BASIC SCIENCE FOR THE CHEST PHYSICIAN

Delivery of genes into the CF airway

Deborah R Gill,^{1,2} Stephen C Hyde^{1,2}

¹Gene Medicine Research Group, Nuffield Division of Clinical Laboratory Sciences, Radcliffe Department of Medicine, University of Oxford, John Radcliffe Hospital, Oxford, UK

²UK Cystic Fibrosis Gene Therapy Consortium, Imperial College London, University of Edinburgh, University of Oxford, Oxford, UK

Correspondence to

Dr Stephen C Hyde, Nuffield Division of Clinical Laboratory Sciences, Radcliffe Department of Medicine, John Radcliffe Hospital, University of Oxford, Oxford OX3 9DU, UK; steve.hyde@ndcls.ox.ac.uk

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ABSTRACT

Gene therapy was suggested as a potential treatment for cystic fibrosis (CF), even before the identification of the *CFTR* gene. Initial enthusiasm has been tempered as it became apparent that reintroduction of the *CFTR* gene into the cells of the lung is more difficult than anticipated. Here, we review the major gene delivery vectors evaluated clinically, and suggest that advances in either plasmid DNA design and/or hybrid lentivirus biology may finally facilitate lung gene transfer with efficiencies sufficient for CF gene therapy to offer clinical benefit.

INTRODUCTION

The potential for gene delivery to treat lung disease gained momentum following the discovery of the cystic fibrosis (CF) gene in 1989. Mutations in the gene encoding the CF transmembrane conductance regulator (CFTR) protein—an epithelial ion channel principally conductive for chloride and bicarbonate—result in an ionic imbalance across the epithelia of affected tissues. In the lung, CF mutations result in the production of abnormally compacted mucus and a reduction in mucociliary clearance. Repeated cycles of bacterial infection and inflammation then lead to deterioration of lung function and ultimately pulmonary failure, which is the cause of death for the majority (>90%) of patients.

The delivery of a therapeutic nucleic acid (DNA or RNA) is an intellectually satisfying concept for an inherited single-gene defect such as CF, with the prospect of correcting many aspects of the complex pathology. The most attractive form of gene therapy is gene repair, in which the specific mutated bases in an individual's genome are targeted and corrected. Recent advances in genome editing, driven by the emergence of the CRISPR/Cas9 system and the maturation of Zinc Finger Nuclease and TALEN technologies,¹ are encouraging, but the relative inefficiencies of these methods and the large number of CF mutations (~2000 at the time of writing, though not all are known to cause disease) currently leaves this approach beyond our grasp for CF. Consequently, the field has mainly focused on gene complementation approaches, where an additional copy of the wild-type CFTR cDNA, under the transcriptional control of a well-characterised exogenous promoter, is delivered to cells homozygous for CFTR mutations. A major conceptual advantage of gene complementation over gene repair is that a single pharmaceutical entity could treat all classes of CF mutations, meaning that a single gene-based drug formulation could be developed to treat all CF

patients. This is in stark contrast with recent successful small-molecule therapeutic agents developed for CF, such as ivacaftor (trade name: Kalydeco, Vertex), that are specific for relatively rare class III CFTR mutations, and are thus restricted to a limited number of CF patients.²

BARRIERS TO LUNG GENE DELIVERY

A crucial dogma of lung cell biology is that the surface airway epithelium is composed of quiescent, terminally differentiated cells types that are slowly replaced by (poorly characterised) stem/progenitor cells. Thus, an effective gene complementation approach for chronic lung diseases such as CF must be capable of being repeatedly delivered to the terminally differentiated cell surface without loss of efficacy, or be capable of permanently altering those stem/progenitor cells that act to populate the lung. Several barriers to successful lung gene transfer have been identified. First, the lung has evolved to keep out pathogens and particulates using a variety of physical and immune mechanisms. Additionally, sputum may be particularly obstructive in CF patients, suggesting that younger CF patients with minimal lung damage and blocked airways should be targeted for gene therapy. Once the gene delivery vector has reached the cell surface, there may be a paucity of receptors available for specific vector uptake, resulting in inefficient gene transfer. Gene delivery vectors may also become inactivated by the immune system and products of inflammation. Tackling these barriers has led to the development of a range of gene transfer vectors (see [figure 1](#)).

CLINICAL DEVELOPMENT OF CF GENE THERAPY

Shortly after the identification of the CFTR gene, multiple clinical trials using the gene complementation approach were initiated, establishing proof-of-concept; 25 Phase I/II clinical trials, involving over 470 patients, have been carried out to date. Viral vectors are based on modified viruses with airway cell tropisms, and tend to be more efficient than non-viral (synthetic) alternatives.

ADENOVIRAL VECTORS

Recombinant *adenovirus* (rAd) was a promising gene delivery vector due to the high gene transfer efficiencies observed in animal models. However, this potential was not fulfilled in clinical trials mainly due to the paucity of adenoviral receptors on the apical lung surface and the severity of the host-immune response to repeated viral delivery.³ In spite of many vector modifications, such as the removal of the all adenoviral genes in 'gutless' vectors, there is currently

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Figure 1 Gene transfer vectors must negotiate physical and immune barriers in the airways before arriving at the apical cell surface to deliver their genetic cargo. Cell receptors on the apical and/or basolateral membrane allow vector binding and entry. Access to basolateral receptors requires temporary disruption of epithelial cell tight junctions. Receptors for recombinant adenoviral vectors (rAd)—CAR (the coxsackievirus and adenovirus receptor) plus a variety of integrins; recombinant adeno-associated viral vectors (rAAV)—heparan sulphate proteoglycan plus integrins and/or sialic acid; and non-viral vectors (plasmid DNA double circle, cationic liposome/polymer orange circles), allow cell entry via Clathrin-dependent receptor-mediated endocytosis. All these vectors promote bursting of the endosome (dashed circles) to release of the vector genome into the cytoplasm. Both the rAd DNA genome and plasmid DNA delivered via non-viral vectors are circular, double-stranded molecules that are trafficked to the nucleus and persist extra-chromosomally. The single-stranded rAAV DNA genome requires conversion and concatemerisation to a linear double-stranded form, which then also exists extra-chromosomally. New serotypes of rAAV with increased affinity for apical receptors have led to improved airway transduction. Receptors for recombinant lentiviral vectors (rLV) are determined by the vector pseudotype, such as the VSV-G protein that binds basolateral heparan sulphate proteoglycan for cell entry via endocytosis. rLV vectors can be pseudotyped with proteins with high affinity for airway cell receptors (F/HN pseudotype uses sialic acid and permits direct cell entry via membrane fusion) leading to increased gene transfer efficiency. After entry, the rLV RNA genome (wavy lines) must be converted to double-strand DNA form (straight lines) prior to integration (red chromosome band) into the host genome.

little support for the further progression of rAd vectors for treatment of chronic lung disease in the clinic.

ADENO-ASSOCIATED VIRUS

Another promising viral vector evaluated for treatment of CF is based on recombinant adeno-associated virus (rAAV). Like gutless adenoviral vectors, rAAV vectors are also DNA-based and devoid of viral genes. Numerous AAV serotypes have been discovered with a variety of tropisms for different tissues. AAV2 was the first serotype to be evaluated clinically for CF, but was ultimately disappointing in a large repeat-administration study powered to detect changes in lung function in CF patients.³ The precise reason for the failure of AAV in these clinical studies is unclear, but new serotypes that are currently under development may offer improved gene transfer efficiencies in the airways. However, AAV is still likely to suffer from problems with effective repeated administration that will need to be overcome for treatment of CF.

NON-VIRAL GENE TRANSFER

Non-viral (synthetic) vectors have been developed to avoid the use of viral proteins, thus minimising the risk of immunogenicity and increasing the chance of effective repeated administration. Proof of principle for repeat administration of a non-viral vector to the nasal epithelium has been demonstrated in CF patients.³ Typically, non-viral vectors comprise circular, plasmid

DNA (pDNA) molecules manufactured from bacteria, which are then complexed with a range of cationic lipids and polymers, known as 'lipoplexes' or 'polyplexes' respectively. Non-viral vectors tend to be less efficient because they lack the specific components required for cell entry that are present in viruses. However, aerosol delivery of pDNA complexed with cationic liposomes to the CF lung resulted in a 25% correction of the CFTR ion transport defect in the lungs.³ One disappointing aspect of this clinical study, and many others using non-viral vectors, was that the observed correction and gene expression was transient. Subsequently, both the level and persistence of gene expression in the lung has been improved by optimisation of the pDNA molecule. Removal of CG dinucleotides from the pDNA and careful manipulation of sequences responsible for gene expression resulted in non-viral vectors with persistent gene expression and minimal inflammation in mouse lung models.⁴ This non-viral formulation has been delivered to the nose and lungs of CF patients in a single-dose pilot study, identifying a safe dose for evaluation in an ongoing Phase IIb multi-dose study (EudraCT: 2011-004761-33, ClinicalTrials.gov: NCT01621867).⁵ In this Phase IIb study, which is the largest CF gene therapy trial performed to-date (enrolling ~130 subjects), participants each received 12 monthly doses of either the non-viral vector or a placebo. Unlike previous studies which have focused on molecular endpoints (typically CFTR channel function and/or vector-derived mRNA), the Phase IIb aims to

evaluate clinical benefit (the primary endpoint being a change in FEV1); the results are anticipated towards the end of 2014.

LENTIVIRUS

A promising lung gene transfer vector, not yet evaluated in the clinic, is based on recombinant lentivirus (rLV). These viruses are RNA-based, and once inside the cell require reverse transcription to DNA, which can then be integrated into the host genome (see figure 1). Genomic integration may be advantageous as it ensures that the vector is passed to daughter cells during division, although it may also be considered a safety risk due to potential genotoxicity. Recombinant lentiviral vectors may be modified by the addition of novel surface proteins (known as pseudotyping) to specifically boost efficiency of airway gene transfer. Importantly, vectors, such as those pseudotyped with the F and HN coat proteins from Sendai virus, result in gene expression for the lifetime of a mouse after a single dose, and can be repeatedly administered without loss of efficacy.⁶ Therefore, these rLV vectors combine the efficiency of viruses with the potential for long-lasting expression after repeated administration to the terminally differentiated airway epithelial cells in the lung. Further development of these vectors, including their large-scale production and purification, is required so that they can be aerosolised to the lungs for treatment of chronic lung disease.

CONCLUSION

CF has been an important pathfinder disease for gene therapy. The challenges faced (and so far overcome) in the lung have led

to the refinement and clinical development of new gene delivery vectors. Discovering if the efficiency of the non-viral pDNA/liposome formulation currently in clinical trial is sufficient for clinical benefit in CF patients will confirm whether more efficient vectors, such as pseudotyped lentiviruses are required. Ultimately, developments in new vector technologies will not only progress gene therapy for CF, but should also benefit applications to treat other lung diseases.

Competing interests The authors hold IP in non-viral and lentiviral gene transfer vectors for the treatment of cystic fibrosis and other diseases.

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