ELSEVIER
Journal of Cystic Fibrosis xx (2018) xxx – xxx
Journal of Cystic Fibrosis
www.elsevier.com/locate/jcf

Original Article

Comparison of *ex vivo* and *in vitro* intestinal cystic fibrosis models to measure CFTR-dependent ion channel activity☆

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Received 8 September 2017; revised 15 January 2018; accepted 6 February 2018
Available online xxxx

Abstract

Background: New functional assays using primary human intestinal adult stem cell cultures can be valuable tools to study epithelial defects in human diseases such as cystic fibrosis.
Methods: CFTR-mediated ion transport was measured in rectal organoid-derived monolayers grown from subjects with various *CFTR* mutations and compared to donor-matched intestinal current measurements (ICM) in rectal biopsies and forskolin-induced swelling of rectal organoids.
Results: Rectal organoid-derived monolayers were generated within four days. Ion transport measurements of CFTR function using these monolayers correlated with ICM and organoid swelling (r = 0.73 and 0.79 respectively). Culturing the monolayers under differentiation conditions enhanced the detection of mucus-secreting cells and was accompanied by reduced CFTR function.
Conclusions: CFTR-dependent intestinal epithelial ion transport properties can be measured in rectal organoid-derived monolayers of subjects and correlate with donor-matched ICM and rectal organoid swelling.

Keywords: Electrophysiology; CFTR; Differentiation; Donor-matched; Organoids; Monolayers; Biopsies

1. Introduction

Human *ex vivo* tissues and *in vitro* cell culture models play a central role in drug development, and have received increasing attention lately as patient stratification and personalized medicine applications [1]. Recent adult stem cell culture technologies have enabled an unprecedented ability to expand primary human tissues *in vitro* [1]. These culture technologies allow for the quick establishment of clinically-annotated disease-specific biobanks which offer new opportunities for disease modeling [2].

One of these exciting opportunities is the 3D culturing of intestinal adult stem cells into organoids [3,4]. We developed a fluid secretion assay by quantitating the swelling of rectal organoids upon incubation with secretagogues [5]. Fluid

Abbreviations: A, amiloride; AUC, area-under-the-curve; cAMP, cyclic AMP; CCH, carbachol; CF, cystic fibrosis; CFTR, cystic fibrosis transmembrane conductance regulator; D, differentiation culture conditions; DMSO, dimethylsulfoxide; ENaC, epithelial sodium channel; FIS, forskolin-induced swelling; Fsk, forskolin; GAPDH, glyceraldehyde 3-phosphate dehydrogenase; HBE, human bronchial epithelial cells; HIS, histamine; IBMX, 3-isobutyl-1-methylxanthine; ICM, intestinal current measurements; Ieq, equivalent short-circuit current; Isc, short-circuit current; LGR5, Leucine-rich repeat-containing G-protein coupled receptor 5; MTECC, Multi Transepithelial Current Clamp; N, non-differentiation culture conditions; NKCC1, Na-K-Cl co-transporter 1; ns, not significant; PAS, periodic acid-Schiff; Rt, transepithelial resistance; RT-q, reverse transcription quantitative; wt, wild type

☆ Part of the data from this study was presented as a poster during the 14th ECFS Basic Science Meeting 29 March–01 April 2017, Algarve, Portugal: P75 Comparison of *ex vivo* and *in vitro* intestinal cystic fibrosis models to measure CFTR-dependent ion channel activity.

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https://doi.org/10.1016/j.jcf.2018.02.007
1569-1993/

secretion is driven by epithelial ion transport, but direct measurement of ion transport in organoids is difficult due to their enclosed luminal compartment. Nonetheless, this organoid swelling assay has been highly instrumental for disease modeling and determining preclinical and individual treatment responses in cystic fibrosis (CF) [6,7] and cholera-induced secretory diarrhea [8].

CF is a rare, genetic disease, caused by mutations in the *CF Transmembrane Conductance Regulator* (*CFTR*) gene that impairs functioning of the cyclic AMP (cAMP)-regulated CFTR ion channel. Reduced CFTR function leads to aberrant transport of anions and fluids across epithelial membranes and results in accumulation of viscous mucus, most notably in the lungs and gastro-intestinal tract, which leads to organ dysfunction [9]. *CFTR* mutations are classified by CFTR expression and function: (I) defective synthesis, (II) reduced trafficking, (III) impaired gating of the channel, (IV) reduced conductance, (V) impaired synthesis and trafficking, and (VI) reduced cell membrane stability [10].

Electrophysiological readouts in Ussing chambers [11] are commonly used to study epithelial ion transport. Intestinal current measurements (ICM) of cAMP-regulated anion secretion can be used to measure residual CFTR function in rectal biopsies [12]. Drug development studies for CF have relied heavily on Ussing chamber studies using differentiated human bronchial epithelial (HBE) cell cultures to measure CFTR activity [13,14]. A major limitation of both approaches is the restricted cell availability due to invasive sampling procedures and limited *in vitro* cell expansion capability [15,16].

The development of assays to study ion transport properties of epithelia, using human stem cells as an unlimited source, are important for studying human diseases and drug development. Recently, intestinal epithelial monolayers from primary mouse and human tissue were successfully grown in Transwell permeable supports [17,18]. Here, our aims were to apply these protocols (i) to generate monolayers derived from rectal organoids with varying CFTR residual function, (ii) to measure CFTR-mediated ion transport in these monolayers with the Multi Transepithelial Current Clamp (MTECC) 24-well system [19,20], (iii) to compare the data with CFTR function measurements in donor-matched rectal biopsies and organoids using the established ICM an organoid swelling assays, respectively [5,12,15,21], and (iv) to modify cell composition and ion channel function by adapting culture conditions.

2. Methods and materials

2.1. Human rectal biopsies and CFTR genotypes

Rectal biopsies were obtained for standard care or participation in a study (approved by the ethics committees of University Medical Center Utrecht and Erasmus Medical Centre Rotterdam). Informed consent to perform CFTR function assays was obtained from all subjects. Twelve subjects were studied in total, including two subjects per mutation class I to IV: class I/class I - E60X/4015delATTT (c.178G > T/c.3883delATTT) and 1811 + 1G > C/1811 + 1G > C (c.1679 + 1G > C/c.1679 + 1G > C), class II - two subjects F508del(p.Phe508del)/F508del, class III - two subjects S1251N (p.Ser1251Asn)/F508del, and class IV - two subjects R117H (p.Arg117His)/F508del. In addition, two carrier subjects (wt/F508del) and two wild type subjects (wt/wt) were studied [22].

2.2. Intestinal current measurements (ICM)

ICM was performed for standard care, diagnostics or clinical studies as described previously [12] and according to Standard Operating Procedure ICM_EU001 (version 2.7, 2011). Short-circuit currents (Isc) were measured in an Ussing chamber system (Physiologic Instruments) using a voltage clamp (DVC-1000, World Precision Instruments). Tracings were recorded with PowerLab (8/30, AD Instruments) and analyzed using LabChart 6 software.

2.3. Rectal organoid culturing

Organoids were generated and cultured as previously described [5,6,21]. Growth medium was refreshed every 2–3 days and organoids were passaged every seven days.

2.4. Organoid swelling assay

The organoid swelling experiments were performed as previously published, with minor adaptations [5,21]. Experiments were performed with undifferentiated organoids or organoids cultured for five days with differentiation medium (organoid growth medium without Wnt3a-conditioned medium, nicotinamide and SB202190) [4]. Organoids were acutely stimulated with forskolin (Fsk, 0.8 and 5 μM), carbachol (CCH, 100 μM) or histamine (HIS, 500 μM).

2.5. Culturing of organoid-derived monolayers

Culturing of monolayers was performed using previously published protocols with slight modifications [18,23]. 24-Well Transwell HTS plates (3378, Corning) were coated with PureCol (Advanced Biomatrix) diluted 1:100 in Dulbecco's Phosphate-Buffered Saline (PBS) supplemented with calcium and magnesium (Gibco) and incubated for 2 h at 37 °C/5% CO_2 after which the PureCol solution was removed. Seven-day old organoid cultures were trypsinized (TrypLE, Thermo Fisher) for 2 × 2 min at 37 °C and mechanically disrupted after each incubation period. 250,000 cells were seeded per insert, with addition of 100 μl and 600 μl organoid growth medium supplemented with Y-27632 (10 μM, Selleck Chemicals), at the apical and basolateral sides respectively. Medium (without Y-27632) was refreshed every 1–2 days. After four days the measured resistance values exceeded 100 $\Omega \cdot cm^2$, indicating confluent monolayers. Electrophysiological measurements were either performed directly or growth medium was replaced with organoid differentiation medium for another five days, until the resistances of the monolayers exceeded 800 $\Omega \cdot cm^2$.

Please cite this article as: Zomer-van Ommen DD, et al, Comparison of *ex vivo* and *in vitro* intestinal cystic fibrosis models to measure CFTR-dependent ion channel activity..., J Cyst Fibros (2018), https://doi.org/10.1016/j.jcf.2018.02.007