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Review

Intestinal organoids for Cystic Fibrosis research☆

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ABSTRACT

Significant progress has been made in the development of CFTR modulator therapy; however, current CFTR modulator therapies are only available for a minority of the CF-patient population. Additionally, heterogeneity in in vivo modulator response has been reported among individuals carrying homozygous F508del-CFTR, adding to the desire for an optimal prediction of response-to-therapy on an individual level. In the last decade, a lot of progress has been made in the development of primary cell cultures into 3D patient-derived disease models. The advantage of these models is that the endogenous CFTR function is affected by the patient's mutation as well as other genetic or environmental factors. In this review we focus on intestinal organoids as in vitro model for CF, enabling for CF disease classification, drug development and treatment optimization in a personalized manner, taking into account rare CFTR mutations and clinical heterogeneity among individuals with CF.

1. Background

The term 'organoid' was first adopted in 1933 to describe a tumorous mass that was extracted from a two-month-old patient [1]. At that time, 'organoids' referred to organ fragments generated by mechanical or enzymatic-dissociation, cultured in vitro on top of coverslips [2]. Later, organoids were generated from established cell lines [3,4] or by fully dissociating organ fragments into single cells [5] that re-aggregated into tissue-like 3D-structures. The discovery and characterization of extracellular matrix proteins (reviewed by Simian et al. [6]), together with the finding that interactions between the extracellular matrix and cells regulated tissue-specific function and morphogenesis [7], further stimulated organoid research in the 70s and 80s of the 20th century.

Recent progress in stem cell biology led to a strong revival of the organoid field, which are now defined as a three-dimensional collection of organ-specific cell types that self-organizes through cell sorting and spatially restricted lineage commitment comparable with the in vivo situation [8]. Embryonic- and induced pluripotent stem cells (IPSCs) are widely used for the generation of organoids, because their stemness and pluripotency facilitate indefinite expansion and because they have the potential to differentiate into almost all cell types of the human body derived from all three vertebrate germ layers [9,10]. The opportunities provided by the pluripotent state of IPSCs come at a cost, as these fully dedifferentiated stem cells require lengthy and complex culture protocols to acquire organoids that consist of a combination of differentiated cell types.

An alternative source of stem cells are adult stem cells (ASC). ASCs are progenitor cells present in epithelial tissues with high regenerative capacity [11]. The behavior and function of these cells depend on their localization in the human body, to which they remain lineage-committed in vitro. The primary role of ASCs is to maintain tissue homeostasis, in the context of normal cell turnover, or tissue repair after damage. The first example of an ex vivo long-term ASC culture showed the use of intestinal crypt-based stem cells, characterized by their expression of the LGR5 (leucine-rich-repeat-containing G-protein-coupled receptor 5) gene [12]. LGR5-positive intestinal epithelial stem cells self-organize into 3D crypt-like structures containing all differentiated cell types when cultured in matrigel and when the stem cell niche environment is mimicked with growth factors to favor tissue self-renewal [13]. This exploratory work using mouse cells was shortly thereafter followed up by human stem cell culture protocols [14]. Most ASC-

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derived organoids can typically be generated within days-to-weeks and can be expanded for over a year. Moreover, these organoids can be stored in living biobanks and retain patient-specific functional characteristics [11,15]. Although it now seems that optimal ASC-culture conditions are fully established, still some challenges remain. For example, the stem cell niche environment is partially mimicked by specific on-site produced conditioned media, for which the generation remains difficult to standardize. Another limitation is that most established organoid models exclusively consist of epithelial tissue and therefore not always represent the whole epithelial compartment according to in vivo tissue composition. Because of the lack of circulation, the maximum size of ASC-derived organoids is relatively small and limited by the diffusion distance of oxygen and nutrients. Also, current organoid systems are not optimal for modeling inflammatory responses in response to infection, since immune cells are lacking in current organoid cultures. In addition, for organoids derived from tissues with a lower cellular turn-over, like mammary gland [16] or alveolar epithelial organoids [17], no long-term culture protocols have been established yet and maturation into functional in-vivo like tissues is still a major challenge. It is therefore important to consider these limitations in the experimental design to take full advantage of the benefits of ASC-derived organoids.

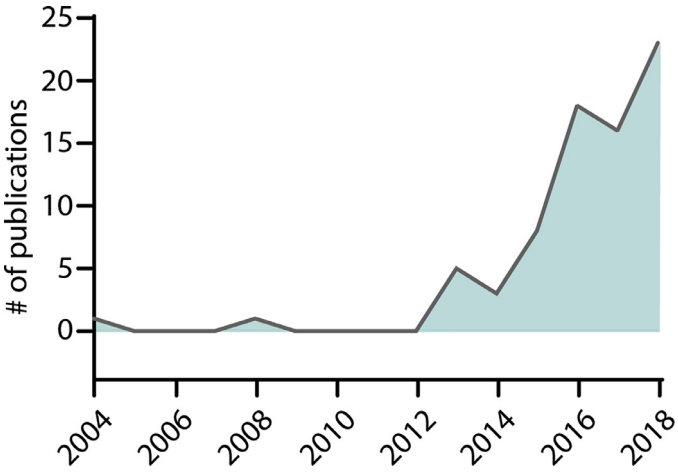

Fig. 1. Number of publications per year using organoid technology for cystic fibrosis research according to pubmed. The following pubmed searches: 'cystic fibrosis[TW] AND organoid[TW]'; 'cystic fibrosis[TW] AND organoids[TW]'; 'CFTR[TW] AND organoid[TW]'; 'CFTR[TW] AND organoids[TW]' were conducted to find all publications related to cystic fibrosis research and the organoid technology. This figure is based on papers using several types of organoids, including organoids generated from patient-derived stem cells, patient-derived induced pluripotent stem cells and animal model-derived stem cells.

1.1. Body of text

A perfect example where ASC-based organoid technology has generated impact is in the field of Cystic Fibrosis (CF) research. Here, patient-derived intestinal organoids have been used for disease modeling, drug screening, and personalized medicine [15]. CF is the most common monogenetic recessive disease in the Caucasian population and is caused by mutations in the *Cystic Fibrosis Transmembrane Conductance Regulator* (*CFTR*) gene. The CFTR protein regulates trans-epithelial secretion of bicarbonate and chloride, and its dysfunction results in aberrant fluid transport and abnormal mucus formation that affects the functionality of multiple organs, including the pancreas, lungs and the intestines.

Liu et al. reported the first use of intestinal organoids in the CF field, showing that mouse organoids exhibit CFTR expression and activity comparable to that of crypt epithelium in vivo [18]. This work was quickly followed up by a study in human CF intestinal organoids that described the first functional readout in human ASC-based organoids [15]. The functional readout enabled measurement of CFTR function, and relied on forskolin-induced swelling (FIS) of whole organoids [15]. Because the CFTR protein is located at the inner (apical) side of the organoids, cyclic adenosine monophosphate (cAMP)-inducing stimuli (e.g. forskolin) lead to ion and fluid transport into the organoid lumen and subsequently into rapid swelling of the organoids [15]. Remarkably, the FIS was completely CFTR dependent, whereas raising intracellular calcium has little to no effect on whole organoid swelling [19]. FIS is highly useful to quantitate and compare CFTR function between CF organoids but later studies found that CFTR function is underestimated in healthy control organoids, as their FIS response is lower due to fluid-filled lumens prior to forskolin stimulation [20]. To compare healthy control and CF organoids, the steady state lumen area (SLA) assay was developed in which the lumen surface area is measured as a percentage of the total organoid surface area, which can also be used to measure drug response [20]. This pioneering work led to today's revival of organoid research in the field of CF (Fig. 1).

CF remains incurable up until today, but the development of CFTR modulators has increased the prospects that CF disease manifestation might be stalled or even prevented, in the near future [21]. Two phase 2 clinical trials have shown that the efficacy of the newest triple combination therapy exceeds the efficacy of the previous developed combinations, but is still only accessible for patients with particular CFTR mutations [22,23]. It has therefore become highly relevant to understand who may benefit from available modulators and who may not. Moreover, many health care systems are reluctant to approve reimbursement for these therapies due to limited cost-efficacy.

In addition to current treatment options, new therapeutic strategies are being explored including the repurposing of current modulators for rare mutations as well as the development of new CFTR-directed therapies [24], like amplifiers, proteostasis modulating compounds, CFTR read-through agents and nonsense-mediated decay inhibiting compounds. Also, bypassing CFTR function with the introduction of artificial anion transporters or by targeting alternative ion channels are attractive new approaches [24], which are especially interesting for patients with mutations not responsive to CFTR modulator therapy.

However, before clinical benefit of new therapeutic agents can be properly predicted, suitable pre-clinical models need to be established, since as much as 80% of all newly developed compounds currently fail in clinical trials [25]. For CF, it is clear that the disease shows a huge variability between patients, for which several reasons can be found (Fig. 2). Firstly in the *CFTR* gene itself, as over 2000 CFTR variants have been reported in the Cystic Fibrosis Mutation Database [26]. The existence of this huge amount of CFTR variants contributes significantly to CF disease heterogeneity [27,28]. According to the latest update of the CFTR2 database [29], as of March 2019, out of 412 CFTR variants studied, 346 were found to be CF-causing, whereas 21 variants were found to be non-CF causing. In addition, 37 variants resulted in variable clinical consequence and 8 variants were of unknown significance [29]. Combining the information from the two CFTR databases, 1700 of the 2000 known variants are estimated to be CF-causing. Besides the identified 412 variants, many rare CF mutations have not been characterized in detail, making it difficult to interpret and translate the molecular genotype(s) into a clinical trajectory [28,30]. Second, other individual variables include genetic modifiers that regulate CFTR or other functions (epithelial or non-epithelial), and interactions with the environment [31,32]. However, it is very challenging to identify and integrate all the disease variables at the individual patient level. In vitro models can enable a more precise measurement of individual CFTR function and modulation by therapy. Such precise CFTR measurements may have the highest likeli-

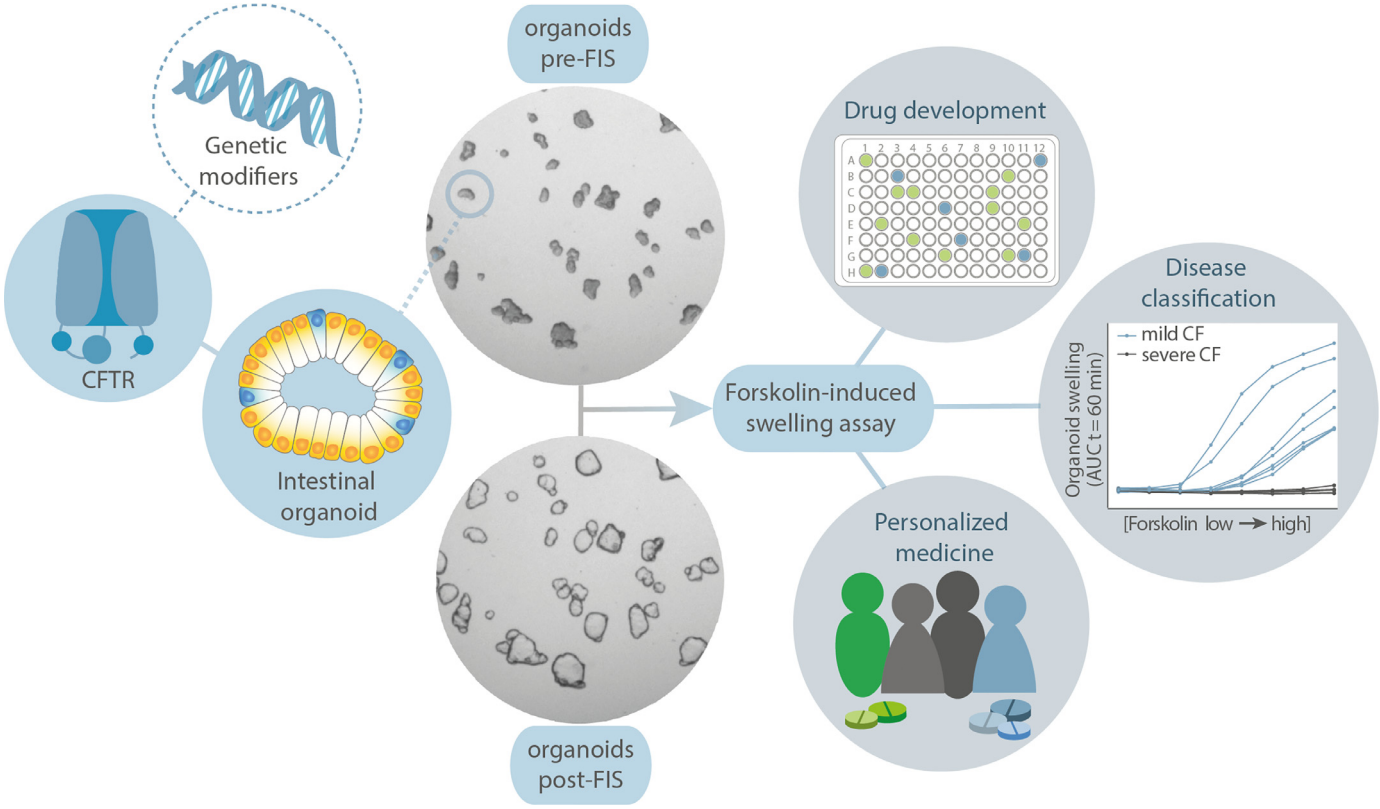

Fig. 2. Besides the CFTR mutation, genetic modifiers can affect CFTR function. These patient-specific characteristics are nicely recapitulated in intestinal organoids and the FIS-assay. This in vitro model therefore better predicts in vivo residual CFTR function and allows for better classifying CF disease. The FIS-assay also accurately estimates in vivo drug efficacy and will help in developing clinically effective drugs as well as defining the most optimal individual therapy (personalized medicine).

hood of predicting clinical benefit from CFTR modulators, and may be further complemented with additional individual factors to improve prediction of therapeutic benefit.

The current pre-clinical high-throughput model that has been used to successfully develop CFTR modulators were Fischer rat thyroid (FRT) cells that ectopically express CFTR cDNA. Shortcomings of this model include that intronic variation is not considered, ultra-rare mutations are difficult to integrate and results are not always accurate. For example, ivacaftor addition caused a 31-fold increase in CFTR-mediated chloride transport in FRT cells expressing G970R-CFTR [33], however, sweat chloride concentration did not change in patients carrying the G970R mutation upon ivacaftor treatment [34]. Pre-clinical models that take into account above mentioned disease contributors, like electrophysiology in the Ussing chamber using patient-derived human bronchial epithelial cells differentiated at air-liquid interface or CF animal models, show high validity for clinical translation, but compromise on screening throughput.

Patient-derived intestinal organoids may therefore provide an alternative to current pre-clinical models by combining high validity together with high-throughput potential. Patient-derived organoids recapitulate human epithelial biology and patient-specific characteristics, like the expression of intronic and intergenic enhancers that regulate CFTR gene expression. Also (epi)genetic signatures contributing to disease severity are recapitulated in organoids and are retained during prolonged organoid culturing [30]. This is especially relevant for capturing patient variation within identical mutation groups or for (ultra)rare mutations because of the low incidence, lack of mechanistic insight and difficulty of clinical trial design [19].

Several patient-derived organoid models have been developed of which the intestinal organoid model and the FIS-assay offer various advantages (Fig. 2). Firstly, CFTR testing can be performed without the need for manipulation, e.g. intestinal organoids do not require pre-incubation with inhibitors since swelling is completely CFTR dependent [19]. Secondly, the swelling assay allows for considerable throughput since the assay can be performed in 96- and 384-well format. The potential of intestinal organoids as pre-clinical model and for personalized medicine has been demonstrated in studies showing that FIS-rates of infant-derived organoids harbouring all kinds of CFTR mutations correspond with clinical phenotypes at 1 year of age as well as in vivo sweat chloride concentration (SCC), enabling accurate estimation of in vivo residual CFTR function on the individual patient level [35]. In addition, in vitro CFTR modulator responses in organoids appeared to correlate significantly with the two most important therapeutic endpoints, change in FEV1 and SCC and displayed excellent accuracy for stratifying drug responders from non-drug responders [36]. Even within the F508del homozygous patient group, intestinal organoids showed varying residual function and drug responsiveness [35,36], comparable to what has been reported in vivo [37], and thus prove highly useful to study individual factors that contribute to individual relations between CFTR genotype, CFTR expression and CFTR function.

1.2. Future directions

An example of a large scale drug screening effort is the Dutch HIT-CF Rainbow project where academically available CFTR modulating drugs and a library of >1400 FDA-approved drug compounds were tested for forskolin induced swelling in organoids of >80 patients with rare mutations, aiming to identify curative treatments for all CF patients. Besides the utilization in drug development, the intestinal organoid model has been used to select patients for clin-

ical trials, with the HIT-CF Europe being a perfect example [38]. In this project, organoids derived from over 500 CF patients with rare CFTR genotypes that do not benefit from current drug development efforts are used to identify potential responders to novel CF therapeutics currently in clinical development for various larger patient groups. Organoids will be employed to identify patients that show high CFTR function restoration, so that selected patients can proceed to clinical trials. Simultaneously, a path for drug access and reimbursement is being explored with the European Medicines Agency.

Next to intestinal organoids, alternative culture models have been developed that may help to understand organ-specific manifestations in CF and to better predict organ-specific drug responses. This has led to the emergence of other epithelial-derived organoids, like hIPSC-derived pancreas [39] or liver [40] organoids. In addition, ASC-derived airway organoids are of special interest as CF mortality is mainly a result of pulmonary failure and enable the assessment of airway-specific fluid transport, mucus viscosity and patient-specific drug responses [41] in the context of airway cell environment. Culturing of airway-derived cells in matrigel results in the formation of spheroids that allow for CFTR function measurement in a comparable fashion to intestinal organoids, via FIS [42]. Also the presence of non-CFTR ion channels, like Na+ channels and TMEM16a in nasal spheroids [43] and bronchial-derived organoids [44] respectively, shows the potential of using airway organoids for the development of alternative non-CFTR targeting therapies. Data from Pranke *et al.*, indicate that in vitro observations in nasal epithelium may also correlate with in vivo outcomes [45], confirming the importance of employing patient-derived in vitro assays as relevant predictors of clinical drug efficacy and disease manifestation.

2. Summary

Combining current knowledge regarding CF classification and the predictive value of intestinal organoids, show the potential of patient-derived organoids for a personalized medicine approach. A personalized approach to diagnostics could not only help deciding on a suitable therapeutic strategy for patients with uncommon mutations, potentially making marketed drugs accessible for these patients, but might also help defining optimal therapies for patients with the same genotype [34]. Ultimately, the goal is to develop a CFTR modulator approach that enables optimal, individual CFTR restoration, regardless of mutation incidence, geographical location or ethnicity.

Declaration of Competing Interest

J.M.B. is an inventor on patent(s) related to the FIS-assay and received financial royalties from 2017 onward. J.M.B report receiving research grant(s) and consultancy fees from various industries, including Vertex Pharmaceuticals, Proteostasis Therapeutics, Eloxx Pharmaceuticals, Teva Pharmaceutical Industries and Galapagos outside the submitted work.

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