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Non-viral therapeutic approaches to ocular diseases: An overview and future directions

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ABSTRACT

Currently there are no viable treatment options for patients with debilitating inherited retinal degeneration. The vast variability in disease-inducing mutations and resulting phenotypes has hampered the development of therapeutic interventions. Gene therapy is a logical approach, and recent work has focused on ways to optimize vector design and packaging to promote optimized expression and phenotypic rescue after intraocular delivery. In this review, we discuss ongoing ocular clinical trials, which currently use viral gene delivery, but focus primarily on new advancements in optimizing the efficacy of non-viral gene delivery for ocular diseases. Non-viral delivery systems are highly customizable, allowing functionalization to improve cellular and nuclear uptake, bypassing cellular degradative machinery, and improving gene expression in the nucleus. Non-viral vectors often yield transgene expression levels lower than viral counterparts, however their favorable safety/immune profiles and large DNA capacity (critical for the delivery of large ocular disease genes) make their further development a research priority. Recent work on particle coating and vector engineering presents exciting ways to overcome limitations of transient/low gene expression levels, but also highlights the fact that further refinements are needed before use in the clinic.

1. Introduction

Inherited retinal degeneration is a primary cause for debilitating impairments in vision among working age people in the developed world [1], and development of effective therapeutics for inherited retinal disease is a primary research goal. Retinal dystrophies are categorized roughly into two categories: 1) retinitis pigmentosa (RP), a broad term encompassing phenotypes that initially affect rod photoreceptors and peripheral vision, and may later be associated with cone cell death, and 2) macular dystrophy (MD), a term encompassing widely varying phenotypes in which macular or central vision is initially affected [2,3]. Though MD can be caused by defects in cone photoreceptors, it can also be caused by defects in the retinal pigment epithelium (RPE). For example, Stargardt's MD is associated with retinal pigment epithelium (RPE) atrophy [4] and subsequent degeneration of the cones in the macular region of the retina and leads to central vision loss. There are also various syndromic forms of inherited retinal diseases (particularly leading to RP) which exhibit degenerative processes not only in the eye but also in other organs [5]. The most common form of syndromic RP is Usher syndrome which comprises hearing loss in addition to visual impairment (for review see [6]).

Inherited retinal diseases are complex. Causal mutations in more than a hundred genes have been identified for inherited retinal degeneration and there are still many unidentified [7]. In addition, most disease genes contain multiple pathogenic mutations leading to a wide variety in the severity and age of onset of the disease [8,9]. Mutations in certain genes like the structural protein peripherin-2 can induce RP as well as MD, depending on the site of mutation involved [10,11] and RP can be inherited in a dominant or recessive manner [5]. There is also a large degree of intra- and inter-familial phenotypic heterogeneity and often incomplete penetrance even within groups of patients carrying the same mutation. This wide variability in phenotypes has led geneticists to look for modifiers or digenic/polygenic disease, topics that are still being explored [12–14]. Many patients with RP develop symptoms in the third or fourth decade of life, however, there are more severe forms of retinal degeneration, such as Leber's congenital amaurosis (LCA), which can lead to complete loss of vision before patients reach puberty (for review see [15]). Gene therapy is a desirable treatment method, but the genetic and phenotypic heterogeneity in inherited retinal degeneration makes the development of a single therapeutic approach a very challenging process.

Gene augmentation therapy is a theoretically straightforward option for the treatment of recessive disease (usually caused by loss-of-function mutations) or a dominant disease associated with haploinsufficiency phenotype [16,17]. In these cases, supplementation with a healthy copy of the gene should be effective, provided

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that the degeneration is not yet severe and that a sufficient amount of gene expression can be obtained from the therapeutic vector. More challenging is designing a treatment for dominantly inherited degenerations caused by gain-of-function mutations. Patients have one healthy and one mutated allele, and the mutant protein interrupts normal function leading to retinal degeneration. Multiple mechanisms (many as yet poorly understood) result in this degeneration, so many different treatment approaches must be considered. A common gain-of-function disease mechanism occurs when mutant proteins are misfolded/aggregated [18,19] or are mislocalized [20] leading to endoplasmic reticulum (ER) stress, and ultimately cell death [21]. Different pharmaceuticals can be used to alleviate ER stress and prolong the life of photoreceptors. Proof-of-principle for this therapeutic approach has been shown in multiple cases, for example, increasing the cellular unfolded protein response via pharmacological induction of the heat-shock protein rescued retinal function and morphology in a P23H rat model of rhodopsin-associated RP [22]. Other dominant mutations cause cell death by other mechanisms, for example some mutations in guanylate cyclase lead to constitutive activity and overproduction of cGMP, causing LCA [23,24]. A key defining feature of these dominant diseases is that reintroduction of the WT allele is not sufficient to completely prevent retinal degeneration.

However, several gene therapy approaches have been tested for dominant diseases. For example, the administration of neurotrophic factors (either as purified proteins or as gene therapy vectors) has been beneficial in some cases [25], however, without the correction of the underlying disease mutations, these treatments merely delay the degenerative process. Thus other research has focused on ways to eliminate the undesirable transcript or mutation. Silencing RNA (siRNA) which is complementary to unwanted transcripts prevents their efficient translation, and knockdown approaches have been tested for dominant phenotypes [26]. Gene knockdown in the retina has been tested using multiple modalities and multiple disease genes, including siRNA (rhodopsin, peripherin-2 and GCAP1) [27–30], and zinc-finger nucleases (for rhodopsin and USH1C) [31,32]. The recent development of additional genome editing systems such as CRISPR/Cas9 that can be used to correct the disease-causing mutation in the native allele also provide great hope for the development of effective treatments for dominant diseases [33]. Preliminary results from the Bakondi group utilizing CRISPR/Cas9 genome editing to correct the RP phenotype in a rhodopsin mutant rat model (S334ter-3) are encouraging (Bakondi B, et al., Gene Editing Corrects The Retinal Dystrophy Phenotype in S334ter-3 Rats, ARVO2015, Abstract number 3183), and results from a complete study are highly anticipated. However, there are several limitations to knockdown technologies that remain to be overcome including the difficulty in designing a knockdown construct which can bind the mutated version but not the healthy sister allele, potential off-target effects [34], and concerns about delivery and expression levels which affect all types of gene delivery strategies. The new technology is intensely studied right now and will hopefully lead to a major breakthrough in future therapy of retinal degeneration.

However, even with the new treatment possibilities, there are still obstacles for any type of gene therapy method including, 1) successful delivery of the therapeutic DNA to the affected tissue, 2) its uptake by the target cells and 3) efficient expression of the transfected vector. All three parts will be discussed in this review for non-viral vectors with a special emphasis on their applications in the eye.

2. Therapeutic approaches for ocular diseases with gene therapy: Pre-clinical testing and clinical trials

The retina is an excellent system to test newly emerging gene therapy vectors due to its relative immune-privilege, the plethora of non-invasive procedures available to assess functional and structural rescue, and the ability to deliver therapeutic agents directly to specific tissues of the eye. Thus gene supplementation therapy in the eye is a mature field and several clinical trials are either completed or underway. Thus far, the ocular gene therapy trials have used viral delivery systems, either adeno-associated virus (AAV) or lentivirus. However, concerns regarding the immune response to viral vectors, limitations in payload capacity, and production costs have spurred investigation of non-viral alternatives. Though these non-viral approaches have not yet been tested in ocular clinical trials, they have been under investigation in animal models for several years and may be poised to enter the clinical trial phase in the near future.

2.1. Current gene therapy clinical trials for retinal degeneration

So far, most clinical trials for gene therapy in inherited retinal degeneration are in phase I or II to test possible toxicity and adverse effects of the applied vectors and gather preliminary data on the efficacy. However, one of the earliest successful therapies which uses AAV to target LCA has successfully completed phase I and II in several different clinical trials (NCT00481546/NCT01496040/NCT00749957/NCT01208389/NCT00643747/NCT00516477) ((see [35,36] for the most recent information) and is now in phase III (NCT00999609). In 1997, a mutation in the RPE-specific gene RPE65 was identified as causative agent for LCA in several patients with a very early onset vision loss [37]. The identification of similar phenotypes in Swedish briard dogs [38], a naturally occurring mouse model of LCA [39] with RPE65 mutations, and an RPE65 knockout mouse line [40] have facilitated the development of a feasible gene therapy vector for RPE65-associated LCA by providing the means to test it. Delivery of the RPE65 gene to RPE cells in the retina of dogs [41,42] and mice [43,44] showed long-term improvement of retinal function and few adverse effects leading to the initiation of the different clinical trials. Patients were treated with AAV containing the RPE65 cDNA and either a ubiquitously expressed promoter or the native RPE65 promoter [45–48]. None of the trials revealed any severe adverse effects, and reported moderate improvement of visual function in some of the patients. Long-term follow-up studies showed that the increase in visual acuity is stable for up to 3 years after treatment [49] despite a progressive retinal degeneration [36]. However, recently released follow-up for up to six years has showed continued diminishment in the areas of improvement [50,51]. Given the already modest nature of the improvement, these results highlight the need for further understanding of the mechanism underlying the disease defect in order to improve on the design of therapy.

Three other retinal disease genes are currently being delivered by AAV in clinical trials. Choroideremia is an X-linked retinal degenerative disease with a prevalence of 1 in 50,000 and is associated with early onset photoreceptor and RPE degeneration and a marked choroidal atrophy (for review see [52]). Mutations in the Rab escort protein-1 (REP-1) are responsible for this disease [53] and cause a defect in membrane association of Rab GTPases which are crucial for proper protein trafficking inside the cell [54]. Two different approaches were tested in a mouse model for choroideremia: a lentiviral vector carrying the elongation factor 1 promoter known to express in ocular cells and the REP-1 cDNA [55] and an AAV2 vector with a ubiquitously expressed promoter and the REP-1 cDNA [56]. Both vectors showed successful transfection of RPE cells in mouse models, but AAV2 was chosen for the phase I/II clinical trial (NCT01461213). Though the trial is ongoing, the 6 patients with advanced retinal degeneration who were initially enrolled showed increased visual acuity after treatment, and the subretinal injection did not cause any detectable damage at 6 months post-treatment, the latest time point assessed so far [57]. Clinical trials for Leber hereditary optic atrophy (LHOA) caused by mutations in the mitochondrial ND4 gene (NCT02161380) [58] and for autosomal-recessive RP caused by mutations in the RPE-specific MERTK gene (NCT01482195) [59] have just been started and are currently recruiting patients. Both trials use AAV2 to subretinally deliver the wildtype version of their respective mutated genes, and results will be eagerly

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