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MINI-SYMPOSIUM: Optimising Cystic Fibrosis Outcomes: Screening and Treating in 2013

Cystic Fibrosis: therapies targeting specific gene defects

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EDUCATIONAL AIMS

- To revise the different classes of mutations causing Cystic Fibrosis
- To inform the reader of new classes of drugs emerging for the treatment of CF
- To discuss the background of new drug development
- To provide *in-vitro* and *in-vivo* data on the safety and efficacy of new drugs

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SUMMARY

Cystic Fibrosis (CF) is caused by a large number of mutations in the *CFTR* gene, leading to specific classes of protein defects. This review discusses these classes, an understanding of which has paved the way for novel treatment strategies. The progress in this field, through from basic research to, in one case, application for license, is described.

BACKGROUND

Cystic fibrosis (CF) is an autosomal recessive condition with a heterozygote carrier rate of 1:25 amongst the Caucasian population and estimated worldwide numbers of 60,000 – 70,000 affected patients.[1] It is caused by a defect in the cystic fibrosis transmembrane conductance regulator (*CFTR*) gene located on the long arm of chromosome 7. This gene encodes the CFTR protein, which is made up of 1480 amino acids.[2] In normal health, this protein crosses the cytoplasm to the cell membrane where it exhibits its functions, the best described of which is as a chloride channel with inhibitory effects on its neighbouring epithelial sodium channel, ENaC. It is therefore critical in chloride and sodium transport across the cell membrane and as a consequence, regulates the hydration of the epithelial surface (water following sodium down its osmotic gradient). Thus, defective CFTR protein leads to absent or decreased chloride secretion, increased sodium and water absorption and airway surface liquid depletion. This impairs mucociliary clearance and results in the classical manifestations of CF: infection, inflammation and eventual bronchiectasis (Figure 1). Respiratory failure is the cause of death in over 90% of CF patients.

Whilst previously a disease of childhood, survival has improved significantly over the past 40 years; this is largely due to emphasis on basic treatments, pulmonary physiotherapy, antibiotics and improved nutrition. Once the gene causing cystic fibrosis was identified in 1989 many believed a cure would not be far off. However, despite ongoing efforts in this area, management to date is based on treating the downstream consequences of mutated CFTR, rather than preventing these by targeting the basic defect. However, over recent years, a better understanding of the basic defect has led to the prospect of gene therapy and new small molecule drugs becoming a reality. The former is currently at the clinical trial stage but as it is not mutation-specific, would be applicable to all CF patients should it show therapeutic promise. It is not discussed further in this article, but the interested reader is directed to an update and review of the field by the UK CF Gene Therapy Consortium.[3] This article focuses on the small molecule drugs under development, which target *specific* classes of CFTR mutation.

CLASSES OF CFTR MUTATIONS

Six classes of CFTR mutations have been identified to date; these are described in Table 1 and illustrated in Figure 2. In general,

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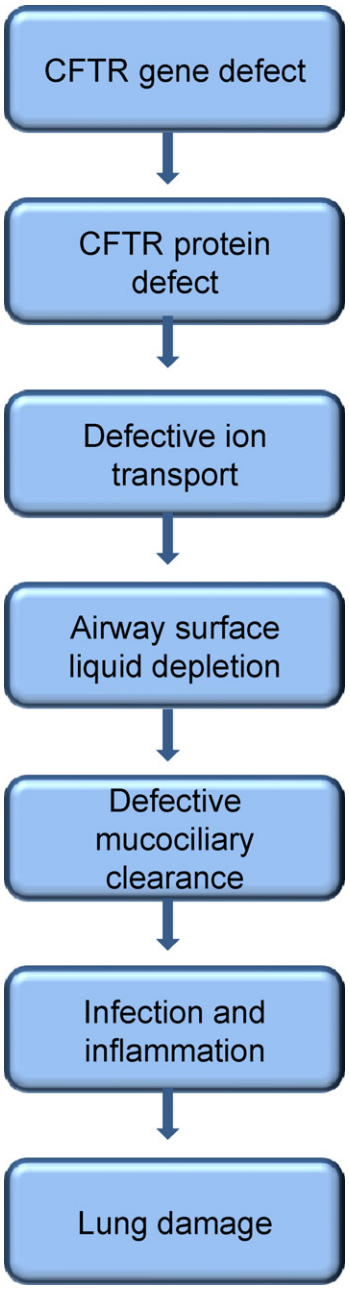

Figure 1. Flow chart to illustrate the downstream consequences of *CFTR* mutations.

genotype/ phenotype correlation is rather poor for lung disease, with the exception of some class IV, V and VI mutations, which may to confer a more variable and sometimes milder disease. The relationship is stronger for pancreatic status, with most patients possessing mutations from classes I-III having exocrine pancreatic dysfunction, whilst many of those in classes IV, V and VI have sufficient function to be clinically pancreatic sufficient. In patients who are compound heterozgotes, the milder mutation usually dominates. One limitation to this classification system is that some mutations lead to protein with features of more than one of these classes; an example is the commonest mutation Phe508del (previously called ΔF508), classically regarded as a class II, misfolding mutation. However, small amounts may escape the intracellular degradation system and any protein that does reach the cell surface also demonstrates features of class III (reduced gating) and class VI (instability and shortened half-life). A further limitation is that of the 1903 mutations identified in the CFTR gene[4] many are, as yet, unclassified. The CFTR2 project will seek to address this.[5]

MUTATION SPECIFIC TREATMENTS

Modern technology has allowed small molecules to be identified through high throughput screening which can simultaneously look at over 100,000 potential drugs. With regards to CF, this technology seeks to identify molecules that improve chloride ion transport in human bronchial CF cells;[7,8] promising 'hits' are then investigated further. It is important to bear in mind that CFTR possesses functions in addition to chloride ion transport and the clinical significance of certain of these is currently unclear.

CLASS I MUTATIONS

Class I CFTR mutations (Figure 2), often called 'nonsense' mutations, contain a premature termination codon (PTC). The result is a shortened and usually unstable, non-functional CFTR protein. Examples of class I mutations include Trp1282X (previously W1282X) and Gly542X. They are highly prevalent amongst Jewish CF individuals, and account for 60% of CF in Israel. Worldwide, class I mutations account for 5-10% of the CF population.[9]

Aminoglycosides were initially shown to allow the ribosome to 'skip' the stop mutation; a random amino acid is inserted at the site of the premature termination codon and full length protein results. *In vitro* studies demonstrated restoration of CFTR function at the cell surface at up to 35% of wild-type levels[10,11] and open-label clinical trials soon followed. Proof of concept was achieved in small trials with nasal[12] or intravenous[13] administration of gentamicin and these were followed by a randomised double blind placebo controlled crossover trial to assess the efficacy of topical gentamicin nose drops on ion transport assessed by nasal potential

Table 1
Classes of protein defects resulting from *CFTR* gene mutations.

CFTR gene mutation class	CFTR protein basic defect
I	Non-sense mutations: A premature termination codon leads to truncated protein with no function [eg. Trp1282X previously termed W1282X]
II	Trafficking mutations: Misfolded protein fails to traffic to the apical cell surface and instead is degraded by intracellular processes [eg. Phe508del, previously termed ΔF508]
III	Gating defect: Protein reaches the apical cell membrane in normal levels, but fails to open in response to intracellular signals [eg. Gly551Asp previously termed G551D]
IV	Decreased conductivity: Protein reaches the cell surface but the abnormal conformation of the pore leads to poor conductance of chloride ions [eg. Arg117His previously termed R117H]
V	Splicing defect: leads to decreased amount of CFTR protein at the cell surface [eg 3849+10 kb C>T]
VI	Functional but unstable with decreased half life at the cell surface [eg Gln1412X previously termed Q1412X]