Progress in Retinal and Eye Research 43 (2014) 108–128

Contents lists available at ScienceDirect

Progress in Retinal and Eye Research

journal homepage: www.elsevier.com/locate/prer

Vector platforms for gene therapy of inherited retinopathies

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ARTICLE INFO

Article history:
Received 3 June 2014
Received in revised form
26 July 2014
Accepted 2 August 2014
Available online 12 August 2014

Keywords:
Gene therapy
Adeno-associated virus
Lentivirus
Adenovirus
Non-viral vectors
Inherited retinopathies

ABSTRACT

Inherited retinopathies (IR) are common untreatable blinding conditions. Most of them are inherited as monogenic disorders, due to mutations in genes expressed in retinal photoreceptors (PR) and in retinal pigment epithelium (RPE). The retina's compatibility with gene transfer has made transduction of different retinal cell layers in small and large animal models via viral and non-viral vectors possible. The ongoing identification of novel viruses as well as modifications of existing ones based either on rational design or directed evolution have generated vector variants with improved transduction properties. Dozens of promising proofs of concept have been obtained in IR animal models with both viral and non-viral vectors, and some of them have been relayed to clinical trials. To date, recombinant vectors based on the adeno-associated virus (AAV) represent the most promising tool for retinal gene therapy, given their ability to efficiently deliver therapeutic genes to both PR and RPE and their excellent safety and efficacy profiles in humans. However, AAVs' limited cargo capacity has prevented application of the viral vector to treatments requiring transfer of genes with a coding sequence larger than 5 kb. Vectors with larger capacity, i.e. nanoparticles, adenoviral and lentiviral vectors are being exploited for gene transfer to the retina in animal models and, more recently, in humans. This review focuses on the available platforms for retinal gene therapy to fight inherited blindness, highlights their main strengths and examines the efforts to overcome some of their limitations.

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[1] Percentage of work contributed by each author in the production of the manuscript is as follows: Ivana Trapani: 40%; Agostina Puppo: 20%; Alberto Auricchio: 40%.

http://dx.doi.org/10.1016/j.preteyeres.2014.08.001
1350-9462/

1. Inherited retinopathies

Untreatable blinding diseases include: i. common conditions like age-related macular degeneration, diabetic retinopathy or glaucoma which are inherited as complex traits in which the disease is the result of the interplay between environmental and genetic factors; ii. inherited retinopathies (IR) which are almost exclusively due to single gene defects and are very rare if considered as single entities but represent all together a significant cause of blindness, affecting about 1:2000 people worldwide (Berger et al., 2010). This review will focus on gene therapy of IR. IR are monogenic diseases caused by mutations in one of more than 200 genes (Cepko, 2012) mainly expressed in photoreceptor cells (PR, rods and cones) and to a lesser extent in the retinal pigment epithelium (RPE). IR are generally classified based on the genetic defect (when identified), the inheritance pattern (either autosomal dominant, recessive or X-linked), the main cell type affected, the onset and type of visual dysfunction, the disease progression rate, and the appearance of peculiar ocular fundus abnormalities (Berger et al., 2010). Among the most frequent and severe forms of IR are retinitis pigmentosa (RP), Leber congenital amaurosis (LCA), Stargardt disease (STGD) and achromatopsia (Lipinski et al., 2013). Retinitis pigmentosa presents either as isolated or as a syndrome. The most frequent syndromic RP is Usher syndrome (USH), which also affects hearing ability (Millan et al., 2011).

2. The eye as a target for gene therapy

Effective treatments for IR are currently unavailable. However, in the past three decades, the identification of many IR-causing genes has paved the way for the development of many gene therapy-based strategies. The eye's unique features make it ideal for gene therapy (Sahel and Roska, 2013). Specifically, it is a small, enclosed compartment, thus enabling small amounts of vector to act, keeping the risk of toxic reactions to a minimum. The eye is also an immune-privileged site given the presence of tight junctions between RPE cells, the blood–retina barrier, local inhibition of immune responses by the unique intraocular microenvironment and systemic induction of immunosuppressive regulatory T cells via eye-specific mechanisms (Caspi, 2010; Willett and Bennett, 2013). These features keep the vector from disseminating systemically and the immune system from reacting against either the vector components or the transgene product. Together these advantages reduce the risk of adverse systemic effects. Retinal cell types are post-mitotic, and thus sustained long-term gene expression can be achieved even in the absence of transgene integration. Various IR animal models are available, which may facilitate preclinical assessment of the efficacy of therapy. The eye's structure allows to evaluate treatment progress; the transparency of the ocular media and the availability of recently developed *in vivo* imaging techniques allow for non-invasive and consistent monitoring of the effects of gene delivery in both animal models and patients. Additionally, given that many ocular diseases are bilaterally symmetrical, we can take advantage of this characteristic to compare the effects of vector/gene delivery in one eye to disease progression in the contralateral eye. Lastly, the eye's accessibility from the exterior allows surgical procedures to be adapted to transfer genetic material into specific ocular compartments and to preferentially target a particular ocular cell type, with minimal risk to patients undergoing surgical procedures. The two most common methods of intraocular delivery are intravitreal and subretinal injections (Liang et al., 2000). Intravitreal injections consist of the release of the therapeutic agent in the vitreous and result in the exposure of the anterior retina. Subretinal injections, alternatively, deposit the vector into a virtual space between the RPE and the PR, inducing a regional and reversible detachment called a “bleb”. The intravitreal injection allows a more widespread distribution of the therapeutic agent over the retina than that of subretinal delivery, using a less challenging and invasive procedure. However, the diffusion of the therapeutic agent to the PR and RPE after intravitreal delivery is limited by several physical barriers, such as the vitreous, the inner limiting membrane (ILM), and the inner retina. Thus, subretinal vector delivery is currently considered to be the most efficient route for targeting PR and RPE cells in the outer retina, the target cells for the treatment of most IR.

3. Gene therapy approaches for IR

Various strategies can be applied to IR gene therapy according to the effect of the mutation underlying the disease. Gene replacement is employed for disorders due to loss-of-function mutations and is based on the delivery of a correct copy of the defective gene without removal of the endogenous mutant one. Gene silencing inhibits the expression of the mutated gene via modification of messenger RNA (mRNA) and is applied to disorders caused by gain-of-function mutations. Given the high number of genes involved in IR and the common apoptotic pathways leading to PR cell death and degeneration, mutation-independent strategies may be designed to either restore photosensitivity by converting surviving retinal cells into photosensors (optogenetics, see Section 5.3.5.) (Mei and Zhang, 2012) or to slow down retinal degeneration (neuroprotection) (Colella and Auricchio, 2010). Neuroprotection prolongs the lifespan of the PR rather than correcting a gene-specific defect. This is typically accomplished by genetically supplying naturally occurring low molecular weight growth factors, which when present at high concentrations, elicit neuroprotective effects. This approach is advantageous because such factors are highly diffusible, and thus the RPE or retinal ganglion cells (RGC) can be converted into factories for secretion of the neurotrophic factor by simple gene transfer techniques. Irrespective of IR mutation-dependent and -independent strategies, vectors are key for successful gene therapy in the eye. There are two major classes of vectors for gene therapy, non-viral and viral. Non-viral vectors include DNA, which can be left naked or combined with either chemicals such as liposomes, polymers and compacted nanoparticles, or physical methods such as electroporation or