High-throughput single organoid swelling assay for personalized evaluation of CFTR modulators in patient-derived rectal organoids

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BACKGROUND

Cystic Fibrosis (CF) has experienced a significant transformation in standard of care over the past decade due to access to novel molecular entities that directly target the dysfunctional protein Cystic Fibrosis Transmembrane Regulator (CFTR). Despite these advances, accurately representing CF disease states at the tissue level in vitro remains a challenge. To address this, we have developed new culture conditions for patient-derived rectal organoids, which, combined with a high-throughput single organoid swelling assay, enable the study and prediction of treatment response in CF patients to different CFTR modulators. Our results demonstrate that in vitro analysis using this system can enhance our understanding of the impact of various treatments on organoid epithelia, enabling the precise tailoring of therapies and supporting treatment decisions for complex CF phenotypes. These advances have the potential to significantly improve patient outcomes and contribute to the ongoing transformation of CF care.

METHODS

Patient-derived human rectal organoids from healthy and CF individuals were grown for 5 days on Gri3D®-3. CF organoids were treated overnight with different CF agents to restore CFTR functionality. The functionality of the CFTR was then assessed using the Forskolin-Induced Swelling (FIS) assay reported by Dekkers et al.1. The organoid arrays were imaged before and after forskolin exposure and the total swelling of each individual organoid was determined based on Doppl’s automated swelling assessment pipeline. To validate the specificity of the assay, non-CF organoids were treated with the CFTR inhibitor CFTRinh-172 prior to FIS.

RESULTS

Homogenously sized human rectal organoids from healthy and CF individuals were grown on microarray cavities, forming a stable gut epithelium with projections and buddings (Fig. 1A). Healthy and CF rectal organoids displayed comparable sizes and shapes, ensuring the reliability of the assay. Transepithelial fluid transport was observed by inducing organoids swelling using forskolin. In CF organoids, this swelling was diminished or abolished but could be partially restored by drugs rescuing the function of the CFTR protein (Fig. 1B). To prove that FIS in human organoids is CFTR dependent, non-CF organoids were pre-treated with a targeted CFTR inhibitor, resulting in a significantly diminished swelling (Fig. 1C,D). CFTR residual function and its rescue by different treatments were analyzed for CF patients using FIS combined to our automated quantification, which precisely tracks each single organoid and determines the % of CFTR recovery compared to the average of a non-CF patient (Fig. 2). We could therefore find the treatment that works most efficiently for rescuing CFTR impairments related to a specific combination of mutations. This provides a personalized approach for developing and optimizing CF treatments for CF patients carrying different CFTR mutations.

REFERENCES


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