ELSEVIER
Journal of Cystic Fibrosis xx (2017) xxx – xxx
Journal of Cystic Fibrosis
www.elsevier.com/locate/jcf

Original Article

Detection of CFTR function and modulation in primary human nasal cell spheroids

John J. Brewington [a,*], Erin T. Filbrandt [a], F.J. LaRosa III [a], Alicia J. Ostmann [a], Lauren M. Strecker [a], Rhonda D. Szczesniak [b], John P. Clancy [a]

[a] Division of Pulmonary Medicine, Department of Pediatrics, Cincinnati Children's Hospital Medical Center, 3333 Burnet Avenue/MLC 2021, Cincinnati, OH, USA
[b] Division of Biostatistics and Epidemiology, Cincinnati Children's Hospital Medical Center, 3333 Burnet Avenue/MLC 2021, Cincinnati, OH, USA

Received 23 February 2017; revised 27 June 2017; accepted 28 June 2017

Abstract

Background: Expansion of CFTR modulators to patients with rare/undescribed mutations will be facilitated by patient-derived models quantifying CFTR function and restoration. We aimed to generate a personalized model system of CFTR function and modulation using non-surgically obtained nasal epithelial cells (NECs).
Methods: NECs obtained by curettage from healthy volunteers and CF patients were expanded and grown in 3-dimensional culture as spheroids, characterized, and stimulated with cAMP-inducing agents to activate CFTR. Spheroid swelling was quantified as a proxy for CFTR function.
Results: NEC spheroids recapitulated characteristics of pseudostratified respiratory epithelia. When stimulated with forskolin/IBMX, spheroids swelled in the presence of functional CFTR, and shrank in its absence. Spheroid swelling quantified mutant CFTR restoration in F508del homozygous cells using clinically available CFTR modulators.
Conclusions: NEC spheroids hold promise for understanding rare CFTR mutations and personalized modulator testing to drive evaluation for CF patients with common, rare or undescribed mutations.

Portions of this data have previously been presented in abstract form at the 2016 meetings of the American Thoracic Society and the 2016 North American Cystic Fibrosis Conference.

Keywords: CFTR; Organoid; Modulator; Personalized model system

1. Introduction

Cystic Fibrosis (CF) is an autosomal recessive disorder affecting >70,000 people worldwide [3,4]. CF is caused by mutations in the gene encoding the Cystic Fibrosis Transmembrane Conductance Regulator protein (CFTR), a traffic ATPase that functions as a chloride and bicarbonate channel [3,5]. CFTR also regulates several epithelial ion transporters, including other chloride channels and the epithelial sodium channel (ENaC) [6]. Over 2000 variants in the CFTR gene have been identified, with multiple characterized defects [7]. CFTR dysfunction results in multisystem disease, with most morbidity and mortality stemming from pulmonary disease due to thick airway mucus, airway obstruction, chronic infection, and inflammation. This process is caused by dysregulation of airway surface liquid (ASL) homeostasis and defective mucus production governed by CFTR [8].

Abbreviations: ALI, air-liquid interface; ASL, airway surface liquid; BMI, body mass index; cAMP, 3′,5′-cyclic adenosine monophosphate; CF, Cystic Fibrosis; CFTR, Cystic Fibrosis Transmembrane Conductance Regulator; CRC, conditional reprogramming culture; ENaC, epithelial sodium channel; IBMX, 3-isobutyl-1-methylxanthine; LAEC, lower airway epithelial cell; NEC, nasal epithelial cell; PBS, phosphate-buffered saline; $ppFEV_1$, percent predicted forced expiratory volume in 1 s

* Corresponding author.
E-mail address: john.brewington@cchmc.org (J.J. Brewington).

http://dx.doi.org/10.1016/j.jcf.2017.06.010
1569-1993/

Novel, small-molecules termed modulators have recently been developed that directly improve CFTR function for limited mutations. Ivacaftor (VX770) is FDA-approved for patients with mutations causing defective CFTR gating or conductance (e.g.: G551D or R117H). Lumacaftor (VX809) is FDA-approved for use in combination with ivacaftor for patients with two copies of the most common CFTR mutation, F508del [9–12]. Additional modulators are in development, potentially expanding both the qualifying patient population and the treatment options for individual patients [13–15].

Current modulators were developed using human lower airway epithelial cells (LAEC) [16]. LAECs from explanted lung tissue are grown at air-liquid interface (ALI) to mature monolayers, allowing examination of ion transport, ASL homeostasis, and mucociliary clearance as proxies of CFTR function [17,18]. This model has driven development of CFTR modulators for common CFTR mutations, but has limitations in patients with rare CFTR mutations. Patient-derived models would allow improved mutation-to-disease correlation and individualized modulator testing [19]. This could be particularly useful for patients with rare or poorly characterized mutations, for whom clinical trials are impractical. Moreover, as novel modulators emerge, such models may help determine the appropriate combination of drugs to optimize clinical outcomes.

Intestinal tissue has been grown as organoids to model CFTR function [20,21]. Acquisition of GI tissue remains invasive, requiring endoscopy or suction biopsy for rectal specimens, with minor safety risks [10,22]. Limited existing work in 3-dimensional culture of nasal tissue is not clearly adaptable to monitoring CFTR function [23–25]. We hypothesized that primary human nasal epithelial cells (NECs) obtained non-surgically could be grown in 3-dimensional culture to yield a patient-derived, swelling-based model of CFTR function. Such a model could provide powerful individualized data from an easily obtained respiratory sample.

2. Methods

This protocol was approved by the Cincinnati Children's Hospital Medical Center Institutional Review Board. All subjects/families provided written assent/consent. Cell cultures from healthy volunteers and patients with a variety of CFTR mutations were studied.

Additional detail regarding ALI culture, electrophysiology studies, immunofluorescence, and protein isolation/detection is provided in the supplemental materials.

2.1. NEC procurement, processing, and expansion

NECs were procured by curettage of the inferior turbinate using a rhinoprobe (Arlington Scientific, Inc.), pooling cells from both nostrils into a 15 mL conical filled with Media A (Table E1) and storing on ice for <24 h. Curettage was chosen based on site expertise.

Curettes were rinsed with media and the collected cells were centrifuged at 360 ×*g* for 5 min. Cell pellets were re-suspended in Accutase (Innovative Cell Technologies, Inc.) and centrifuged at 360 ×*g* for 5 min. The resulting pellet was re-suspended in Media A and placed into VitroCol (Advanced BioMatrix) coated petri dishes pre-seeded with irradiated mouse embryonic fibroblast feeder cells (Globalstem). Cells were maintained in Media A for five days, then Media B until confluent, changing media daily. Once confluent, media was removed and cells were exposed to 0.1% trypsin (Sigma-Aldrich) for 5 min to remove them from the dish. This mixture was centrifuged at 360 ×*g* for 5 min and the supernatant was removed. Cells were then passaged to a new dish using the protocol above or to spheroid or monolayer culture. All experiments utilized cells of passage 1 or 2.

2.2. Spheroid culture

Growth factor-reduced matrigel (Corning, Inc.) was thawed on ice. Cells were suspended at 500,000 cells/mL in 100% matrigel, vigorously but carefully pipetting to generate a single-cell suspension while avoiding the introduction of air bubbles. This mixture was seeded in 100 μL aliquots into 16 mm four-well plates (NUNC) or 35 mm glass-bottom dishes (MatTek Corporation), creating a spherical "drop" of matrigel using a 200 μL pipette tip with the distal 3–4 mm trimmed at an angle. The plates were incubated at 37 °C and 5% CO_2 for 30 min, until the matrigel set. Media C was then added to the well to cover the matrigel drop. Cells were maintained in Media C until mature (presence of a lumen and a slightly thickened spheroid wall, suggesting a pseudostratified epithelium; typically 7–10 days), changing media daily.

2.3. Spheroid stimulation and measurement

Spheroid plates/dishes were placed in an incubated chamber (37 °C, 5% CO_2) on an Olympus IX51 inverted microscope. Spheroid images at time 0 (n = 10–20 spheroids per condition for all experiments described) were taken at 20× magnification using Slidebook 5.5 (3i, Intelligent Imaging Innovations) software. Spheroids were stimulated by adding forskolin/IBMX diluted in PBS directly to the media (final concentration of 10 μM/100 μM), and spheroid swelling was monitored with time-lapse imaging for 1 h. At completion, post-stimulation images of all spheroids were captured at time 60 min.

Images were analyzed by manually delineating the luminal area of each spheroid using MetaMorph 7.7 software; examples are provided in Fig. E1. Staff performing the analysis were blinded to mutation, condition, and pre- or post-stimulation timing of each image; total analysis time was approximately 20 min per experimental condition. Spheroid area data was imported into Microsoft Excel 2010 and percent change from time 0 to 60 min was calculated for each individual spheroid.

2.4. Statistical analyses

All measured spheroids were included in the data analysis. Paired *t*-tests or ANOVA were used to compare continuous data, including change in spheroid area, I_{sc}, and densitometry using Microsoft Excel 2010 software. An alpha (p) value < 0.05 was used to determine statistical significance. For repeated-measures analysis, variance components were estimated by restricted

Please cite this article as: Brewington JJ, et al, Detection of CFTR function and modulation in primary human nasal cell spheroids, J Cyst Fibros (2017), http://dx.doi.org/10.1016/j.jcf.2017.06.010