Organoids as a Personalized Medicine Tool for Ultra-Rare Mutations in Cystic Fibrosis: the Case of S955P

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Background: More than 2,000 CFTR mutations have been described, which can be grouped according to their functional defect into 7 classes. For most of them, neither the associated disease liability nor the underlying basic defect are known. This knowledge is essential for disease prognosis and CFTR-based therapeutics, since functional defect-specific corrective therapies (CFTR modulators) have been already approved.
Aims: As a case study for personalized medicine we aimed to characterize novel ultra-rare mutation S955P (p.Ser955Pro), identified in Czech patient with F508del (p.Phe508del) on the other allele.

Materials and Methods: Rectal biopsies were analyzed in micro-Ussing chamber (Fig. 1). Crypt isolation, organoid culturing and FIS assay were performed (Fig. 2). CFTR protein was detected in organoids with anti-CFTR CFF antibodies (Fig. 3). Novel cell line (S955P-CFTR CFBE cells) was generated. Western blot analysis of organoids and S955P-CFTR CFBE cells was performed (Fig. 4). CFTR function was assessed in S955P-CFTR CFBE cell monolayers in micro-Ussing chamber (Fig. 5). Single-channel recordings by patch-clamp was carried out in S955P-CFTR CHO cells (Fig. 6). Statistical analyses were performed on GraphPad Prism 7.0 using two-tailed paired student's t-tests, with p ≤0.05 considered as significant.

Fig. 1.

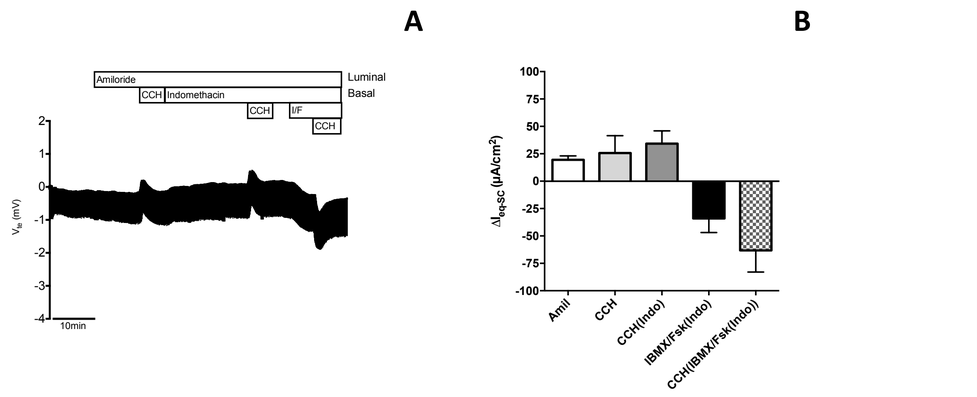

Fig. 1. (A) Recording of the effects of cholinergic (CCH, 100 µM, basolateral) and cAMP- dependent (IBMX/Fsk (I/F), 100 µM/2 µM, basolateral) activation on transepithelial voltage (Vte) in rectal biopsies from patient (F508del/S955P). Experiments were performed in the presence of Amiloride (Amil, 20 µM, luminal) and/or Indomethacin (Indo, 10 µM, basolateral), as indicated in the panels. **(B)** Summary of activated equivalent short-circuit currents (ΔIeq-sc); data represent the mean of measurements on three rectal biopsies ± SEM. CF2 - ΔIeq-sc,IBMX/Fsk (Indo) = −34.1 ± 22.2 µA/cm2; ΔIeq-sc,CCH(IBMX/Fsk(Indo)) = −63.2 ± 34.1 µA/cm2 (18% of WT CFTR function).

Fig. 2.

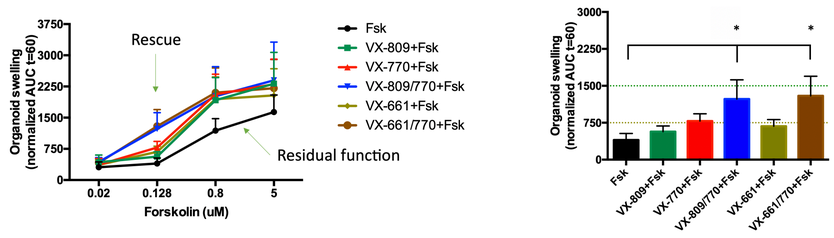

Fig. 2. FIS assay on intestinal organoids from individual (F508del/S955P). Quantification of FIS in organoids for all treatments at forskolin (Fsk) concentrations of 0.02, 0.128, 0.8 and 5 µM, expressed as the AUC of organoid surface area increase (baseline = 100%, t = 60 min). Quantification of organoid swelling for all treatments at [Fsk] = 0.128 µM. The dashed yellow and green lines represent the established thresholds for medium and high clinical benefit potential for treatments, respectively. Data represent the mean of measurements on 5–8 replicate wells per condition ± SEM. Asterisks indicate degree of significant difference calculated by unpaired one-way ANOVA using Fisher's LSD test. * - p value < 0.05

Fig. 3.

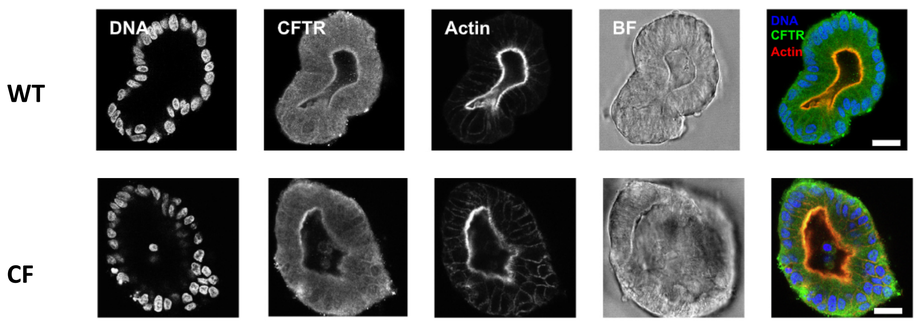

Fig. 3. Confocal microscopy slices of intestinal organoids (wt, CF). CFTR was immunostained with the 528 antibody (1:250, CFF). Nuclei and F-actin were labelled with Methyl green (1:750) and Phalloidin-TRITC (1:250) respec-tively. Corresponding brightfield images (BF) are also shown. Scale bar represents 20 µm. Images were acquired with a Leica TCS SP8 confocal microscope. (I.A.L. Silva)

Fig. 4

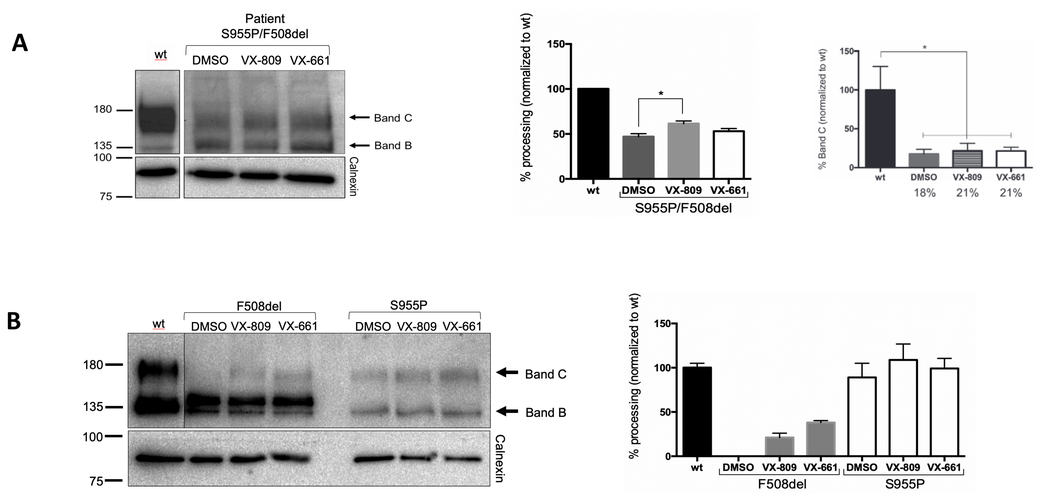

Fig. 4. (**A**) WB analysis of CFTR protein expressed in intestinal organoids from a non-CF control (wt), and from individual CF2, under basal conditions or following treatment with VX-809 (3 µM) or VX-661 (5 µM) for 24 h (n = 3). (D) Quantification of fully-glycosylated protein (band C) for each condition, following densitometry to calculate the percentage of mature CFTR (band C) vs total CFTR expressed (=%processing). (E) Quantification as in D, but the percentage of mature CFTR (band C) was estimated vs mature CFTR (band C) in wt (% band C). **(B)** WB analysis of CFTR protein expressed in CFBE cells stably expressing S955P, F508del or wt-CFTR. CFBE cells were also analyzed following treatment with VX-809 (3 µM) or VX-661 (5 µM) for 24 h (n = 3). For each condition, densitometry was used to calculate the percentage of mature CFTR (band C) vs total CFTR expressed (=%processing). Data were normalized to the efficiency of processing of wt-CFTR and are shown as mean ± SEM. Asterisks indicate degree of significant difference calculated by unpaired t-test. * - p value < 0.05. (I.A.L.Silva).

Fig. 5.

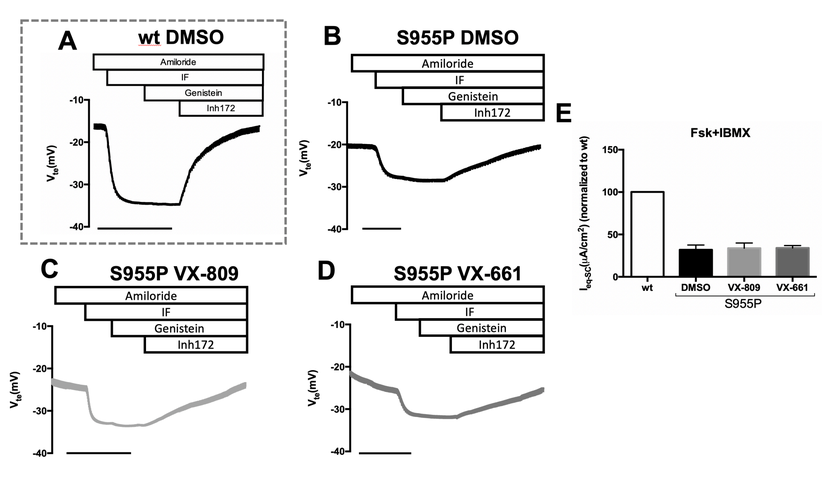

Fig. 5. CFBE cells stably expressing (A) wt-CFTR or (B–D) S955P-CFTR, were pre-incubated for 24 h with DMSO (0.1% v/v) (A, B) as vehicle control, (C) 3 µM VX-809, or (D) 5 µM VX-661 and analyzed under CFTR potentiation with genistein. (E) Graph summarizing equivalent short-circuit currents (Ieq-sc) after apical stimulation with forskolin+IBMX (I/F 2 µM and 100 µM, respectively) obtained in A-D. A low Cl− Ringer solution was used at apical side to stablish a Cl− gradient. Negative transepithelial voltage (Vte) deflections were observed following the addition of apical I/F which were fully reverted by addition of CFTR inhibitor Inh172 (30 µM). S955P-CFTR shows impaired CFTR function (~18% WT). (I.A.L.Silva).

Results: Western blot analysis of organoids and S955P-CFTR CFBE cells excluded defect in CFTR intracellular trafficking (Fig. 4). Also, relatively high residual function of CFTR (~18% vs non-CF) was confirmed in rectal biopsies, CFBE cells and intestinal organoids (Fig. 1, Fig. 2, Fig. 5). Normal channel conductance but decreased open-probability (50% vs wt-CFTR) was confirmed by single-channel study (Fig. 6). These data are consistent with **S955P being a class III mutation**. The fact that in organoids S955P-CFTR appears at much lower levels than wt-CFTR (Fig. 3) suggests its unstability, thus **possibly also being a class VI mutation**. Analysis of intestinal organoids using CFTR modulators showed that combination of **VX-770/VX-809 or VX-770/VX-661 resulted in significant responses** which is in the range of possible clinical benefit (Fig. 2).

Conclusion: Altogether these data illustrate how complementary *in vitro* and *ex vivo* studies can contribute to understand the basic defect of ultra-rare CFTR mutations and to determine their responsiveness to CFTR modulator drugs for possible translation into clinical use.

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Fig. 6.

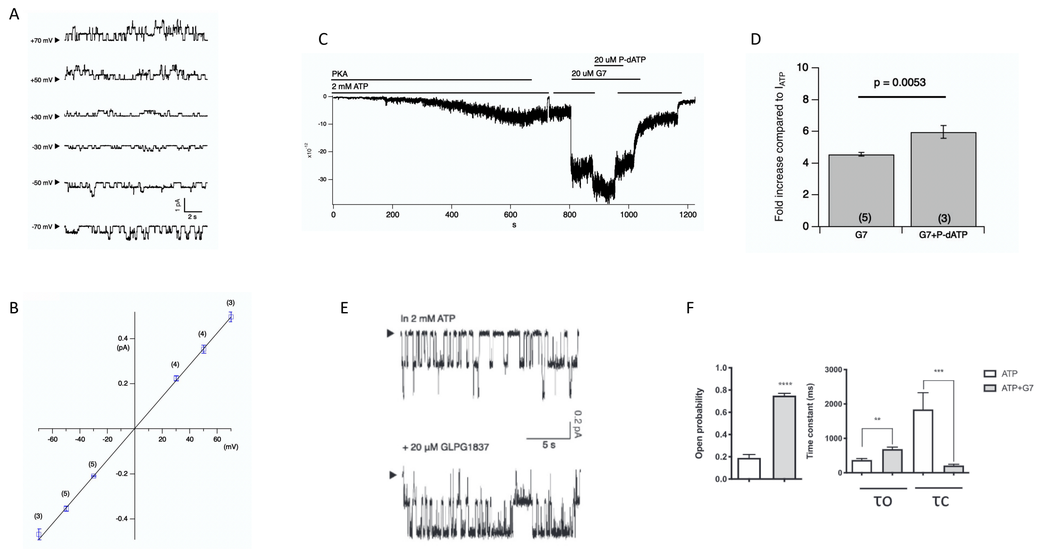

Fig. 6. (A) Single-channel CFTR current traces at different membrane potentials in the presence of 2 mM ATP. (B) Single-channel I-V relationships of S955P-CFTR (blue points) vs WT-CFTR (fitting from WT (Yeh et al., 2014)). (C) GLPG1837 further increases macroscopic S955P-CFTR currents in the presence of P-dATP (a high affinity ATP analogue). (D) Comparison of residual currents after incubation with GLPG1837 (G7) shows an increase in 955P-CFTR gating of ~4-fold and ~6-fold in the absence or presence of P-dATP, respectively. (E) Single-channel behavior of GLPG1837- potentiated S955P-CFTR. Comparing these two 20-s traces, longer open events and shorter closed events after potentiation with GLPG1837 were noted. (F) Summary of the additive effect of GLPG1837 on single-channel kinetics of S955P-CFTR. Po, open time (τo), and closed time (τc) were as follows: 0.19 ± 0.03; 368 ± 46 ms, and 1841 ± 490 ms for ATP alone (n = 4); and 0.75 ± 0.02, 689 ± 56 ms, and 212 ± 35 ms for ATP + GLPG1837 (n = 3). Error bars represent the SEM of the mean. Asterisks indicate degree of significant difference calculated by unpaired t-test. ** - p value < 0.01, *** - p value < 0.001. (TC.Hwang, JT. Yeh).