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COMMENTARY

Epithelial Cell Chloride Channel Activity Correlates with Improved Airway Function in Cystic Fibrosis Patients with the Major Mutant: Delta F508

Commentary on the article by Sermet-Gaudelus *et al.* on page 628

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Cystic fibrosis (CF) is a lethal disease, caused by mutations in the gene, the cystic fibrosis transmembrane conductance regulator (CFTR) (1). Normally, the CFTR protein is localized at the apical membrane of epithelial cells of the respiratory tract, sweat ducts, gastrointestinal tract, and the reproductive organs. These multiple organs are affected in cystic fibrosis (2). CF-related airway disease is the major cause of mortality, characterized by mucus obstruction in the large airways, persistent pulmonary infections, intense neutrophil-dominated airway inflammation, and bronchiectasis (3).

The role of CFTR in epithelial tissue physiology. CFTR functions as a phosphorylation and nucleotide regulated chloride channel on the apical (or luminal) surface of epithelial cells (4, 5) and its role in fluid transport in various tissues depends on the driving force on chloride ions in each particular cell type. A number of hypotheses have been proposed to link the basic defect in CFTR protein to the dysregulated host defense observed in the airways of CF patients. Certain hypotheses evoke a direct role for CFTR in the regulation of airway inflammation (6) and others implicate a potential role for CFTR in the modification of cell surface receptors for bacterial binding (7). The relative contribution of each of these potential defects to the development and persistence of CF airway disease remains unclear. However, at present, there is consensus that the disruption of CFTR-dependent hydration of the airway surface represents a primary trigger for CF airway pathogenesis in cystic fibrosis (8, 9).

The severity of CF airway disease, the extent of bacterial infection and the consequences of bacterial colonization on lung function are highly variable among CF patients (10–12). Several factors may influence CF airway disease severity including: the nature of the primary CFTR mutation (13), the influence of secondary genetic modifiers (11, 12), expression of alternative chloride channels (14), and possibly the effect of environmental factors such as nutrition and hypoxia on the immune system (15). Clearly, an improved understanding of the factors modifying CF airway disease severity may provide us with additional clues regarding the key defects that promote persistent infection and inflammation.

The major mutation results in a misfolded protein. Presently, there are greater than 900 CFTR mutations documented to cause cystic fibrosis. Several different mechanisms are thought to underlie the molecular basis for disease. Certain types of mutation in the gene lead to defects in CFTR protein synthesis. Other types of mutations cause the CFTR protein to exhibit defective chloride channel function on the plasma membrane (4). Commonly, CFTR mutations can affect the folding and processing of the protein, such that its trafficking and/or stability at the cell surface are reduced (16). It is important to bear in mind that these broad groupings of mutations are not necessarily exclusive.

The major CF mutation, ΔF508, leads to the production of a misfolded protein. CFTRΔF508 protein is recognized by the quality control machinery of mammalian cells and retained in the endoplasmic reticulum (16). Hence, this protein fails to mature and is not complex glycosylated in the Golgi apparatus. Biochemically, this defect is readily detected in immunoblots of cell lines heterologously expressing the protein. Wild type CFTR protein migrates as a 170-kD protein (Band C) whereas CFTRΔF508 migrates primarily as a 140–150 kD protein (Band B) as it lacks complex glycosylation. The first comparative immunohistochemical studies of CFTR and CFTRΔF508 protein in human tissues, showed that CFTRΔF508 protein was mislocalized relative to the wild type protein in sweat ducts. In contrast to wild type CFTR protein localization at the apical membrane of sweat ducts, CFTRΔF508 failed to reach the cell surface and was retained primarily in an intracellular compart-

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ment, most likely the endoplasmic reticulum (17). Since that original article, several other laboratories have corroborated these results in other CF affected tissues, including primary cultures of respiratory epithelial cells (18). Most patients with this mutation, lack sufficient regulated chloride channel function on the cell surface and exhibit severe disease.

The misfolding defect of CFTRΔF508 can be rescued and chloride channel function restored at the cell surface. It is well known that the processing defect of CFTRΔF508 can be partially rescued in cell cultures through various experimental maneuvers directed toward altering association with molecular chaperones involved in its maturation and/or degradation. Alternatively, strategies aimed at modulating CFTRΔF508 folding, *i.e.* by reducing temperature to 26°C from 37°C (19) or by treating the cells with the chemical chaperone; glycerol (20), have also been effective in promoting partial maturation of CFTRΔF508 protein. Importantly, rescue of the processing defect restores correct trafficking of CFTRΔF508 to the cell surface where it exhibits either near normal or partial function as a cAMP activated chloride channel (20, 21). These studies have provided the rationale to search for appropriate strategies with which to promote proper CFTRΔF508 folding and trafficking to the cell surface. Promising compounds such as phenylbutyrate are currently in Phase II clinical trials.

Disease severity is variable in patients homozygous for CFTRΔF508. Recent studies have shown that in some patients, a fraction of the total cellular CFTRΔF508 protein may bypass the cellular quality control machinery to reach the apical membrane of respiratory epithelia. These novel findings are dependent on refinements in *ex-vivo* and *in-vivo* assays of chloride channel expression and function. For example, nasal potential difference (NPD) measurements allow the assessment of sodium and chloride ion transport properties of the nasal epithelium *in-situ* (22). Specifically, it is possible using this method to assess cAMP activated chloride electrodiffusion through CFTR as an isoproterenol evoked change in transepithelial potential difference. It was shown recently using this method that a mild clinical phenotype in certain patients harboring CFTRΔF508 on both alleles correlated with improved isoproterenol-evoked PD measurements (23). These findings were interpreted to suggest that disease amelioration in these patients may reflect limited but biologically effective surface expression of the CFTRΔF508 protein. Furthermore, in contrast to the previously cited immunohistochemical studies in sweat ducts, Kalin *et al*. (24) have shown apical expression of CFTRΔF508 protein by immunofluorescence in biopsies of airway submucosal glands obtained from certain patients homozygous for this mutation. These authors suggest that there is likely to be both tissue and patient specific expression of this mutant protein. Therefore, there is a strong rationale to address the hypothesis that variation in CFTRΔF508 expression on the surface epithelium accounts for some of the variability in disease variability in the CF patient population (25).

In this issue of *Pediatric Research*, Sermet-Gaudelus *et al.* (26) investigate whether clinical heterogeneity in ΔF508 patients is linked to heterogeneity in functional cAMP-regulated Cl^- channel expression. In the study they use the Cl^- sensitive fluorescent dye SPQ (6-methoxy-N-3'-sulfopropylquinolinium) to measure cAMP activated Cl^- fluxes in freshly isolated nasal epithelial cells from ΔF508 patients with mild and severe pulmonary disease. The data demonstrate a positive correlation between enhanced cAMP-activated Cl^- flux and a milder pulmonary phenotype as determined by FEV1 and the Schwachman score. This study is the first to use SPQ to measure Cl^- fluxes and correlate them with pulmonary disease phenotype in ΔF508 patients. Previous studies using the more accepted measurement of Cl^- channel function in CF patients, nasal potential difference (NPD) have also documented a positive correlation between a milder pulmonary phenotype in ΔF508 patients and increased cAMP-regulated Cl^- flux (23, 27, 28).

Both the NPD and SPQ measurements have advantages and disadvantages associated with their use in measuring functional Cl^- channels in cystic fibrosis patients. As yet only a small number of papers have been published on using SPQ for measuring cAMP-activated Cl^- currents in nasal cells from CF patients (26, 29, 30). Therefore further studies comparing this method with more standard electrophysiological measurements such as NPDs and sweat tests will be required to determine whether it might be a useful diagnostic tool. There are also a few technical difficulties associated with the procedure itself. The loading of dye into cells can be highly variable also leakage of the dye from cells during the course of the experiment can cause changes in fluorescence that may interfere with the interpretation of the data. However, one advantage of SPQ measurements in cells isolated from patients is that the procedure is less time consuming for the subject than the measurement of nasal potential differences. Unlike SPQ fluorescence measurements, NPDs have been used quite extensively as an aid in diagnosis (31) and for measuring the successful correction of the CF defect in gene therapy trials for CF (30). Despite this there are problems with variability in NPD measurements between institutions (32).

In conclusion, the study by Sermet-Gaudelus *et al.* is the first to use SPQ to demonstrate a link between cAMP-activated Cl^- flux in nasal epithelial cells and a milder pulmonary phenotype in homozygous ΔF508 individuals. Similar results have been obtained in other studies using different methods (23, 27, 28). Together, these findings suggest that Cl^- channel function in nasal epithelia may be a useful predictor of the severity of pulmonary disease in ΔF508 patients. Further studies comparing SPQ measurements in isolated nasal epithelial cells with other more standard assays, such as nasal potential difference measurements, will be required to determine whether the method described by Sermet-Gaudelus *et al.* will prove useful in more extensive studies of genotype-phenotype relationships in CF or to monitor the efficacy of therapeutic maneuvers. Interestingly, this study also shows that there is no correlation between cAMP regulated chloride conductance and the transcription of the CFTR gene. Similar results were obtained in a study by Beck *et al*. (33). This suggests that the increase in functional CFTR in ΔF508 patients with a milder pulmonary phenotype is due to the modification of some posttranslational step. These modifications may include: enhanced trafficking of CFTR protein to the plasma membrane, increased stability of the CFTR protein in the plasma membrane, or an up-regulation

in the activities of other Cl^- channels (14). Hence, there are likely to be multiple genes that influence the severity of disease. Currently, a comprehensive study of CF-affected twins and siblings is underway to create an extensive clinical database and DNA collection that will enable assessment of genes that may modify the course of CF disease.

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