



Intestinal organoids to model cystic fibrosis

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CF can be studied using patient-specific intestinal organoids. CFTR protein function in intestinal organoids correlates with clinical disease severity and drug testing in organoids could aid in finding drugs for people with rare CFTR mutations. <https://bit.ly/2GryRl0>

Cite this article as: van Mourik P, Beekman JM, van der Ent CK. Intestinal organoids to model cystic fibrosis. *Eur Respir J* 2019; 54: 1802379 [<https://doi.org/10.1183/13993003.02379-2018>].

Introduction

Recent advances in adult stem cell biology have resulted in the development of organoid culture technologies using a variety of tissue sources such as intestine, lung and kidney [1]. Organoids are three-dimensional, multicellular structures that recapitulate tissue features of the parental organ and are usually grown from donor tissue fragments [1]. As organoids are functional expressions of individual genomes, these cultures are particularly useful to understand how genetic factors contribute to individual disease. As such, they are used to study hereditary diseases such as cystic fibrosis (CF), and more common diseases such as cancer where genetics can influence disease severity and drug efficacy [2, 3].

Intestinal organoids have been on the forefront of these developments, as culture methodology was first developed for this tissue source [4]. For CF, human intestinal organoids can be grown from intestinal crypt fragments that are isolated from rectal biopsies. Taking rectal biopsies is typically an innocuous procedure that can be performed in all age groups (including newborns) without a need for anaesthesia [5, 6]. Intestinal organoids can be generated with high individual success rates, which facilitates access to tissue that can be expanded, stored in liquid nitrogen and can be thawed and continuously cultured for >6 months while preserving individual functional phenotype [7]. While many labs worldwide have adopted organoid culturing [8], few laboratories have expertise in using intestinal organoids to study CF. Here we focus on applications of intestinal organoids for CF and emphasise important future directions that can help to develop effective therapies for all people with CF.

How does it work?

Genetic variability of the cystic fibrosis transmembrane conductance regulator (CFTR) gene can result in misfolding and malfunction of the CFTR protein. This subsequently dysregulates epithelial ion and fluid transport, resulting in CF disease manifestations. CFTR plays a dominant role in rapid fluid secretory responses in the large intestine that are evoked by cyclic (c)AMP inducing stimuli, and its activation can lead to secretory diarrhoea. Chloride is the main driver of this rapid fluid secretory response [9, 10].

CFTR is expressed on the apical membrane that lines the internal lumen of rectal organoids. CFTR activation by cAMP-raising agents such as forskolin leads to chloride transport into the organoid lumen

Received: Dec 15 2018 | Accepted after revision: April 07 2019

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that is accompanied by luminal water secretion through osmosis (figure 1). Two functional assays have been developed to quantify CFTR function in intestinal organoids that rely on the luminal chloride secretion and coupled water transport.

First, incubation of organoids with forskolin leads to rapid luminal fluid secretion through CFTR activation that causes whole organoid swelling within 60 minutes. The forskolin-induced swelling (FIS) phenotype is absent in human and mouse organoids lacking functional CFTR gene products (e.g. two class I mutations or *Cftr* knockout), and is inhibited by chemical CFTR inhibitors, supporting full CFTR-dependency of the FIS readout [10]. The resulting swelling of organoids is quantified using live confocal microscopy after organoid labelling with calcein green. The relative size increase of all organoids in a well is calculated over time using 10-minute intervals. A relative swelling curve is generated from these data and an area under the curve of this relative swelling is calculated to compare conditions (e.g. different drugs, different donors) in a single graph [11].

Second, steady-state differences in luminal phenotype exist between healthy control and CF organoids independent of forskolin. Healthy control rectal organoids have large fluid-filled lumens, suggesting the presence of functional CFTR and physiological cAMP signalling during standard culture conditions leading to luminal salt and fluid transport [12]. CF organoids do not have lumens that are easily recognised upon visual inspection. This phenotypical difference can be quantified before a FIS assay is performed by manually drawing in the lumen of calcein-labelled organoids in the baseline images, and subsequently expressing the lumen area as percentage of total area. Whereas CF organoids have steady-state lumen areas (SLA) between 0% and 10% of total organoid area, healthy control SLA is between 40% and 80%. While SLA is mostly CFTR-driven, some variability in this phenotype exists at the 0–10% range independent of CFTR genotype.

FIS and SLA are complementary assays. FIS has considerable throughput and quantifies CFTR function at levels associated with CF disease and treatment thereof with current CFTR modulators [7, 10]. As FIS

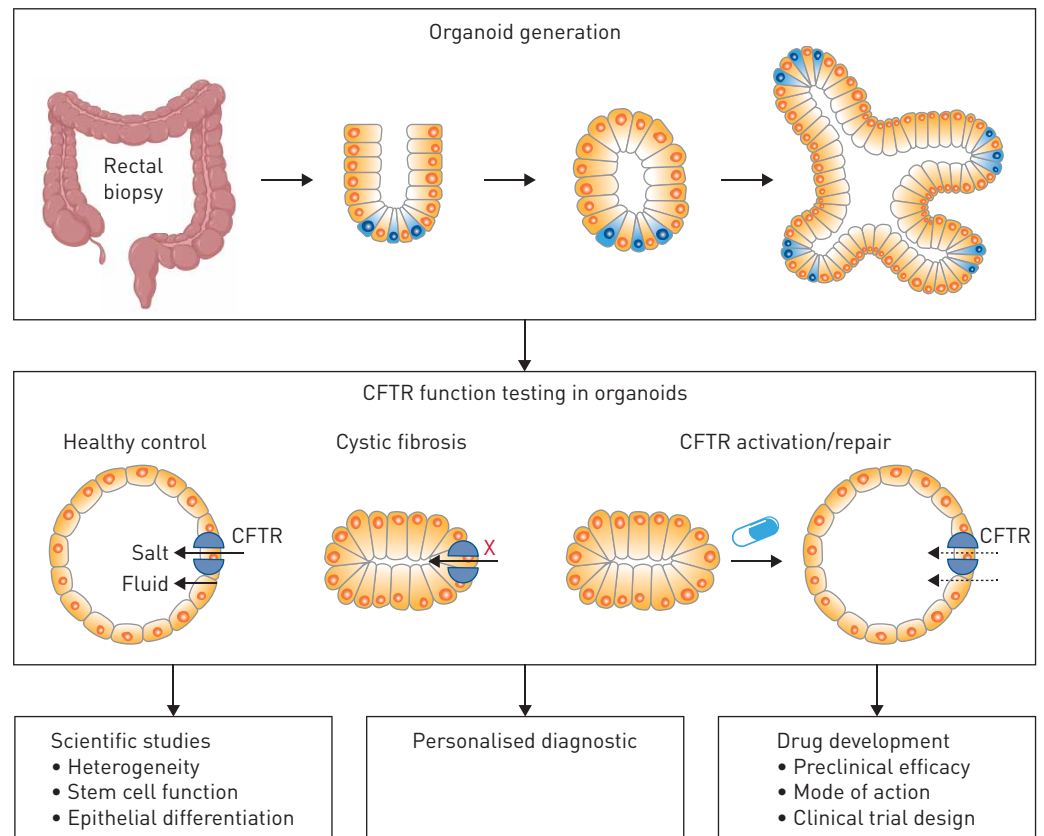


FIGURE 1 Potential applications of intestinal organoids to study cystic fibrosis. Intestinal organoids can be generated from rectal biopsies. In organoids, robust cystic fibrosis transmembrane conductance regulator [CFTR] function measurements can be performed based on phenotypic differences between organoids and the observation that repair and activation of CFTR causes swelling of organoids. This model can be used in preclinical and clinical studies with the potential to obtain patient-specific information on disease severity and CFTR-modulator drug response. Figure partially created using BioRender.

measures relative size increase, it requires that the initial starting sizes are comparable. This is true for CF organoids, but at larger SLA (~>25%), relative size increases are underestimated when two-dimensional area measurements are performed [7]. Therefore, FIS of CF and healthy controls are not directly comparable. SLA facilitates comparison between CF and healthy control organoids, but has limited resolution to discriminate at lower CFTR function levels associated with severe CF disease [7].

Both FIS and SLA have only recently been developed, and technological expertise is currently available in a select number of laboratories. The CFTR dependency of FIS has been confirmed by independent other CF [13, 14] and non-CF laboratories in intestinal [15] and non-intestinal tissues [16]. To further standardise multicentre research, biopsies can also be shipped to a centralised laboratory for processing, which is feasible within 48–72 h after obtaining the biopsy. Growing organoid structures for FIS experiments takes ~4 weeks, and at that point organoids can be stored in liquid nitrogen for future use. When in culture, FIS and SLA assays can be performed weekly, and repeated measurements over several weeks can be performed to ensure reliability of measurements.

What is the current use of organoids in studying cystic fibrosis?

Intestinal organoids are used to better understand how CFTR function or manipulation thereof by therapeutic interventions can impact on individual clinical phenotype (figure 1). Additionally, the recently funded HIT-CF project (www.hitcf.org) will use organoids to design clinical trials by pre-treatment stratification of *in vitro* responders.

Clinical studies indicate that organoid FIS is relevant to characterise CFTR function of rare CFTR variants. Many of the currently known 2000 CFTR variants have not been characterised, which complicates the prediction of individual disease phenotype. Moreover, limited data regarding drug effects on rare genotypes prevents access to existing and new CFTR-mutation specific treatments. Pilot studies indicate that organoids could help both as individual biomarker of CFTR function to predict individual disease phenotype [17], as well as response to therapy [18], which could help characterise rare mutations for treatment response [7].

Organoids and clinical disease phenotype

Organoid FIS reflects residual CFTR function and correlates with predicted phenotypic characteristics of the CF genotype [7, 17] and other biomarkers of CFTR function, such as sweat chloride concentration (SCC) [7, 17] and intestinal current measurements (ICM) [17, 19]. While nasal potential difference (NPD) is another important clinical biomarker of CFTR function, no data on the correlation between organoid FIS and NPD are currently available.

The first study to directly compare organoid FIS and clinical phenotype prospectively included 34 newborns with CF. Newborns were clustered into low FIS or high FIS. Low FIS in organoids was related to increased pulmonary and pancreatic disease parameters at the age of 1 year [17]. Interestingly, in cases where SCC and ICM disagreed, FIS appeared to correctly align with the clinical indicators [17]. This study suggests that the full CFTR dependency of FIS and the 48 datapoints we typically gather (by titrating forskolin and repeating measurements) to type individual CFTR residual function may provide a higher accuracy and precision as compared to other biomarkers of CFTR function. The precise measurement of CFTR function also suggests that a further refinement of residual function classification is possible. Since these early studies are small, it is unclear how well the model predicts long-term clinical risk. Follow-up studies in larger cohorts are needed to validate the use of organoids for predicting disease phenotype in individuals with rare mutations, and predicting phenotypic variability between individuals with identical CF-causing mutations.

Organoids for studying drug efficacy

Preclinically, organoids are being used to identify and develop CFTR-modulating drugs and explore mechanisms associated with differences in CFTR function. Pharmaceutical companies use intestinal organoids in their drug development pipeline [20, 21]. During initial high-throughput screening, many different chemical structures are discovered that could work as CFTR-modulating drugs. Organoids can be a useful tool to efficiently validate lead compounds, because of their medium/high throughput and sensitivity to drug effects in combination with the fact that they express endogenous CFTR. Organoids are used to test the potency of single drugs, but also to compare the efficacy of different combination treatments [20–22].

Moreover, studies by DEKKERS and co-workers [7, 10, 23] in organoids indicated that both the CFTR mutations and additional patient-specific genetic differences modify response to CFTR modulators. By studying the effects of a range of drugs on different genotypes, the optimal CFTR modulating drug and their potency for each genotype can be identified for further clinical studies [23, 24].

Several studies have highlighted the translational potential of organoids. Genotype-specific effects of ivacaftor and lumacaftor/ivacaftor in organoids correlate with clinical trial data at group level [7]. The failure of a phase III clinical trial with ataluren in subjects with stop-codon mutations was preceded by a report on the absence of drug activity in the organoid model [25, 26]. These group-based observations indicate that organoids can help to characterise the response of mutations to particular treatments. Such an approach would complement current CFTR “theratyping” efforts that mostly rely on cell-line expression systems in which CFTR mutations are introduced. Based on quantitative and qualitative differences in therapy response between organoid subgroups (e.g. comparing average p.Ala455Glu/p.Phe508del, p.Asn1303Lys/p.F508del or class I/p.Phe508del responses), mutation-specific phenotypes with different therapeutic vulnerabilities can be deduced [23]. These theratyping efforts have no direct relationship to the individual patient, but rely on the coupling of individual CFTR genotype to the cell-based data.

Recent proof of concept in two patients with ultra-rare mutations showed clear *in vitro*–*in vivo* correlation in response to treatment with ivacaftor [7]. A subsequent study in 24 individuals with a range of genotypes found that intestinal organoid FIS after treatment with the drugs curcumin, genistein, ivacaftor and lumacaftor/ivacaftor correlated with *in vivo* responses in pulmonary function and sweat chloride concentrations on the individual level. Moreover, receiver operating characteristic curves generated from these data suggest that individual clinical response of CFTR-modulating drugs can be predicted with good-to-high accuracy [18]. These initial studies indicate that organoids provide a functional readout that integrates both the patient-specific CFTR mutations and additional genetic variation that modifies the response to treatment. If these results are validated in subsequent studies, this would suggest that a direct test on the patient organoid can be used to characterise the response to treatment, independent of any *a priori* knowledge on the CFTR genotype.

How do organoids compare to other *ex vivo* or *in vitro* cell models?

Cell-based approaches have mostly been used to type the average therapeutic response of a mutation or genotype. The Fischer rat thyroid (FRT) cell line, in which CFTR mutations are introduced in a standardised manner using defined cDNAs, can be used for high-throughput drug screening, and has helped to extend ivacaftor treatment to new CFTR mutations [27]. The strength of this model is its definition of standardised test components, and that patients do not have to undergo additional procedures. However, FRT cells are nonhuman, which influences CFTR protein folding [28] and pharmacological treatment responsiveness [29, 30]. To complement this mutation-oriented approach, several models have been developed using patient-derived cells.

Only a few studies directly compare drug outcomes in *ex vivo* or *in vitro* cultured cells and *in vivo* parameters of treatment. ICM on *ex vivo* rectal biopsies is validated for measuring residual CFTR function [31, 32], and sensitively detects CFTR-modulator treatment effects after *in vivo* drug treatment [33, 34]. However, only one therapy can be tested per biopsy, making personalised drug assessment burdensome.

CFTR function in human bronchial epithelial (HBE) and nasal epithelial (HNE) cells is assessed using electrophysiological studies on air–liquid interface cultures, which mimics the airway environment [35]. High-throughput screening is not feasible due to cell senescence after a limited number of passages, and repeatedly obtaining HBE cells from living donors is an invasive process. HNE cells can be obtained by nasal brushing, and initial studies show that electrophysiological measurements in brushed HNE cells correlate with *in vivo* CFTR function and might be predictive of individual treatment response [36, 37].

Compared to the aforementioned models, intestinal organoids have both limitations and advantages. Because healthy organoids are pre-swollen, FIS cannot compare CF to healthy control CFTR function. Moreover, although both intestinal and airway cells are derived from the endoderm, it is unclear whether intestinal epithelial CFTR function is directly related to airway epithelial CFTR function, which might influence treatment response [29, 30]. Nevertheless, the potential to biobank and repeatedly test intestinal organoids facilitates robust personalised medicine approaches. Future studies are needed to elucidate which model is superior in terms of feasibility and predictive capacity.

NPD and SCC are established *in vivo* CFTR function measurements that are both used for diagnosis of CF and as a biomarker in phase I and II clinical trials to evaluate CFTR function improvement after drug treatment [38]. These methods have been thoroughly validated and are available in many centres. However, NPD and SCC cannot be used preclinically, which limits their use for personalised medicine approaches. For an in-depth overview of studies on ICM, NPD and SCC see DE BOECK *et al.* [39].

Future use of organoids

Current studies with organoids support the possibility of additional classification of residual function. CFTR function is probably a biological continuum that is mostly dependent on CFTR genotype and

further modified by individual genetic and environmental factors. The first clinical validation studies showed how relatively large differences in residual function (albeit all in the CF domain) correspond with clinical manifestations [17]. Whether more subtle differences in organoid swelling (e.g. between organoids from people with identical CF-causing mutations; or in conditions of borderline CF and CFTR-related disease) also associate with clinical phenotype still need further long-term clinical follow-up studies.

The current focus of organoid studies in CF is on pretreatment selection of patients with ultra-rare CFTR variants in a Europe-wide consortium study (www.hitcf.org). Additionally, this study highlights the possibility of using the organoid technology to study pharmacy-overarching drug combinations. Future studies should define more precisely how assay sensitivity and specificity change in relation to the magnitude of treatment effects *in vivo* and how short term predictions relate to long-term outcomes. Implementing organoids in dose-optimisation studies might help to further individualise treatment with upcoming CFTR-modulating drugs.

Conclusion

Rectal organoids are *in vitro* primary cell cultures that enable the individual typing of CFTR function and response to CFTR modulators. The first clinical studies indicate that FIS of rectal organoids help to characterise the disease phenotype and drug response of rare CFTR mutations. Additional studies are underway to further validate relations between *in vitro* CFTR function and *in vivo* clinical phenotype in the context of CF diagnosis, prognosis and therapeutic response.

Conflict of interest: P. van Mourik has nothing to disclose. J.M. Beekman has a patent on organoid swelling for personalised diagnosis in CF with royalties paid by Hubrecht Organoid Technology. He received financial support to cover travel costs and preparation time of scientific lectures from Vertex. He also received funding from Galapagos for CFTR modulator studies. C.K. van der Ent reports grants from Vertex Pharmaceuticals Inc, Gilead Sciences and Galapagos, grants and other funding from ProQR, and other funding from TEVA pharmaceutical industries, outside the submitted work.

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