High-throughput single organoid swelling assayfor personalized evaluation of CFTRmodulatorsin patient-derived rectal organoids

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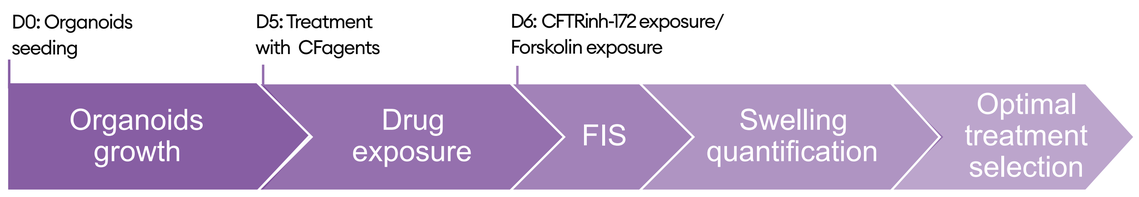
BACKGROUND

Over the past decade, the standard of care for Cystic Fibrosis (CF) has advanced significantly, primarily due to new molecular treatments targeting the dysfunctional Cystic Fibrosis Transmembrane Regulator (CFTR) protein. Despite these advancements, accurately replicating CF disease states *in vitro* remains a challenge.[1] To address this, we have developed new culture conditions for patient-derived rectal organoids[2], and paired them with a high-throughput single organoid swelling assay. This combination allows us to study and predict patient responses to different CFTR modulators.[3]

Our results demonstrate that this system enhances our understanding of how treatments impact organoid epithelia, enabling precise therapy tailoring and better treatment decisions for complex CF phenotypes. These advances have significantly improved patient outcomes and continue to transform CF care.[3]

METHODS

Patient-derived rectal organoids from healthy and CF individuals were grown for 5 days, and CF organoids were treated overnight with various CF agents to restore CFTR functionality. Functionality was assessed using the Forskolin-Induced Swelling (FIS) assay.[5] Organoid arrays were imaged before and after forskolin exposure, and swelling was measured using Doppl's automated assessment pipeline. To validate specificity, non-CF organoids were treated with the CFTR Inhibitor CFTRinh-172 before FIS. For CF patients, lung function, as expressed by forced expiratory volume in the first second (FEV1), and changes in sweat chloride following modulator treatment, were compared with organoid responses.



RESULTS

Homogeneously sized rectal organoids from healthy and CF individuals are grown on microcavity arrays, forming a stable gut epithelium with projections and buddings (Fig. 1A). Both types have comparable sizes and shapes, ensuring assay reliability. Transepithelial fluid transport is observed by inducing organoid swelling with forskolin. In CF organoids, this swelling is reduced or abolished but can be partially restored by drugs rescuing the function of the CFTR protein (Fig. 1B). To confirm FIS dependence on CFTR, non-CF organoids are pre-treated with a CFTR inhibitor, significantly reducing swelling (Fig. 1C,D).

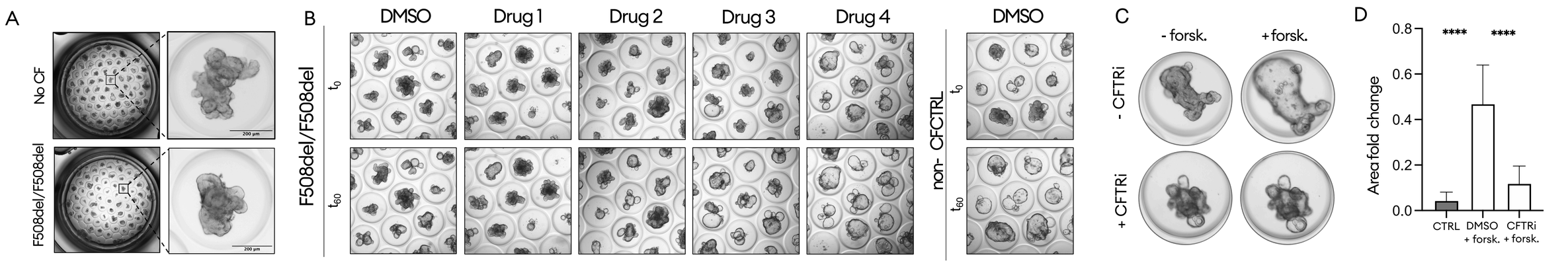


Figure 1: FIS and assay specificity. A. Representative Brightield (BF) images of human rectal organoids from wildtype CFTR and F508del/F508del subjects cultured for 5 days. B. Representative BF images before (t0) and ager 60 min (t60) of forskolin stimulation for CF and non-CF organoids pre-treated overnight with DMSO or four different CF drugs. C. Representative BF images of non-CF organoids before and after forskolin stimulation with/without CFTRinh-172. D. Area fold change quantification of non-CF organoids. CTRL: untreated, not exposed to forskolin. DMSO + forsk: treated with DMSO before forskolin. CFTRi + forsk: treated with CFTRinh-172 before forskolin.

We analyze CFTR residual function and its rescue by various treatments using FIS and automated quantification, tracking each organoid and determining CFTR recovery as a percentage compared to a non-CF average (Fig. 2). This allows us to identify the most effective treatment for specific CFTR mutations, providing a personalized approach for developing and optimizing CF treatments. Remarkably, we observed a positive correlation between organoid CFTR activity rescue and post-treatment FEV1 and sweat chloride in all CF patients (Fig. 2). These results highlight the great potential of rectal organoids in identifying individuals likely to benefit from novel therapies targeting specific CFTR variants, thus offering valuable insights for therapeutic decision-making.

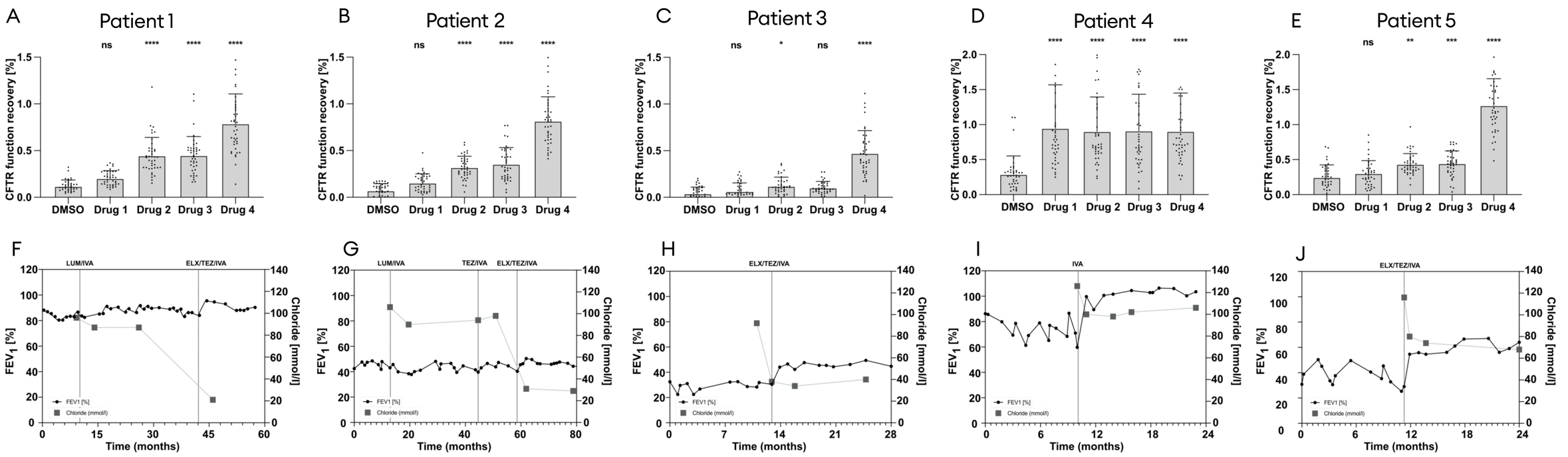


Figure 2: FIS quantification of five different CF patients upon different treatments. Doppl's automated swelling quantification for CF patients with different CFTR mutations: A,B. F508del/F508del, C. F508del/1898+G>A, D. 1677del/R334W, E. F508del/2347delG. The CFTR function recovery [%] was determined measuring the total swelling for each organoid and normalizing it to the average swelling of non-CF control organoids. Statistical significance was determined compared to the DMSO (baseline) control. *: p<0.05, **: p<0.01, ***: p<0.001, ****: p<0.0001, ns: non-significant. One-way ANOVA multiple comparison analysis (Tukey correction). F-J. Evolution of lung function and sweat chloride before and after treatment with CFTR modulators. Participants' sex and age are indicated in parenthesis. The dotted lines indicate the start of modulator treatment. F, female; M, male; FEV1, forced expiratory volume in the first second.

SUMMARY

- We tested CFTR modulators in CF patients using patient-derived rectal organoids and a high-throughput single organoid FIS assay.
- New culture conditions were developed for healthy rectal organoids, enabling reliable analysis of swelling.
- This approach offers a method to measure CFTR functionality and drug response, aiding in the development of personalized CF treatments.

References

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ACKNOWLEDGMENTS

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