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REVIEW ARTICLE

Lung gene therapy—How to capture illumination from the light already present in the tunnel

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Abstract Gene therapy has been considered as the most ideal medical intervention for genetic diseases because it is intended to target the cause of diseases instead of disease symptoms. Availability of techniques for identification of genetic mutations and for *in vitro* manipulation of genes makes it practical and attractive. After the initial hype in 1990s and later disappointments in clinical trials for more than a decade, light has finally come into the tunnel in recent years, especially in the field of eye gene therapy where it has taken big strides. Clinical trials in gene therapy for retinal degenerative diseases such as Leber's congenital amaurosis (LCA) and choroideremia demonstrated clear therapeutic efficacies without apparent side effects. Although these successful examples are still rare and sporadic in the field, they provide the proof of concept for harnessing the power of gene therapy to treat genetic diseases and to modernize our medication. In addition, those success stories illuminate the path for the development of gene therapy treating other genetic diseases. Because of the differences in target organs and cells, distinct barriers to gene delivery exist in gene therapy for each genetic disease. It is not feasible for authors to review the current development in the entire field. Thus, in this article, we will focus on what we can learn from the current success in gene therapy for retinal degenerative diseases to speed up the gene therapy development for lung diseases, such as cystic fibrosis.

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Introduction

In 2008, three research teams independently reported the success in clinical trails of gene therapy treating a rare form of retinal degenerative diseases called Leber's congenital amaurosis (LCA).[1–3] LCA represents a group of inherited blindness with childhood onset.[4] The clinical success has been achieved in treating LCA2, one form of the disease, which is caused by mutations in the retinal pigment epithelium-specific 65-kDa protein gene (*REP65*). *REP65* encodes a protein providing the isomerohydrolase activity for the retinal pigment epithelium to produce 11-*cis*-retinal from all-*trans*-retinyl esters during the visual cycle for regenerating the visual pigment after exposure to light. Without this gene function, 11-*cis*-retinal, the natural ligand and chromophore of the opsins of photoreceptor cells, cannot be regenerated, thus rendering the opsins incapable of capturing light or transducing it into electrical responses for initiating vision. Although this defect in light transduction has an immediate impact on visual function, retinal cell degeneration is delayed in patients, thus making target cells available for gene therapy. The three teams tested the same therapeutic approach in patients by subretinal injection of recombinant adeno-associated virus vector 2 (AAV2) expressing the *RPE65* complementary DNA (cDNA). Patients with treatment showed improvements in visual function without serious adverse events. In 2012, three patients received the same treatment in their other eye and all three demonstrated improvements in visual and retinal function in their second eyes after the treatment, which was administered one-and-a-half to three-and-a-half years after their first eyes were treated.[5] Readministration of the same gene therapy vector caused no harmful immune reactions in patients. In 2014, a gene therapy trial for another retinal degenerative disease, choroideremia, was shown to be successful.[6] Choroideremia is an X-linked recessive disease that is caused by mutations in the *CHM* gene, which encodes the Rab escort protein 1 (REP1). The same gene therapy vector, AAV2, was used in this study. In addition to the eye gene therapy success, progress has been made in other fields as well. For example, as a milestone for using gene therapy as medicine, European Union approved Glybera as the first gene therapy drug for a form of lipoprotein lipase deficiency.[7–9] In this case, AAV1 was used to deliver a naturally occurring functional variant of the LPL gene associated with lower rates of cardiovascular disease and increased efficiency in fat metabolism. These clinical successes provide the proof of concept that the power of gene therapy can be harnessed to benefit human beings.

However, gene therapy developments for other diseases, such as cystic fibrosis (CF) lung disease, are not as successful for eye diseases.[10–13] CF is the most common monogenic fatal disorder in the Caucasian population and it is caused by recessive mutations in the gene encoding the cystic fibrosis transmembrane conductance regulator (*CFTR*).[14–16] Although the disease affects multiple organs, including the lung, pancreas, intestine, gall bladder and reproductive organs,[13,17] lung failure due to chronic infection and inflammation is currently responsible for most morbidity and mortality. Therefore, CF gene therapy studies to date have been aimed at treating the pulmonary manifestations. When the cystic fibrosis gene was identified in 1989, it appeared that this disease can be used as an ideal model for the development of gene therapy for lung diseases since airway epithelial cells where the *CFTR* gene is expressed are readily accessible to gene therapy vectors. Yet, all the CF clinical trials conducted so far did not show any evidence of significant therapeutic benefits brought to CF patients.[18–35] Basic research in lung gene therapy developments later identified major barriers to vector delivery and sustained therapeutic gene expression.[10,13,36] Thus, it is useful to look into what is fundamental to the successful gene therapy development for eye diseases to make lung gene therapy fruitful.

In this review article, we will first visit the early developments in CF lung therapy and look into the major challenges encountered in the lung gene therapy field. We will then review the key factors that are critical to the eye gene therapy progress to explain the possible rationale for the clinical success. We will finally discuss strategies that can be translated from the eye gene therapy field to speed up the lung gene therapy development.

Early stages of lung gene therapy developments

Because CF is a monogenic disease and the target cells in lung airway are easily accessible to gene therapy vectors, when the gene was identified, an illusion was created suggesting that lung gene therapy for CF would be available in a few years. The initial excitements inspired many scientists racing in conducting clinical trials. Both viral and non-viral gene therapy vectors were tested. One of the early clinical studies was conducted by Zabner et al in 1993 to examine the safety profile of an adenoviral (Ad) vector with nasal applications.[18] Adenoviruses contain a linear double-stranded DNA and have been widely used as tools for gene delivery because of their ability to infect both dividing and non-dividing cells with a high efficiency, especially epithelial cells. The early generations of Ad vectors were developed by deleting the E1 region within the viral genome to prevent viral proliferation in transduced cells and/or other regions such as E3 or E4 to increase the DNA carrying capacity. There are more than 50 serotypes of adenoviruses identified so far.[37] In this study, a serotype 2 adenoviral vector expressing the human CFTR cDNA was administered to a defined area of nasal epithelium in three patients. Although this initial study showed some functional correction in nasal epithelial cells with no vector-related adverse effects, more extensive studies later demonstrated with similar methods that there was no significant functional correction in nasal epithelia.[19,21] The Ad vectors have also been tested in the lung[24–26] and none of the studies demonstrated functional correction or efficacy in patients.

In addition to the early generations of Ad vectors, recombinant adeno-associated virus (AAV) vectors have also been tested in CF patients.[28,38,39] AAV is a replication-defective parvovirus that depends on a helper virus, either adenovirus or herpes virus, for its propagation during lytic infection.[40] It has a small single-stranded DNA genome (about 4.7 kb). The advantage of AAV as a gene therapy