EPITHELIAL CELL SPECIFIC PROPERTIES AND GENETIC COMPLEMENTATION IN A ΔF508 CYSTIC FIBROSIS NASAL POLYP CELL LINE


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(Received 24 January 1995; accepted 22 March 1995)

SUMMARY

Analysis of vectorial ion transport and protein trafficking in transformed cystic fibrosis (CF) epithelial cells has been limited because the cells tend to lose their tight junctions with multiple subcultures. To elucidate ion transport and protein trafficking in CF epithelial cells, a polar cell line with apical and basolateral compartments will facilitate analysis of the efficacy of different gene therapy strategies in a "tight epithelium" in vitro. This study investigates the genotypic and phenotypic properties of a CF nasal polyp epithelial, AF508 homozygote, cell line that has tight junctions pre-crisis. The cells (ECFNPE14o−) were transformed with an origin-of-replication defective SV40 plasmid. They develop transepithelial resistance in Ussing chambers and are defective in cAMP-dependent Cl− transport as measured by efflux of radioactive Cl−, short circuit current (Isc), or whole-cell patch clamp. Stimulation of the cells by bradykinin, histamine, or ATP seems to activate both K+− and Ca2+-dependent Cl− transport. Measurement of ΔCl− efflux following stimulation with A23187 and ionomycin indicate a Ca2+-dependent Cl− transport. Volume regulatory capacity of the cells is indicated by cell swelling conductance. Expression of the CF transmembrane conductance regulator mRNA was indicated by RT-PCR amplification. When cells are grown at 26°C for 48 h there is no indication of cAMP-dependent Cl− as has been previously indicated in heterologous expression systems. Antibodies specific for secretory cell antigens indicate the presence of antigens found in goblet, serous, and mucous cells; in goblet and serous cells; or in goblet and mucous cells; but not antigens found exclusively in mucous or serous cells. Gene complementation studies with an episomal vector containing wild-type CF transmembrane conductance regulator cDNA showed correction of the cAMP-dependent Cl− transport defect. This cell line contributes unique phenotypic features to the store of transformed CF epithelial cells already available.

Key words: CF airway epithelial cells; ΔF508 CFTR; secretory cell; transformation; ion transport; gene therapy.

INTRODUCTION

Cystic fibrosis (CF) is the most common lethal genetic disease in the Caucasian population affecting about 1 in 2500 individuals in the population (1,27). This disease impairs the electrolyte transport properties of epithelial cells in the airways, sweat glands, pancreas, and other organs (1). Isolation of the gene responsible for CF, the cystic fibrosis transmembrane conductance regulator (CFTR), was an important step in defining the underlying mechanisms controlling the relationship between CFTR function and genotype. The most common mutation associated with CF, a three base pair deletion that results in a phenylalanine deletion at codon 508 (ΔF508), is found in ~70% of all CF chromosomes (19,20). Due to its prevalence, the ΔF508 CFTR protein does not reach the plasma membrane (3,25,30), but that this transport defect can be overcome to some extent by lowering the cellular temperature (7,25). Because these studies were carried out in heterologous cell systems or in non-airway epithelial cells, it is important that these findings be investigated in human airway epithelial cells. Through the development of transformed CF airway epithelial cell lines it has been possible to begin to address some of these questions in a systematic fashion.

Numerous human airway epithelial cell lines (both non-CF and CF) have now been developed in our laboratory (4−6,13,14,22) and those of others (2,17,18,31,33). However, very few of these cell lines maintain tight junctions and cell polarity post-crisis or over multiple generations. At present, there are two normal cell lines transformed in vitro that maintain their ability to form tight monolayers and maintain vectorial ion transport over greater than 30 subcultures (5,6,16). Unfortunately, there are no CF airway epithelial cell lines that retain these properties. Because cell polarity and vectorial ion transport may be an important element in expanding our understanding of the effects of genotype on CFTR function as well as the pathology of CF, we have utilized a pre-crisis transformed CF cell line with tight junctions to investigate certain phenotypic properties that might be associated with cell polarity and a more differentiated epithelium.

An immortalized cell line (ECFNPE14o−) was established from a primary culture of CF nasal polyp epithelial cells by transfection...
with an origin-of-replication defective simian virus 40 (SV40) plasmid (4-6,12-14). Analysis indicates that the cells are homozygous for the ΔF508 mutation. This cell line is defective in cAMP-dependent Cl⁻ transport, but has intact Ca²⁺-dependent Cl⁻ transport as measured by efflux of ³⁶Cl⁻. Furthermore, agents that function through cell surface receptors that activate this Ca²⁺-dependent Cl⁻ transport pathway are effective in simultaneously stimulating K⁺ transport as has been indicated in previous studies.(21). They also develop transepithelial resistance in Ussing chambers. Characterization of the cells with antibodies specific for secretory cell antigens indicates that cells express antigens suggestive of goblet cell origin. Gene therapy studies using an episomal vector containing wild-type (wt) CFTR cDNA showed correction of the cAMP-dependent Cl⁻ transport defect.

MATERIALS AND METHODS

Cells and cells transformation. Nasal polyp epithelial cells from a patient with CF were isolated and grown in modified serum-free LHC-9 medium (MLHC-8e) on tissue culture plastic that had been precoated with fibronectin/vitrogen/bovine serum albumin (FN/V/BSA). Cells were transformed as described previously with the linearized pSVori-plasmid containing a replication-deficient SV40 genome (4-6,12-14,22). Transfected cultures were grown in MLHC8e medium at 37° C under 5% CO₂ in air until cells with altered growth characteristics appeared. Transformants were isolated by trypsinization and expanded for further study. All colonies on the petri dishes were pooled and designated ΣCFNPE14o-. After six subcultures the cells were transferred to Eagle's minimal essential medium (MEM) supplemented with 10% fetal bovine serum (FBS) and antibiotics.

Immunocytochemical staining. Cells were grown to confluence on coated well slides. After washing, fixing, and drying the slides were rehydrated and stained for immunofluorescence. Primary antibodies used were against the SV40 large tumor antigen (Oncogene Science, Uniondale, NY), the AE1/AE3 anticytokeratin antibody (Boehringer Mannheim, Indianapolis, IN), the E9 monoclonal antibody against the junctional complex adhesion protein cellCAM120/80 (29).

For light microscopic immunocytochemistry, slides were fixed with 4% paraformaldehyde. Staining was by a modification of a biotin/avidin procedure. Primary antibodies used in these studies included monoclonal antibodies that recognize antigens expressed by goblet, serous, and mucous cells (A1D3), goblet and mucous cells (A1E11, B6G6, B6E8), or goblet and serous cells (B8C3) and serous cells alone (A2E7, A3B7) or mucous cells alone (A1FB, A8E4) (10).

ΔF508 mutation analysis. Genomic DNA was prepared from ΣCFNPE14o- cells at different passages and PCR amplified with the GeneAmp kit. Oligonucleotide primers CF8C (5'-ATAGGAAACACCAATGATAT-3') or CF7C (5'-ATAGGAAACACCAAAGATGA-3') (antisense) (bp 1649-1668 of wtCFTR cDNA) and CF1B (5'-CCTTCTCTGTGACCTCTATCA-3') (sense) were used for allele-specific amplification of ΔF508 or wild type CFTR DNA, respectively. The AAA in primer CF7C

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