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Nanoparticle-based technologies for retinal gene therapy

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ABSTRACT

For patients with hereditary retinal diseases, retinal gene therapy offers significant promise for the prevention of retinal degeneration. While adeno-associated virus (AAV)-based systems remain the most popular gene delivery method due to their high efficiency and successful clinical results, other delivery systems, such as non-viral nanoparticles (NPs) are being developed as additional therapeutic options. NP technologies come in several categories (e.g., polymer, liposomes, peptide compacted DNA), several of which have been tested in mouse models of retinal disease. Here, we discuss the key biochemical features of the different NPs that influence how they are internalized into cells, escape from endosomes, and are delivered into the nucleus. We review the primary mechanism of NP uptake by retinal cells and highlight various NPs that have been successfully used for *in vivo* gene delivery to the retina and RPE. Finally, we consider the various strategies that can be implemented in the plasmid DNA to generate persistent, high levels of gene expression.

1. Introduction

Gene replacement therapy holds great promise for the treatment of many inherited retinal diseases. This approach directly targets the root of the disease, rather than treating symptoms, and is therefore theoretically the closest approach to a cure. In practice however, gene replacement therapy is far from perfect. Besides potential safety concerns, practical limitations exist. These include limited uptake and distribution of the gene expression vector, attenuated expression of the therapeutic gene over time, and the difficulty of treating patients after the onset of degeneration. When it comes to gene therapy, the two major gene delivery methods are viral (e.g., adeno-associated virus (AAV)) and non-viral (nanoparticles (NPs)). Each system comes with its own set of advantages and disadvantages. While AAV-based therapies typically have better transfection efficiencies than NP-based systems [1], NP technology offers a unique set of advantages. NPs are easy to synthesize and their molecular structures can be easily manipulated due to accessible functional groups. Furthermore, they generally have a low production cost compared to AAV systems, can accommodate large vector sizes, and possess a favorable safety profile (low immunogenicity and no risk of insertion mutagenesis) (reviewed in [2]). An additional layer of complication is conferred by the content of the plasmid DNA itself, and great effort has been placed on optimizing the DNA content of gene delivery plasmids to optimize persistence and levels of gene expression after delivery.

The overall effectiveness of a NP-based gene delivery system is dependent on three key factors: (1) cellular uptake of NPs, (2) escape of NPs from endosomal vesicles into the cytosol, (3) transfer of the plasmid DNA to the nucleus. NPs that have been formulated for gene therapy fall into one of several categories: (1) metal NPs; (2) lipid NPs; (3) polymer NPs. They differ in size, charge, shape, and structure, but all possess a mechanism to enter the cell, avoid or escape from endosomes, and deliver the plasmid cargo into the nucleus for gene expression. In this review, we discuss retinal diseases that are suitable for gene therapy. Next, we highlight the mechanisms (e.g., endocytosis, phagocytosis) through which most NPs are taken up by cells in the retina, followed by a discussion of the key features of the different NP technologies that have been evaluated as vehicles for gene transfer to the retina. Finally we

Abbreviations: AAV, adeno-associated virus; NP, nanoparticles; LCA, Leber's congenital amaurosis; RPE, retinal pigment epithelium; OS, outer segment; SNP, single nucleotide polymorphism; ERG, electroretinogram; CME, clathrin mediated endocytosis; CvME, caveolae mediated endocytosis; ONL, outer nuclear layer; SLN, solid lipid nanoparticle; PLA, poly-lactic acid; PLGA, poly-lactic-co-glycolic acid; PEG, polyethylene glycol; HSA, human serum albumin; GNP, gelatin nanoparticle; K5, plasminogen kringle 5; RGC, retinal ganglion cell; INL, inner nuclear layer; OLM, outer limiting membrane; GFAP, glial fibrillary acidic protein; CNV, choroidal neovascularization; PAMAM, polyamidoamine; PPI, polypropylimine; LAA, lipoamino acid; RDS, retinal degeneration slow; S/MAR, scaffold/matrix attachment region; SAF, scaffold attachment factor.

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assess the influence the plasmid content has on therapeutic efficacy.

2. Ocular gene therapy approach for retinal diseases

Retinal diseases can be entirely genetic or caused by a combination of genetic and environmental factors. Of the latter, the most prevalent include diabetic retinopathy and age-related macular degeneration, in which the genetic component is not necessarily causative, and mutations in associated genes only contribute to risk of developing the disease (reviewed in [3,4]). On the other hand, most monogenic hereditary diseases can be traced in patients' genealogical pedigrees, and whole genome sequencing of samples from the patient and family members accelerates the identification of causal mutations and subsequent evaluation of disease mechanisms. Retinal degenerative diseases can be broadly categorized into two major groups depending on whether the disease initially targets rod (rod-cone dystrophy) or cone photoreceptor cells (cone-rod and cone dystrophies) (reviewed in [5]). Rod photoreceptors are responsible for night vision and are the dominant cell type in the peripheral (extramacular) region of the retina (>90%), whereas the macular (central) region of the retina is densely packed exclusively with cone photoreceptor cells. Patients with rod-cone dystrophies such as retinitis pigmentosa initially present with night-blindness followed by progressive loss of peripheral vision [6]. As the disease progresses to the advanced stages, patients are left with a small visual field that eventually disappears. Patients with cone-rod dystrophies such as Stargardt disease and Leber's congenital amaurosis (LCA) on the other hand present with rapid loss of central vision early in life followed by a progressive loss of peripheral vision [7–9].

To date, almost 300 unique genes have been associated with major retinal diseases including RP, LCA, and Stargardt disease (http://sph.uth.tmc.edu/retnet/disease.htm). These mutations are often in proteins directly responsible for a critical photoreceptor or retinal pigment epithelium (RPE) function, or in other proteins within the same functional network. Usher syndrome, for example, is associated with early onset retinitis pigmentosa and is caused by homozygous or compound heterozygous mutations within 10 different proteins (MYO7A, harmonin, CDH23, PCDH15, SANS, CIB2, usherin, VLGR1, whirlin, and clarin-1) (reviewed in [10,11]), all of which are thought to interact with one another within a protein complex that maintains photoreceptor structure and mediates material transport between photoreceptor inner and outer segments (OSs). While homozygous mutations in any one of the Usher-associated genes will cause retinitis pigmentosa, the onset of the disease, its progression, and the degree of severity can vary significantly from gene to gene and mutation to mutation. The heterogeneity of the disease is linked to the different functional roles of each protein within the complex. Similarly, mutations in genes involved in key developmental or physiological functions in the retina are also known to cause retinal degeneration, for example, mutations in retinal genes associated with photoreceptor development (e.g., CRX and CRB1) and phototransduction (e.g., GUCY2D) cause cone-rod dystrophy that is characteristic of LCA (reviewed in [12]). Mutations in genes expressed in the adjacent RPE can also cause retinal degeneration. Loss-of-function mutations in RPE genes such as RPE65 and LRAT (both involved in retinoid cycle) and in MERTK (regulates photoreceptor OS phagocytosis by the RPE) are known to cause LCA. The monogenic nature of many hereditary retinal diseases makes them a highly desirable target for gene therapy. But for each disease-associated gene, there can be up to hundreds of clinically-verified pathogenic single nucleotide polymorphisms (SNPs) scattered across the entire length of the gene. Therefore, for autosomal recessive retinal diseases, the most economical and straightforward strategy is the restoration of a fully functional version of the protein via DNA-based gene-therapy. In cases when the disease is caused by a dominant genetic mutation, gene-editing strategies (e.g., zinc-finger nucleases) can be implemented to correct the DNA mutation at the chromosomal level [13,14]. Alternatively, shRNA knockdown of the mutant message with concurrent expression of a knockdown-resistant copy of the wild-type gene [15,16] is a widely tested approach.

From a technical perspective, retinal gene therapy is feasible and is aided by the systemic isolation and immune privilege of the retina [17]. Gene-therapy vectors (and their carriers) delivered directly to the retina are less susceptible to elimination through the immune system and systemic excretion, and therefore they usually have a longer half-life, improved effectiveness, and higher bioavailability compared to vectors delivered systemically. In addition, potential side effects from non-specific delivery of the gene to other organs, such as the liver or kidney, can also be avoided. Although this local delivery improves safety profiles (since the systemic immune system is not activated), this is somewhat counteracted by the fact that intraocular delivery of therapeutics is highly invasive. Targeting cells in the outer retina (photoreceptors and RPE cells) requires subretinal injections [18,19]. Although advancements have been made in the injection technique, repeated injections are prone to complications and should be avoided. Therefore, the success of a gene therapy strategy is contingent on its ability to achieve, from a single injection, persistent, high levels of gene expression and phenotypic correction in the target retinal cell type. Optimizing these parameters is the goal of preclinical gene therapy trials, and with the availability of non-invasive tools for accessing retinal morphology (e.g., optical coherence tomography) and function (electroretinography; ERG), gene-therapy plasmids and packaging methods can be easily evaluated for their effectiveness in treating animal models of the disease prior to human use.

3. NP vs. viral strategies for ocular gene therapy

Assessments of non-viral NP-based gene-therapies often begin with a discussion comparing NPs to viral systems. This is due largely to the success of AAV gene therapy studies: as an example the first successful clinical application of retinal gene-therapy was achieved using AAV-based delivery of the *RPE65* gene for the treatment of LCA in 2008 [20–22]. Patients from these studies retained a high level of visual function up to three years after treatment and did not develop any major health complications [23–25]. AAV-based therapies are currently being developed for various retinal diseases, including rod-cone dystrophy, Stargardt disease, and juvenile retinoschisis (reviewed in [26]). As a result of this success, AAV-based therapies have become the benchmark for other gene-therapy approaches. However, a major drawback to AAVs as a carrier is that they can only accommodate relatively small sized genetic cargo (<5 kbp) [27]. The most recent advancements in AAV technology have extended the total transgene capacity to 10 kbp with the use of dual AAV vectors, wherein the gene is delivered in two fragments (<5 kbp each AAV) and subsequently recombines in the infected cells using homologous recombination or trans-splicing strategy, or both [28]. On the other hand, NPs can easily accommodate plasmids with sizes up to 20 kbp [29]. In addition to the size limitations of AAVs, there are also some issues with safety. Studies have shown that AAV vector DNA was detected in the serum of injected animals within 15 min after subretinal injection [30]. Viral genomes were also detected in nasal and lacrimal fluids. Other studies show that intravitreal injection of AAV carrying a GFP reporter gene into mice and dogs induced GFP expression in the optic nerve and brain [1,31]. The threats are compounded by