Commentary

Taking stock of gene therapy for cystic fibrosis

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Abstract

The identification of the cystic fibrosis (CF) gene opened the way for gene therapy. In the ten years since then, proof of principle *in vitro* and then in animal models *in vivo* has been followed by numerous clinical studies using both viral and non-viral vectors to transfer normal copies of the gene to the lungs and noses of CF patients. A wealth of data have emerged from these studies, reflecting enormous progress and also helping to focus and define key difficulties that remain unresolved. Gene therapy for CF remains the most promising possibility for curative rather than symptomatic therapy.

Keywords: cationic lipids, CFTR, cystic fibrosis, gene therapy, recombinant viruses

The cloning of the CF gene with subsequent characterisation of its protein (CFTR) [1] opened the way for gene and protein therapy. The idea was simply to administer the normal CF gene or protein, as though it were a drug, to the organ most affected yet apparently quite accessible: the lungs. In theory this should result in the restoration of normal cellular function and thus prevent or treat the disease. A vigorous research effort followed the identification of the gene; the first reports of *CFTR* gene transfer *in vitro* appeared in 1990 [2], only one year later. Further studies *in vitro* [3] were followed by gene transfer *in vivo* to the airway epithelial cells of transgenic CF mice [4,5], confirming that it was possible to achieve some functional restoration of the ion transport defects in these cells after transgene expression. Within four years, four clinical studies of gene therapy in CF patients had been reported; since then there have been over 20 phase I studies.

Several of these trials used adenoviral vectors to transfer *CFTR* cDNA to adult volunteers with CF; many of these trials have now been reported [6–13]. Some early studies reported acute inflammation, which was probably related to the local instillation of a high viral load. There has been evidence of gene transfer as determined by the detection of mRNA or CFTR protein, although this was not a consistent finding. Electrophysiological correction has been suggested in some studies, but only one study was controlled and this showed limited evidence of gene transfer at the highest dose, with no evidence of functional correction. Two clinical trials with adeno-associated virus

AAV = adeno-associated virus; CF = cystic fibrosis.

(AAV) as the gene transfer agent have also been reported [14,15]. No significant side effects have been observed and some evidence of correction of the chloride defect has been suggested.

Liposome–plasmid complexes have been tested in several clinical trials [16–21]. Of these, five were double-blind placebo-controlled studies. Three trials with a similar design showed a partial correction of the chloride transport defect in the nose [16–18]. In a further study, complexes were delivered to the lungs by nebulisation [20] with an approximate 20% restoration of chloride transport towards normal values. Administration was associated with a transient febrile reaction that did not occur in the control group, who received only the lipid; this reaction might have been attributable to the bacterial origin of the DNA [22]. None of these studies showed any correction of the sodium defect, and vector-specific mRNA was only inconsistently found.

A wealth of data have emerged from all of these studies, reflecting enormous progress but also helping to focus on the key difficulties. The principal issue is to improve the efficiency of gene transfer, but questions relating to the level of efficiency needed and the target cell population also need addressing. The key problem of efficiency can be considered in terms of either improving vectors or overcoming biological barriers. An intrinsic function of the lining epithelium of the airways is to prevent penetration by foreign materials and invading organisms. Thus, a complex series of epithelial barriers, including a mucous layer that inhibits gene transfer [23], a glycocalyx, an apical cell membrane and tight junctions between the cells, conspire to keep out intraluminally delivered materials, including both viral and non-viral vectors. This problem is compounded in CF by the presence of thick, infected sputum, also known to inhibit gene transfer [24], and plugging of the small airways with mucus. The use of adjunctive mucolytic agents or the abrogation of tight junction barrier function either with detergents or with antibodies against intrinsic tight junction components, are just two novel strategies being investigated to overcome these barriers.

In addition, much effort is being devoted to the modification of vectors. For adenovirus, AAV and other viruses, the appropriate receptors are largely confined to the basolateral cell membrane. Thus, attempts are now being made to adapt viral entry by using apically sited receptors such as the UTP-binding P2Y2 receptor. Novel viruses are increasingly being investigated, and cationic liposomes and polymers are increasingly being coupled to peptides, sugars and viral particles to try to increase gene transfer efficiency. The vexing question of how much effective gene delivery and expression is required to achieve clinical benefit remains unresolved, but the level is likely to be low. If gene transfer is achieved in only a certain proportion of cells within the airway epithelium, then studies suggest that 6–10% of cells need to be corrected to reverse the chloride defect and 100% for the sodium defect [25,26]. If, in contrast, delivery occurs to every cell, then levels of approximately 5% of normal CFTR mRNA can correct the chloride defect and reverse the intestinal pathology in CF mice [27]. These goals have not yet been reached but the relatively low levels that might be needed are encouraging.

CF affects the conducting airways rather than the alveoli. These include both the larger bronchial regions lined by a pseudostratified columnar epithelium and containing numerous submucosal glands, and the small bronchiolar regions lined by a simple columnar epithelium devoid of glands. A central question for CF gene therapy is which cell type and which region (large or small airways) to target. Although ciliated superficial epithelium is abundant and displays the ion transport defects in patients with CF, the submucosal glands are the cells expressing the highest CFTR levels in the lung and might well need to be targeted for clinical benefit. This raises considerations of delivery, because topical application is unlikely to reach these cells. Further, most results suggest that small airways are both the initial and the major site of disease in CF. Again, the effective delivery of cDNA to these areas is difficult with present nebuliser technology and remains an important strategic issue.

A final important issue relates to repeated application. All of the current vectors are likely to produce episomal gene transfer, and the current duration of expression after a single application is measurable in weeks. This does not matter as long as multiple applications are feasible. In this regard, cationic liposomes seem to have an important advantage, with a third application having been shown to be as effective as the first in a recent trial of multiple applications of liposome-mediated gene therapy to the nasal epithelium of CF patients [28]. The repeated administration of viral vectors results in the production of neutralising antibodies that limit reapplication efficiency, and considerable effort is being expended to reduce this problem.

Gene therapy will probably prove to be most beneficial if given very early, before the onset of established infection or inflammation in the lungs. Thus, questions about the execution and design of trials in the paediatric population are likely to become the focus of new efforts [29]. The rigorous measurement of gene transfer efficiency *in vivo* and the development of markers – both real and surrogate – of clinical benefit also remain important challenges.

In conclusion, *CFTR* gene therapy has proved to be safe so far and has produced a proof of principle with respect to CFTR function in the target organ in humans, an encouraging position in a young field. Nevertheless, it is not yet a clinically effective treatment for CF lung disease.

The inevitable pessimism that follows unrealistic expectations of a rapid cure has dogged the recent media view of gene therapy. Gene therapy is clearly at the typical stage of new drug development where considerable and unglamorous effort needs to be expended to move from proof of principle to clinical product. Encouraging progress in gene therapy can clearly be found both within the CF field and in parallel areas. Thus, a clinical study of intramuscular injection of an AAV vector expressing factor IX in adults with severe haemophilia B [30] demonstrated prolonged expression of the protein and positive changes in clinical endpoints, including circulating levels of factor IX and the frequency of infusion of factor IX protein. The questions that remain to be answered for successful CF gene therapy have now been clearly defined, and gene therapy for CF remains the most promising possibility for curative rather than symptomatic therapy.

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