAV containing the RPE65 transgene evoked a significant improvement in photopic and scotopic ERGs in the treated animals compared to controls (100).

Initial results from three separate clinical trials of gene therapy for RPE65 deficiency have recently been reported with details from 18 patients with follow-up periods ranging from 90 days to 1.5 years. Each study involved the treatment of three young adults by subretinal injection of an AAV2 vector expressing RPE65, using either a tissue-specific or constitutive promoter (96-98). Although there were differences in the trial protocols, all three studies detected improvements in retinal sensitivity. In one subject in particular, there was a dramatic improvement in visual capability in dim light. Furthermore, there was an improvement in visual function within a short period of days to weeks following gene delivery. This improvement persisted for up to 1.5 years, evidence that it was achieving physiologically relevant levels of gene expression and supporting the durability of the gene product. However, no improvement could be detected by ERG in any of the patients. There were no vector-related serious adverse events, toxicity, or any major immune response. These encouraging results have stimulated further studies in which the optimal dosage and time-frame of the treatment are being evaluated.

Stargardt disease (STGD) is the most common juvenile macular dystrophy. Over 400 mutations in the Abca4 gene have been identified so far. Abca4 localizes to the outer segments of photoreceptors and functions as a retinoid transporter. Abca4–/– knock-out mice have been shown to accumulate retinoids between the RPE and photoreceptors, resulting in lipofuscin deposits which cause a slow photoreceptor degeneration (101, 102). Significant improvement in the retinal function of the Abca4−/− mouse has been achieved after subretinal administration of rAAV2/5 encoding Abca4 (103). Recently, EIAV-based lentiviruses encoding Abca4 have been delivered to the subretinal space of newborn Abca4−/− mice, resulting in a reduction in the levels of lipofuscin deposits (104).

Achromatopsia is a congenital disorder with clinical characteristics of photophobia, color blindness and poor visual acuity due to lack of functional cone photoreceptors (105). At present, mutations in three cone-specific genes, CNGB3, CNGA3 and GNAT2, have been associated with this disease (106). Subretinal administration of rAAV vectors encoding GNAT2 resulted in an improvement of both cone-mediated ERG responses and visual acuity in the Gnat2cpfl3-null mouse model (107).

7. SUMMARY AND PERSPECTIVES

Significant progress has been made in understanding the molecular pathogenesis of several retinal and choroidal neovascular disorders as well as inherited degenerative diseases. This progress has revealed several new targets for therapeutic interventions. However, there are still many unresolved problems. Many diseases have complex pathways which complicate any treatment. On the other hand, the complexity of diseases can offer also several alternative targets for therapeutic interventions. Phenotypes of retinal diseases vary from early and severe to late and progressive. Slowly progressive diseases have a much wider therapeutic window than those with more acute, aggressive course or those with an earlier onset. In addition, chronic diseases need long-term therapy and the therapy should be available early enough before the retina has been permanently damaged. Biodegradable implants or prodrugs, simple derivatives of drugs converted to their active parent drug chemically or enzymatically could provide effective drug delivery system for chronic ocular diseases. With gene therapy vectors, it is necessary to solve the current problems in vector production, tissue-spesific vector targeting, risk of insertional mutagenesis, immunological consequences and biosafety. Most likely the new generation vectors in the future will be tailor-made for specific therapeutic applications and for individual patients. Most of the work in ocular gene therapy field is still at the preclinical phase, and only three vectors have reached the phase 1/2 clinical trials. However, first of the clinical trials with gene therapy strategies especially in LCA patients have delivered promising results. While safer and more efficient gene transfer vectors are being continuously developed, patients with these formerly blinding and untreatable diseases may have a cure in sight in the near future.

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