Cystic Fibrosis Theratyping: Matching Mutations to Medications

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Introduction

Cystic Fibrosis (CF) is an autosomal recessive disease caused by a mutation in the gene for the Cystic Fibrosis Transmembrane conductance Regulator protein (CFTR)[1]. CFTR is an anion channel that mediates Cl^- and HCO_3^- transport in epithelia.

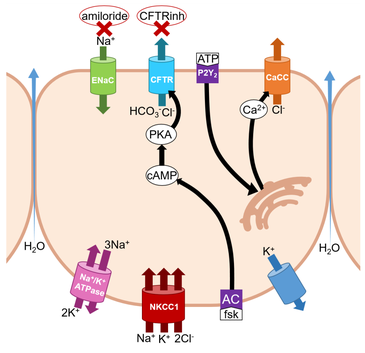

Fig. 1. A model of chloride secretion in an airway epithelial cell. On the basolateral cell membrane, the NaKCC cotransporter moves Na^+, K^+, and 2 Cl^- ions into the cell, while Na^+/K^+ ATPase transports 2 K^+ into the cell and 3 Na^+ out of the cell. The basolateral K^+ channel conducts K^+ out of the cell along its concentration gradient. Forskolin stimulates adenyl cyclase to increase the concentration of cAMP, which activates protein kinase A that phosphorylates CFTR, transporting Cl^- out of the cell through the apical membrane.

CFTR activity can be blocked with CFTR inhibitors. The epithelial sodium channel (ENaC) transports Na^+ into the cell through the apical membrane and can be selectively blocked with amiloride. The stimulation of the $P2Y_2$ receptor by ATP causes a release of Ca^{2+} from the endoplasmic reticulum, which activates calcium activated chloride transport (CaCC).

In 2012 the FDA approved the first CFTR modulator for the treatment of the underlying chloride channel defect in Cystic Fibrosis. Theratyping, the process of matching mutations to medications, is a new approach for personalized medicine in Cystic Fibrosis[2]. As of today, there are more than 2000 known CFTR mutations; among those about 350 are known as disease-causing mutations, and many occur in only a few cases[3]. Instead of simply evaluating an individual's genotype, theratyping allows for categorizing mutations as responders or nonresponders to specific CFTR correctors and potentiators.

Conditional reprogramming technology (CR) is a method for generating 2D planar airway cultures from CF patients. CR involves plating cells on irradiated fibroblast feeder cells in the presence of a Rho kinase (ROCK) inhibitor, which can expand cells 10,000 fold[4]. In contrast, EpiX™ is a feeder-free technology that has the potential to expand airway epithelial cells more than 10^{12} fold[5]. We see a potential in EpiX™ technology for supporting CF theratyping and advancing precision medicine for cystic fibrosis.

Objective

To develop planar cultures of nasal and bronchial epithelial cells to study normal and mutant CFTR function measurements in individuals.

Methods

Conditional reprogramming of nasal cells

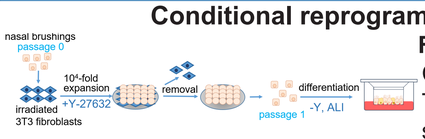

Fig. 2. Cells were obtained from 14 CF patients using nasal brushings. The cells were expanded in serum-supplemented F medium (Ham's F12:DMEM 3:1) using conditional reprogramming technology on irradiated 3T3 fibroblast feeder cells in the presence of a ROCK inhibitor (10 μM Y-27632). The nasal epithelial cells were harvested by differential trypsinization (passage 1). To allow for differentiation, nasal cells were grown on human placental collagen coated Snapwell inserts for 21-28 days in serum-free ALI medium (supplemented DMEM:LHC Basal Medium 1:1) at an air liquid interface. These cells were then used in Ussing assays.

Expansion of nasal cells using EpiX™ technology

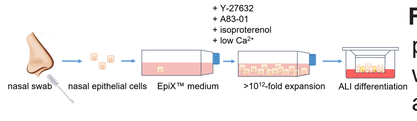

Fig. 3. Cells were obtained from 5 CF patients using nasal swabs. The cells were expanded for 3-4 passages over a period of 28 days in collagen 1 coated cell culture flasks suspended in EpiX™ medium, supplemented with ROCK inhibitor (5μM Y-27632), TGF-β inhibitor (1μM A83-01), and isoproterenol (3μM) under low Ca^{2+} conditions. Cells were then plated on uncoated Snapwell inserts and differentiated in V-ALI medium for 21-28 days at an air-liquid interface to be used in Ussing assays.

Ussing assays

Cells plated in Snapwell inserts are mounted into Easy Mount Ussing chambers (Physiologic Instruments) filled with bicarbonate buffered Krebs Henseleit solution, separating the chamber into two halves to measure Cl^- transport by the CFTR channel across the apical membrane. There are four electrodes, two voltage measuring and two current passing. The transepithelial voltage is clamped to zero mV, and the resulting short-circuit current generated by the transepithelial ion transport is measured. Data is collected using the USB DataQ710 acquisition system in combination with the WinDaq recording and playback software.

Amiloride is added to block Na^+ absorption by ENaC, the epithelial sodium channel. Administration of forskolin, a cAMP agonist, is followed by mucosal addition of the CFTR modulator VX-770 to stimulate CFTR Cl^- transport. CFTR inhibitors (CFTR inhibitor 172, PPQ-102, GlyH101) are applied to fully block and quantify CFTR-mediated Cl^- currents. At the end of the experiments, ATP is added to measure the magnitude of the calcium-activated Cl^- conductance, CaCC.

CFTR function data as measured by the activation and subsequent inhibition of the CFTR currents is then analyzed and compared between groups using t-tests and ANOVA. Graphs are illustrated using the software SigmaPlot.

Results

Range of normal CFTR currents in cells from non-CF individuals

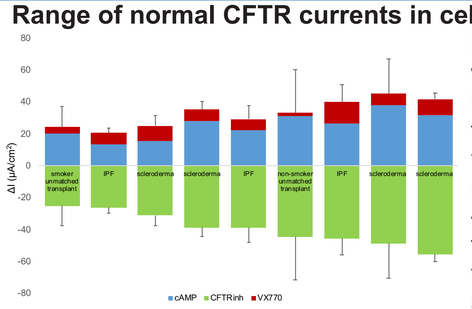

Fig. 4. CFTR chloride transport was measured in P0 human bronchial epithelial cells (HBEs) from 9 non-CF individuals with various conditions. Currents ranged from -25.0±5.2 (n=6) to -48.7±8.9 (n=6), which is a 1.9 fold variation. The smoker was on the low end and the scleroderma subject was on the high end.

Histological comparison of HBEs from non-CF individuals

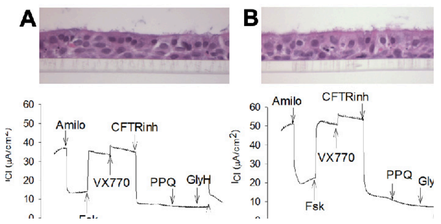

Fig. 5. Cross sections of bronchial epithelial P0 cultures at day 22 stained with hematoxylin and eosin. Cells are from subjects with (**A**) IPF and (**B**) scleroderma. Corresponding representative current traces are below.

CFTR function in CR nasal cells from 14 CF patients

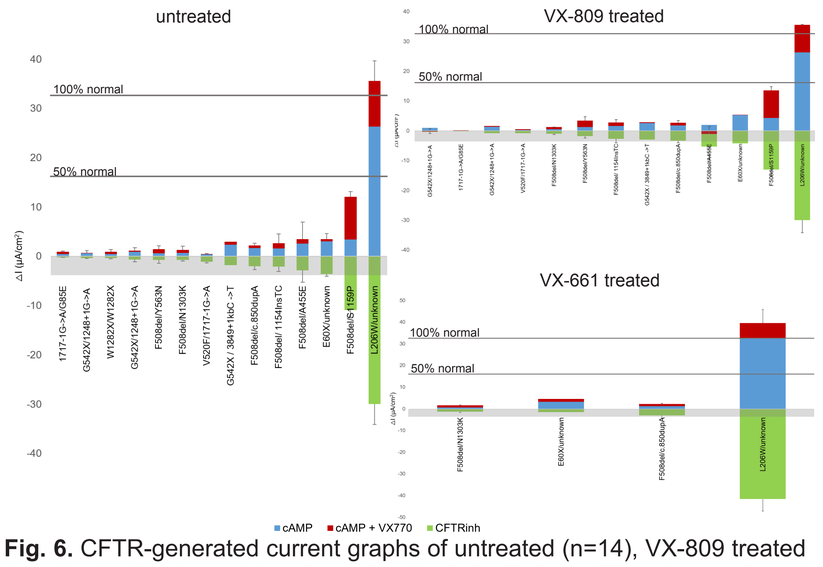

Fig. 6. CFTR-generated current graphs of untreated (n=14), VX-809 treated (n=13), VX-661 treated (n=4) nasal epithelial cells from CF patients cultured with the CR method at P1. 10% normal CFTR function is defined as 3.3 μA/cm² and surpassing this categorizes the genotype as a responder. The shaded area is less than 10% normal CFTR function. Of the 6 F508del heterozygotes, only F508del/S1159P responded to VX-770 and VX-809 treatment, and F508del/A455E responded to VX-809. Additionally, the L206W/unknown genotype responded to all three treatments. E60X/unknown responded to VX-809.

CFTR function in EpiX™ nasal cells from 5 CF patients

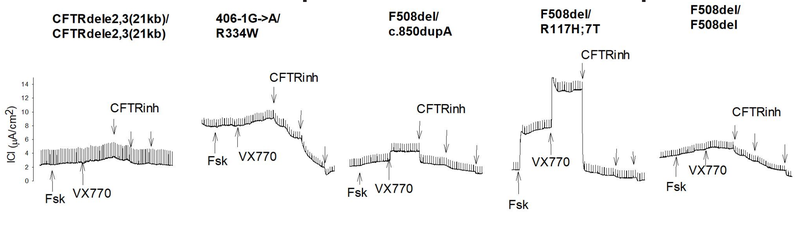

Fig. 7. Representative current traces of CFTR Cl^- currents stimulated by forskolin and VX-770 of VX-809 treated cells of 5 different CF genotypes expanded using EpiX™ technology. Each genotype displays a unique current pattern. CFTRdele2,3(21kb)/CFTRdele2,3(21kb) has a stop mutation, 406-1G->A/R334W has one splice and one substitution mutation, F508del/c.850dupA has one stop and one deletion mutation, F508del/R117H;7T has a deletion and a substitution and poly T mutation, and F508del/F508del has two deletion mutations.

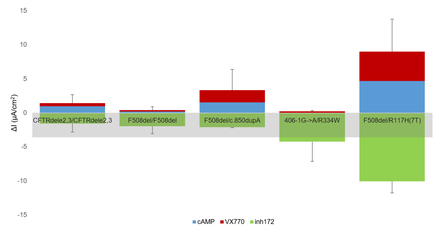

Fig. 8. CFTR activity of untreated nasal epithelial cells from 5 CF patients expanded with EpiX™ technology. The shaded area represents less than 10% normal CFTR function (3.3 μA/cm²).

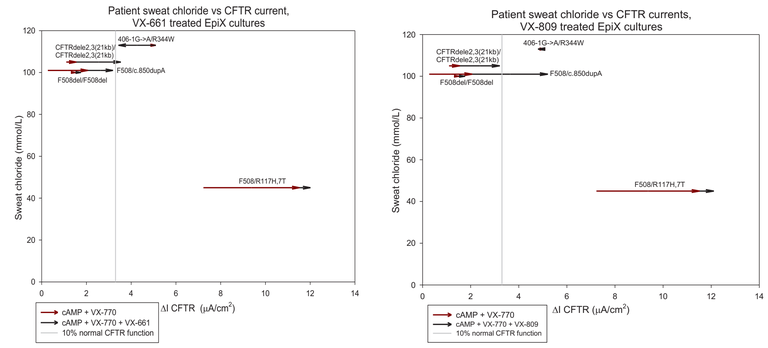

Fig. 9. Vector plots of sweat chloride vs change in CFTR current in VX-661 and VX-809 treated EpiX™ expanded cells from 5 CF patients. Responders are genotypes that surpass 10% normal CFTR function (3.3 μA/cm²). F508del/R117H,7T and 406-1G->A/R334W had greater than 10% normal CFTR function both before and after treatment. F508del/c.850dupA responded to VX-809 treatment and CFTRdele2,3/CFTRdele2,3 responded to VX-661 treatment.

Conclusion

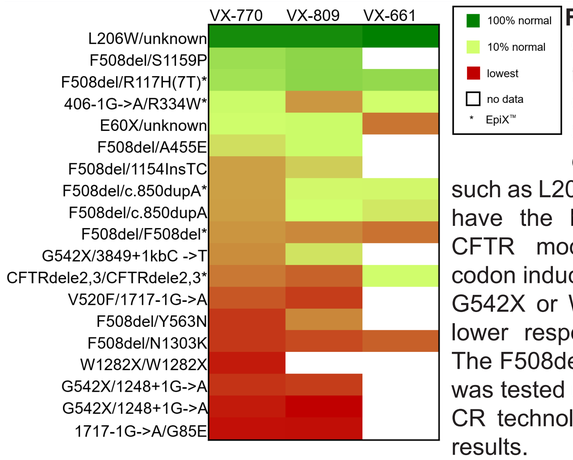

Fig. 10. Heat map of CFTR genotypes and their responses to CFTR modulators. Substitution and deletion mutations, such as L206W or F508del tend to have the highest responses to CFTR modulators, while stop-codon inducing mutations such as G542X or W1282X tend to have lower responses to modulators. The F508del/c.850dupA genotype was tested using both EpiX™ and CR technologies, yielding similar results.

- There is significant variation (1.9 fold) in CFTR function among non CF individuals
- Both CR and EpiX™ technologies are effective in creating 2D planar cultures of airway epithelial cells for ex vivo CFTR theratyping
- EpiX™ technology can generate planar airway cultures with cells obtained by a simple nasal swab
- 5 out of 19 CF CR cultures are identified as responders (larger than 10% normal CFTR activity, 100% = 33μA/cm²): F508del/S1159P, F508del/A455E, L206W/unknown, E60X/unknown, F508del/c.850dupA
- 4 out of 5 CF EpiX™ cultures are identified as responders (larger than 10% normal CFTR activity, 100% = 33μA/cm²): F508del/R117H,7T, 406-1G->A/R334W, F508del/c.850dupA, CFTRdele2,3/CFTRdele2,3

References

1. Rommens JM Iannuzzi MC, Kerem B, Drumm ML, Melmer G, Dean M, Rozmahel R, Cole JL, Kennedy D, Hidaka N. Identification of the cystic fibrosis gene: chromosome walking and jumping. Science. 1989;245(4922):1059-65. doi:10.1126/science.2772657. PubMed PMID: 2772657
2. Clancy JP, et al, CFTR modulator theratyping: Current status, gaps and future directions. J Cyst Fibros. 2018. doi:10.1016/j.jcf.2018.05.004.
3. Cystic Fibrosis Mutation Database – CFMDB. http://www.genet.sickkids.on.ca/cftr/app. Updated April 25, 2011. Accessed July 24, 2019.
4. Liu X, Ory V, Chapman S, Yuan H, Albanese C, Kallakury B, Timofeeva OA, Nealon C, Dakic A, Simic V, Haddad BR, Rhim JS, Dritschilo A, Riegel A, McBride A, Schlegel R. ROCK inhibitor and feeder cells induce the conditional reprogramming of epithelial cells. Am J Pathol. 2012 Feb;180(2):599-607. doi: 10.1016/j.ajpath.2011.10.036. Epub 2011 Dec 18. PubMed PMID: 22189618
5.Zhang C., Lee HJ, Shrivastava A, Wang R, McQuiston TJ, Challberg SS, Pollok BA, and Wang T (2018) Cell Reports 25, 598-610.e5. DOI: 10.1016/j.celrep.2018.09.072