68* Renal cyst formation as tool for CFTR research

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Madin Darby canine kidney (MDCK) epithelial cells are a valuable model system to investigate renal cyst development and growth. These cells endogenously express CFTR, form polarized epithelia on filter supports and cysts in collagen gels. In previous work, we demonstrated that CFTR blockers retard cyst growth predominantly by inhibiting fluid accumulation within the cyst lumen (Li et al. Kidney Int. 2004; 66:1926–38). Here, we investigate the utility of renal cysts for CFTR research using MDCK cells stably expressing wild-type human CFTR (WT-MDCK) or the most common CF mutation F508del (F508del-MDCK) and the thiazolidinone $CFTR_{inh}$-172, a potent, specific inhibitor of the CFTR Cl^- channel. After 6 days growth in the continuous presence of forskolin (10 μM), WT-MDCK cells formed more cysts with larger volume compared to parental MDCK cells ($p < 0.01$, Student's unpaired t-test). By contrast, F508del-MDCK cells formed fewer cysts with smaller volume ($p < 0.05$). $CFTR_{inh}$-172 (10 μM) reduced both the number and volume of cysts formed by WT-MDCK cells ($p < 0.01$ vs. untreated WT-MDCK). Because cyst enlargement is determined both by cell proliferation and fluid accumulation within the cyst lumen, we studied the proliferation of the three MDCK cell lines with the same conditions used for cyst formation assays. At day 6, no differences in cell numbers were observed ($p > 0.05$). Our finding that either a loss-of-function mutation or a specific CFTR inhibitor attenuates greatly renal cyst formation suggests that renal cyst formation might serve as a valuable model system to explore the effects of CFTR modulators.

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69 Activation of chloride secretion in human airway epithelial cells by a new pyrrolo[2,3-b]pyrazine

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CFTR is expressed at apical membrane of airway epithelial cells and plays a pivotal role in regulation of volume and composition of airway surface liquid. CFTR defect causing cystic fibrosis, development of CFTR pharmacological activators is an important challenge for protein therapy.

Among a library of 6-phenylpyrrolo[2,3-b]pyrazines, we report the impact of the substitution of 4-hydroxyphenyl (RP107) by 2-fluorophenyl (RP193). EC50 of compounds, performed by iodide efflux experiments on CHO-CFTRwt cell line with 1μM forskolin, shows a comparable affinity: 110±1nM for RP193 vs. 152±1.2 nM for RP107. Patch clamp experiments reveal linear and time independent chloride current elicited by RP193, and fully blocked by the CFTR inhibitors: 2′-deoxyadenosine-methylglyoxal adducts (100pM) and CFTRinh-172 (10μM). We tested RP193 on primary culture of human bronchial epithelial cells (HBEC), a more realistic model of airway epithelium. Non-CF lungs fragments were obtained from patients who underwent lobectomy for lung cancer. Human bronchi were dissected free of lung parenchyma. HBEC were harvested using enzymatic isolation procedures, seeded on permeable membrane collagen and grown at air-liquid interface. Cultures were mounted in Ussing chambers and baseline short circuit current (Isc) was measured. In presence of amiloride 10μM and forskolin 1μM, sequential addition of RP193 (1 to 60μM) induce an increase in Isc. These results demonstrate efficiency of RP193 on human CFTRwt chloride channel.

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70 G622 amino acid has a crucial role in pharmacological activation of CFTR chloride channel by benzo[c]quinolizinium compounds

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To explore the mechanism of action of benzo[*c*]quinolizinium compound, the potent activator 5-butyl-6-hydroxy-10-chlorobenzo[*c*]quinolizinium chloride (MPB-91, Derand et al., 2001) was tested on CFTR-G622D (CF mutation) and CFTR-E621D/G.

Site directed mutagenesis on GFP-CFTR-wt was used to obtain CFTR variants: G622D, E621D and E621G. Patch-clamp experiments, in whole cell configuration, were performed on Cos-7 transiently transfected to examine CFTR chloride channel activity. As GFP-CFTR-wt, the three mutants Cl^- channels elicit a time and voltage independent current in presence of 10 μM Fsk and are blocked by 10 μM of $CFTR_{inh}$-172. Ratio of global conductance (G) G_{Fsk}/G_{basal} of GFP-CFTR-G622D is 1.7 fold less than GFP-CFTR-wt [respectively 1.92±0.15 (n = 13) and 3.23±0.43 (n = 12)]. Western blot analysis shows a diminished trafficking of CFTR-G622D proteins. As expected, GFP-CFTR-wt channels are stimulated by 100 μM of MPB-91. Interestingly, activation of E621D and E621G but not of G622D channels is recorded in presence of MPB-91.

Our study shows: (1) G622D, E621D and E621G CFTR channels are functional and global conductance of G622D channels is reduced; (2) G622D proteins show an altered trafficking which could explain the weaker cAMP Cl^- currents; (3) G622D channels are refractory to benzo[*c*]quinolizinium activation.

The neutral amino acid G622 is well conserved in CFTR sequence of many species and among the ABC transporter family (Callebaut et al., 2004). Our findings suggest that G622 has a crucial role in pharmacological activation of CFTR by MPBs and in the direct interaction of the compound with the channel.

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71 Ezrin and cAMP/PKA have different compartmentalization in CFBE41o- and 16HBE14o- cells

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NHERF1 overexpression in CFBE41o- (CFBE) cells induces a significant increase of both apical membrane deltaF508CFTR expression and of PKA-dependent activation of chloride efflux. We hypothesize that this NHERF1-dependent rescue of deltaF508CFTR chloride secretion could be driven by the interaction of the actin cytoskeleton with the ezrin-NHERF1-CFTR multiprotein complex. As NHERF1 binds only active ezrin, we analyzed the distribution of active P-T567-ezrin with respect to total ezrin in both cell lines. In differential detergent extraction the ratio of active to total ezrin was lower in CFBE with respect to 16HBE14o- (HBE) cells. Confocal analysis demonstrated that active phospho-ezrin is localized at the apical membrane in HBE while being mislocalized to the basolateral membrane in CFBE cells. As ezrin is also an AKAP (A-kinase anchoring protein) protein involved in PKA compartmentalization in the vicinity of CFTR protein, we investigated the compartmentalization of cAMP and PKA. Confocal analysis demonstrated that PKA is localized at the apical membrane in HBE cells while being cytoplasmic in CFBE cells. Further, FRET (Fluorescence Resonance Energy Transfer) analysis showed that cAMP is localized in the membrane regions of HBE cells while being cytoplasmic in CFBE cells. These data show that in CFBE cells the known altered deltaF508CFTR localization is associated with a mislocalization of the signal transduction molecules involved in its activation, suggesting that the trafficking defect in CF cells includes its multiprotein regulatory complex (deltaF508CFTR-NHERF1-actin-ezrin-PKA).

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