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Posted Date: 30 June 2023

doi: 10.20944/preprints202306.2182.v1

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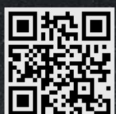

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Review

Pathogenic Relationships in Cystic Fibrosis: CFTR, SLC26A9 and Anoctamins

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Running Title: CFTR, SLC26A9 and anoctamins in CF

Abstract: The Cl^- transporting proteins CFTR, SLC26A9, and anoctamin (ANO1; ANO6) appear to have more in common than anticipated initially. They all participate in the pathogenic process and clinical outcome of renal and airway diseases. In the present review we will therefore concentrate on recent findings concerning electrolyte transport in airways and kidney, and the role of CFTR, SLC26A9, and anoctamins (ANO1 and ANO6). A particular focus will be on the airway diseases cystic fibrosis and asthma, as well as renal alkalosis and polycystic kidney disease. In essence, latest findings are summarized that demonstrate CFTR as the only relevant secretory Cl^- channel in airways under basal (non-stimulated) conditions and after stimulation by secretagogues. For proper CFTR function, expression of ANO1 and ANO6 appears to be a prerequisite. Evidence is summarized suggesting that the Cl^- transporter SLC26A9 may have a reabsorptive rather than a Cl^- secretory function in the airways. In renal collecting ducts bicarbonate secretion takes place due to synergistic tasks of CFTR and the Cl^-/HCO_3^- transporter SLC26A4 (pendrin), which is likely to be supported by ANO1. Finally, in autosomal dominant polycystic kidney disease (ADPKD), the secretory function of CFTR in renal cyst formation might have been overestimated, while ANO1 as well as ANO6 turned out to be crucial in ADPKD, and therefore represent novel pharmacological targets for the treatment of polycystic kidney disease.

Keywords: TMEM16A; TMEM16F; anoctamin; SLC26A9; CFTR; pendrin

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1. Introduction

A vast number of reports describe functional changes induced by either knockout or knockdown or by overexpression of the cystic fibrosis transmembrane conductance regulator (CFTR). Although CFTR is the essential Cl^- channel for transepithelial Cl^- secretion, many of the morphological and functional changes occurring during change of CFTR-expression are thought to be caused indirectly by up- or downregulation of intracellular signaling pathways or by metabolic changes, which affect the function of other, independent proteins. Along this line, physical protein-protein interactions are reported, resulting in functional coupling to partner proteins (e.g. the epithelial Na^+ channel ENaC or the Cl^-/HCO_3^- exchanger pendrin), thus controlling ion transport and other cellular and tissue functions. Meanwhile, large "interactomes" exist for CFTR, which lead to the somewhat provocative question, whether CFTR "acts with everything?" [1-6]. Indeed, CFTR is a true hub for kinases and crosstalk of cAMP and Ca^{2+} [7]. In this short review we will focus specifically on the interplay of CFTR with the Cl^- transporter SLC26A9, the Ca^{2+} activated Cl^- channel anoctamin 1 (ANO1) and the phospholipid scramblase anoctamin 6 (Ano6) in airways and kidney.

Airway secretion of bicarbonate (HCO_3^-) by CFTR and additional apical transporter

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Early studies demonstrated that disruption of cftr in mice causes organ disease typical for cystic fibrosis (CF), such as meconium ileus, distal intestinal obstructions with mucus accumulation, blockage of pancreatic ducts and lacrimal gland dilatation, along with some developmental defects [8]. These initial studies were confirmed in a number of subsequent transgenic models for cystic fibrosis. However, a central aspect of CF pathology, namely the chronic inflammatory airway disease was hardly detectable in CF mice [9]. However, in a number of studies with a CF pig model, human CF pathology could be nicely reproduced [10,11]. In contrast to transgenic F508del-cftr mice, CF pigs demonstrated reduced airway surface pH, impaired bacterial killing and adhesive mucus that disrupts mucociliary transport [12-15]. It was concluded that dysfunctional CFTR leads to a lack of HCO_3^- secretion, thus causing acidification of the airway surface liquid (ASL), followed by mucus abnormalities, attenuation of airway defense, inflammation and a typical CF lung phenotype [14-17]. However, in another porcine CFTR-knockout model acidic ASL pH could not be detected [18,19]. In this study, micro pH-electrodes measurements were used to assess ASL-pH directly in small airways of lung sections from acutely sacrificed newborn piglets [18]. Pathological changes in these CFTR-/- lungs were not detected. Along this line, another study reported mucus accumulation preceding pulmonary infection in children with CF [20]. Moreover, using a novel luminescent technology integrated with fiberoptic probes, an acidic airway surface liquid pH could not be detected in children with cystic fibrosis [21]. This raises the question whether HCO_3^- may also use a secretory pathway that is different to CFTR.

SLC26A9 is expressed in the apical membrane of airways from CFTR-knockout piglets, but not in airways expressing CFTR-F508del

Recently, expression of the Cl^-/SLC26A9 was found to be essentially absent in human F508del-CFTR/F508del-CFTR airways [22], which corresponds to the well-known inhibitory effect of F508del-CFTR on membrane expression of SLC26A9 [23,24]. In contrast, SLC26A9 is well expressed in the apical membrane of airway epithelial cells in non-CF lungs and in lungs from CFTR-knockout piglets [22]. Membrane expression of SLC26A9 in the absence of CFTR was also shown in cell culture, whereas coexpression with F508del-CFTR in cultured cells abrogates biosynthesis, trafficking and function of SLC26A9 [23-25]. We therefore speculate that normal ASL-pH measured in airways of CFTR-/- piglets is due to normal location and function of SLC26A9, which suggests that HCO_3^- can be secreted by SLC26A9 to the luminal side of airways.

Transport of HCO_3^- by SLC26A9 has been proposed in some studies [26-28], but was not found by other laboratories [29-32]. Additional tissue factors like epithelial polarization or coexpression with additional proteins like CFTR may affect SLC26A9 transport function. Along this line, the vast majority of SLC26A9 expressed in non-polarized cells remains in the cytosol, while it is nicely expressed in the apical membrane of polarized cells [33]. Coexpression with wtCFTR or complete absence of CFTR (CFTR knockout piglets) allows proper plasma membrane location of SLC26A9, in contrast to airways of CF-patients expressing a F508del-CFTR allele [22]. Like other SLC26A proteins (SLC26A3,4,6,8), also SLC26A9 may interact physically with CFTR via R (regulatory) and STAS (Sulphate Transporter and AntiSigma factor antagonist) domains, and probably through PDZ-domain interaction [29,34-38]. A recent study showed a contribution of SLC26A9 to airway bicarbonate secretion using the novel SLC26A9 inhibitor S9-A13. Online recordings of ASL pH in primary human nasal epithelial cells under thin film conditions indicated a sustained decrease in ASL-pH by S9-A13, while subsequent activation of CFTR was unable to re-alkalinize ASL-pH [33]. These initial results should now be confirmed by additional studies *in vivo* to confirm SLC26A9-dependent bicarbonate transport in airways. It will also be interesting to learn to what extend SLC26A9 contributes to airway HCO_3^- secretion, when compared to SLC26A4 (pendrin), which probably secretes most HCO_3^-, particularly during inflammation [39]. Along this line, the main task of SLC26A9 in airways and particularly in alveoli could be actually reabsorption of Cl^- rather than Cl^- secretion (reviewed in [40].

CFTR causes constitutive basal Cl^- secretion in the airways

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Airways demonstrate a basal Cl^- secretion in the absence of secretagogues and cAMP or Ca^{2+}-dependent stimulation. It should be noted that during patch clamp recordings a spontaneous basal CFTR activity was not observed in the absence of PKA- or PKC-dependent stimulation. Previous studies provided conflicting data as to the origin of basal Cl^- secretion. While some studies suggested a spontaneous activity of CFTR causing basal Cl^- secretion [18,41-43], others suggested SLC26A9 as the responsible transporter [23,25,29,44,45]. Previously separation of both conductances (CFTR and SLC26A) was difficult due to the lack of specific inhibitors for SLC26A9, off-target effects of CFTR [46] and because intracellular trafficking and activity of SLC26A9 depends on expression and function of CFTR [23,24,47-49].

In the study by Jo et al the SLC26A9-inhibitor S9-A13 exerted no inhibitory effect on airway Cl^- transport *in vitro* or *ex vivo*, while $CFTR_{inh}172$ inhibited both basal and cAMP-induced Cl^- secretion [33]. Interestingly, neither inhibition of adenosine receptors nor inhibition of adenylate cyclase blocked basal Cl^- secretion, asking for additional mechanisms. It is possible that increase in protein kinase C (PKC) activity, e.g. through ATP-release and binding to purinergic receptors, may keep some CFTR active at basal cAMP levels and PKA-phosphorylation [50-53]. Moreover, the Hanrahan lab recently demonstrated expression of SLC26A4 (pendrin) in ciliated primary nasal and bronchial airway epithelial cells, where it enhances Cl^- secretion by stimulated CFTR [39]. The molecular mechanism of STAS/R- domain interaction had been shown earlier for activation of CFTR by SLC26A6 [35]. As discussed in the next section, the Ca^{2+} activated Cl^- channel ANO1 may contribute maintenance of a basal CFTR activity by a Ca^{2+} dependent mechanism.

Relationship between CFTR and anoctamins

Early studies showed cAMP/PKA and increase in intracellular Ca^{2+} as two independent second messenger pathways that lead to epithelial Cl^- secretion [54]. The pharmacological tools to discriminate both Cl^- conductances, however, were rather non-specific and thus our team could not clearly keep these conductances apart [55-57]. We and others also reported that CFTR seemingly “inhibits” endogenous Ca^{2+} activated Cl^- currents (CaCC) in *Xenopus* oocytes, bovine pulmonary artery endothelium and isolated parotid acinar cells [58-62]. After molecular identification of the Ca^{2+} activated Cl^- channel (CACC) as anoctamin 1 (ANO1), it was found that CFTR does not inhibit ANO1 but that ANO1 currents and CFTR currents are not additive, i.e. do not add up to the sum of both currents [63].

In fact, Ca^{2+}- enhancing agonists such as purinergic or muscarinic ligands activate mostly CFTR-dependent secretion in airways [50,64,65], while ANO1 currents rapidly inactive due to mechanisms outlined in previous reports [66-69]. Therefore, after inhibition or in the absence of CFTR CACC is very short-lived and in the intestine there is not even an apical CACC (ANO1) [70-73]. Inactivation of ANO1 is reasonably well understood [66] [74] and therefore raises the question of whether direct pharmacological activation of ANO1 by ETX001 (ETD-002) can be successful in restoring Cl secretion in the airways of CF patients [75]. It should also mentioned that ANO1 is expressed at only very low levels in airways[76].

Activation of ANO1 in CF may be even counterproductive as ANO1 is a proinflammatory factor, enhances mucus production and mucus secretion, and supports pain sensation [77-81]. Moreover, during inflammatory airway diseases such as asthma and CF, ANO1 is upregulated in pulmonary arterial vessels where it supports airway constriction [76,82]. Finally, ANO1 supports the release of inflammatory cytokines such as IL-8 and accumulation of pulmonary CD-45 positive cells [77]. A phase 1 clinical trial with ETD-002 has been finished more than a year ago, but so far, no outcome has been reported.

Crosstalk between CFTR and ANO1

Studies reported attenuated expression of ANO1 in the apical membrane of airway epithelial cells, when coexpressed with F508del-CFTR [56,83]. We found evidence for an interaction of ANO1 and CFTR through PSD-95/Dlg/ZO-1 (PDZ) domain proteins, similar as described for SLC26A9 [23]. The functional interaction between ANO1 and CFTR is based on the crosstalk of intracellular Ca^{2+}

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and the intracellular cAMP signaling pathway. Crosstalk is facilitated by exchange protein directly activated by cAMP (EPAC1) and Ca^{2+} -sensitive adenylate cyclase type 1 (ADCY1). Assembly of such a local signalosome also depends on the presence of G-protein coupled receptors (GPCRs) [57,84].

Reduced plasma membrane expression of CFTR in the absence of ANO1

Cell-specific knockout of ANO1 in ciliated airway epithelial cells abolished Ca^{2+} activated Cl^- currents and largely reduced Ca^{2+}-dependent Cl^- secretion in mouse airways. Moreover, Ca^{2+} dependent Cl^- transport was abolished in intestinal epithelial cells from epithelial-specific ANO1-knockout mice [56]. However, we also reported the surprising finding, that in parallel to the loss of ANO1-dependent transport, also CFTR-dependent Cl^- transport was lost in these ANO1-knockout animals [56] (Figure 1). In both airways and intestine, we found that expression of CFTR in the apical membrane was largely attenuated, if not abolished. It should be noted that expression of ANO1 in mouse airways is very low, while clear expression of ANO1 is detected in colonic epithelial cells, which however, is mainly located in the basolateral membrane [73,85].

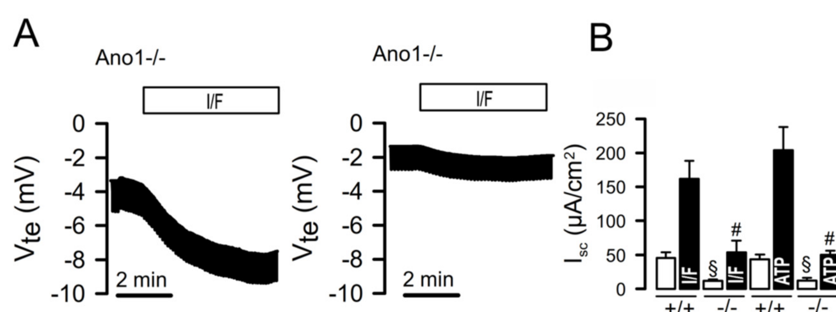

Figure 1. *Attenuated CFTR-dependent Cl^- secretion in in mice with intestinal epithelial knockout of ANO1.* **A**) Using chamber recordings obtained under open circuit conditions, as described in [56]. Stimulation of colonic epithelia with IBMX (100 µM) and forskolin (2 µM) induced a pronounced voltage deflection, which was strongly attenuated in a colonic tissue obtained from a mouse lacking expression of ANO1. **B**) Calculated equivalent short circuit currents indicate strongly attenuated CFTR-dependent (I/F-stimulated) and Ca^{2+} - dependent (ATP (100 µM) -stimulated) Cl^- secretion. Mean ± SEM (number of experiments). [§]significantly reduced when compared to basal Isc in +/+ tissues (ANOVA). [#]significantly reduced when compared to stimulated Isc in +/+ tissues (ANOVA). For methods see [56].

How are these findings be explained? From earlier studies we know that ANO1 tethers the endoplasmic reticulum (ER) near the plasma membrane (PM), via binding to the inositol trisphosphate receptor (IP_3R). Due to this, IP_3 - mediated Ca^{2+}-release from the ER and store-operated Ca^{2+} influx are strongly improved in airway sub-apical or colonic sub-basolateral membrane compartment [86-88]. Along this line it is of note that extended synaptotagmin-1 (ESYT1), another ER-PM tether was found to further enhance PM-expression of ANO1 and Ca^{2+} signaling [89]. PM-expression of CFTR requires exocytosis due to high local Ca^{2+} levels facilitated by ANO1. Exocytosis is further supported by ANO6. Using knockout mice for ANO1 and ANO6 and a number of human cell lines we and others showed that both ANO1 and ANO6 are important for PM-insertion and activation of CFTR [76,77,90-93]. In this context, Ca^{2+}-dependent activation of PKC could play a role [50,94]. Enhanced sub-membranous Ca^{2+} may further support CFTR activity by via Ca^{2+}-activated adenylate cyclases and EPAC. Both anoctamins are equally important for mucus secretion by goblet cells and release of lysozyme and other antimicrobial factors by Paneth cells [95]. We may speculate that ANO1 serves local Ca^{2+} signaling and not or at least not primarily Cl^- secretion.

Patients carrying a loss of function mutation in ANO1 lack of CFTR currents

The first two patients expressing the ANO1-variant c.897+3_897+6delAAGT were reported recently. These patients express a dysfunctional ANO1 and lack of Ca^{2+} activated Cl^- currents [96].

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The two reported siblings presented in early infancy with reduced intestinal peristalsis and recurrent episodes of hemorrhagic diarrhea. Analysis of isolated primary airway epithelial cells obtained from one of the patients reproduced the results obtained earlier in tissue specific ANO1-knockout mice [56]: Apart from the absence of Ca^{2+} activated Cl^- transport, CFTR Cl^- currents were also completely absent possibly due to a lack of expression of CFTR in the apical membrane. Moreover, analysis of cells obtained from a heterozygous sibling showed reduced Cl^- secretion [96]. Rather surprising, the patients did not show a CF-like lung phenotype, although sweat tests were positive, indicating defective CFTR Cl^- conductance. This is even more surprising given the fact that both Ca^{2+} - activated ANO1 and cAMP-activated CFTR Cl^- conductances were absent. Cytokine levels measured in sputum samples obtained from one of the ANO1-patients were largely reduced when compared to cytokine levels measured in samples from two CF patients (Figure 2). While this may provide further evidence for the pro-inflammatory role of ANO1 [81], it also raises questions regarding the true contribution of apical Cl^- conductance for CF pathology [78].

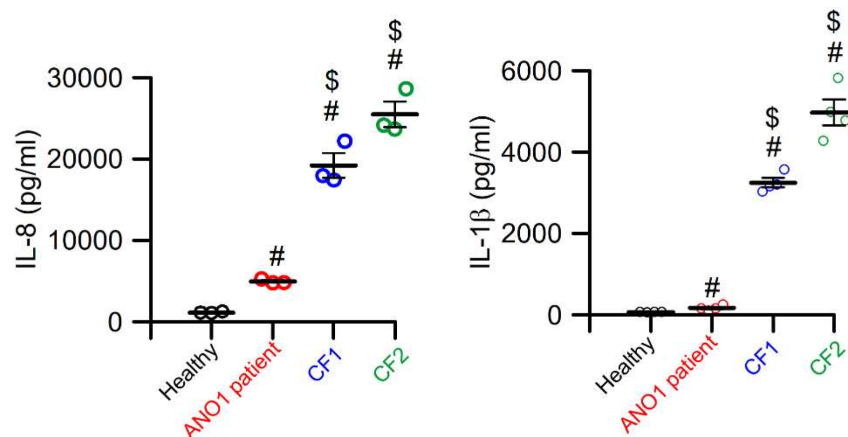

Figure 2. Cytokines in a sputum sample from a patient carrying the ANO1 los-of-function variant c.897+3_897+6delAAGT are strongly reduced when compared to samples from CF patients. Sputum samples were obtained from a healthy volunteer, the patient carrying the ANO1 variant c.897+3_897+6delAAGT, and from two CF patients and the concentration of the cytokines IL-8 and IL-1ß were determined. Although the ANO1-patient lacks of CFTR function in addition to the defect in ANO1 Ca^{2+} -dependent Cl^- secretion, cytokines were strongly reduced when compared to CF patients carrying known CFTR mutations. Mean ± SEM (number of measurements). #significantly enhanced when compared to the healthy volunteer (ANOVA). $significantly enhanced when compared to the ANO1 patients (ANOVA). For methods see [97].

CFTR and ANO1/ANO6 in cell death

Within the pathogenic relationships of CFTR with other proteins, the phospholipid scramblase ANO6 was found to have a role in CFTR-dependent cell death [98]. CFTR had been proposed to release glutathione (GSH) from airway epithelial cells to be enriched in the apical airway surface liquid, which will neutralize reactive oxygen species (ROS) [99-101]. Apparently GSH-efflux does not change cytosolic GSH content [102] and we were unable to detect different ROS levels depending on expression of CFTR [103]. However, we observed an enhanced activity of ANO6 in the presence of wtCFTR. As in most other cell types, ANO6 is also expressed in airway cells epithelial cells where it can scramble plasma membrane phospholipids, which leads to cell death [104]. The importance of ANO6 for regulated cell death is also demonstrated in ANO6 knockout mice. In these animals the number of apoptotic cells within the intestinal epithelium was strongly reduced [103].

In vivo inoculation with *P. aeruginosa* or *Staphylococcus aureus* induced lipid peroxidation in lungs of CFTR-knockout mice and wild type animals. Exposure of human airway epithelial cells to *P. aeruginosa* induced an increase in reactive oxygen species (ROS) and caused lipid peroxidation and cell death. *P. aeruginosa* induced cell death, was independent of expression of wt-CFTR or F508del-

CFTR [105]. In contrast, knockout of ANO1 clearly reduced cell death, probably because ANO1 supports Ca^{2+} - dependent activation of ANO6 and phospholipid scrambling [106].

Bicarbonate is secreted in renal collecting ducts, which requires CFTR, Pendrin and possibly ANO1

CFTR is expressed in tubular epithelial cells of human kidneys, where it affects different transport functions [107]. Early studies suggested a role of CFTR for renal bicarbonate (HCO_3^-) transport [108,109], which was later confirmed for many other epithelial organs [17]. HCO_3^- excretion was found to be largely reduced in people with CF, particularly when patients were challenged with the hormone secretin, which binds to its receptor and increases intracellular cAMP. A defect in renal bicarbonate excretion can lead to metabolic alkalosis occasionally observed in CF patients. Detailed studies in mice lacking expression of CFTR or the HCO_3^- transporter SLC26A4 (pendrin) finally uncovered the molecular mechanism [110,111]. Physical interaction of CFTR with pendrin and/or Cl^- recycling via CFTR drives tubular release of HCO_3^- through apical pendrin and urinary excretion. This process takes place in ß-intercalated cells of the renal collecting duct, which coexpress all CFTR, pendrin and receptors for secretin [110]. ANO1 is colocalized together with pendrin (and CFTR) in the apical membrane of renal ß-intercalated cells and may support the activity of CFTR [110] (Figure 3).

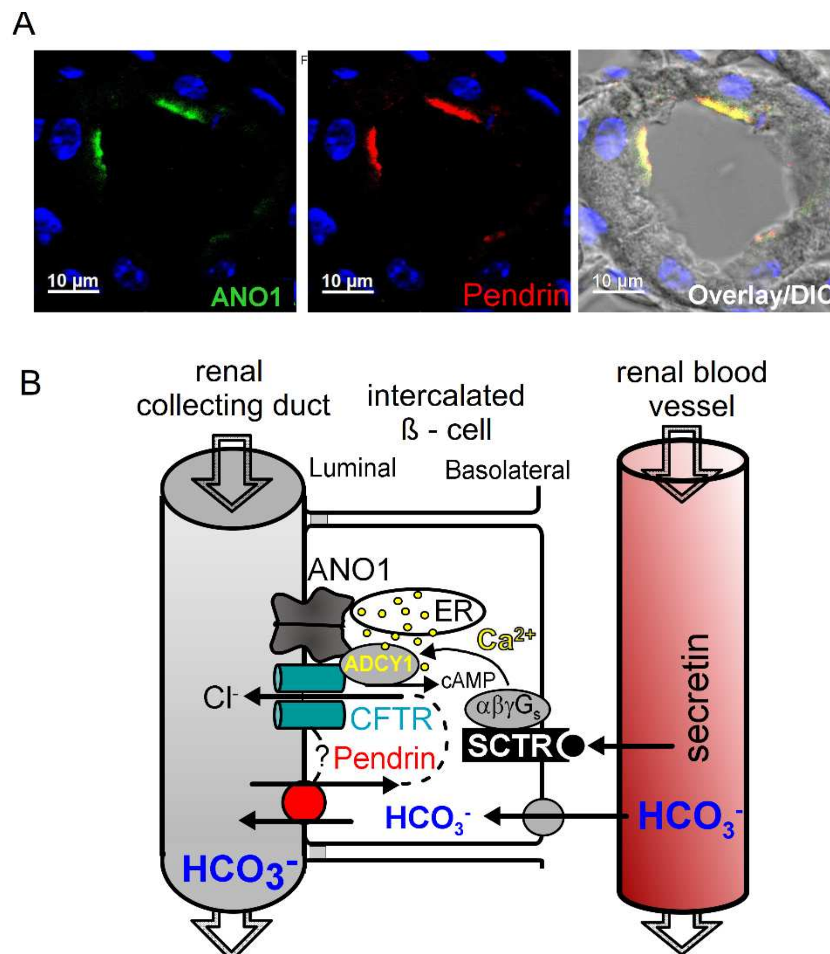

Figure 3. *ANO1 is colocalized with pendrin in the apical membrane of ß-intercalated cells.* **A)** Immunocytochemistry demonstrating colocalization of ANO1 and pendrin in the apical membrane of ß-intercalated cells of mouse collecting duct. For methods see [110]. **B)** Model showing the molecular mechanisms for HCO_3^- excretion by collecting duct ß-intercalated cells. Blood HCO_3^- is taken up into ß-intercalated cells and is transported by pendrin into the collecting duct lumen in exchange with Cl^- which recycles via colocalized CFTR. In addition CFTR may directly interact with

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CFTR (?). Colocalized ANO1 tethers the endoplasmic reticulum (ER) to the apical membrane and facilitates efficient Ca^{2+} signaling in the apical compartment which supports insertion of CFTR into the apical membrane and its activation. Increase in blood secretin leads to activation of basolateral secretin receptors (SCTR), which further activates CFTR and HCO_3^- excretion. For methods see [110].

CFTR and ANO1 in polycystic kidney disease: which one counts?

Autosomal Dominant Polycystic Kidney Disease (ADPKD) is the most common monogenic kidney disease affecting approximately 1:1000 individuals often resulting in end-stage renal disease [112]**.** Mutations in either the PKD1 (~78%) or PKD2 (~15%) gene [113] cause formation of multiple renal cysts which originate from renal tubule epithelial cells, predominantly principal cells of the collecting duct [114,115]. The cysts grow continuously over years and cause compression of the adjacent intact nephrons, which results in a decline of renal function [116]. Two key features are identified for cyst growth: change from an absorptive towards a secretory epithelium and abnormal proliferation of cyst epithelial cells [117]. It is assumed that the major secretory force for cyst fluid secretion is apical cAMP-dependent Cl^- secretion and several studies suggested CFTR as the essential Cl^- channel [118-120].

However, recently Cabrita et al. demonstrated that cyst growth in ADPKD is prevented by pharmacological and genetic inhibition of the calcium activated chloride channel ANO1 [121]. Loss of PKD1 increased expression of ANO1 and CFTR and induced Cl^- secretion in murine kidneys. Importantly, upregulated ANO1 enhanced intracellular Ca^{2+} signaling and proliferation of PKD1-deficient renal epithelial cells. In contrast, increase in Ca^{2+} signaling, cell proliferation and CFTR expression was not observed in PKD1/ANO1 double knockout mice. In a sophisticated renal collecting duct M1 cell organoid model and in primary renal epithelial cells, cell proliferation and Cl^- secretion was also dependent on enhanced expression of ANO1 [122,123]. Knockdown of PKD1 or PKD2 increased basal intracellular Ca^{2+} levels and enhanced purinergic Ca^{2+} release from endoplasmic reticulum. Ca^{2+} signals, proliferation, and Cl^- secretion were largely reduced by knockdown or blockade of ANO1. ANO1 is therefore central to enhanced Ca^{2+} release from IP_3-sensitive ER Ca^{2+} stores, and is a central player in ADPKD caused by mutations in PKD1 and PKD2. The data strongly suggest pharmacological inhibition of ANO1 to slow down progression of ADPKD.

Concerning disease progression, male gender is a major risk factor [124,125]. Talbi et al. found that kidneys from PKD1 knockout mice had a more pronounced phenotype in males compared to females. Proliferation of cells from the cyst epithelium was enhanced in male when compared to female kidneys. This was paralleled by higher basal intracellular Ca^{2+} concentrations in cells isolated from PKD1 knockout males. These results again suggest enhanced intracellular Ca^{2+} levels contributing to enhanced proliferation and cyst development in male kidneys. Notably, incubation of renal cells with dihydrotestosterone enhanced basal Ca^{2+} levels and ATP-stimulated ANO1 currents [126]. Similar results were obtained in a mouse model for autosomal recessive polycystic kidney disease (ARPKD) [127]. Finally, polycystic kidneys are under constant oxidative stress, which causes lipid peroxidation **[128]. Lipid peroxidation has been shown to activate** ANO1 and to drives cyst growth [129].

Targeting ANO1 or CFTR in ADPKD?

Inhibitors of Cl^- currents such as duiphenylamine-2-carboxylate and knockdown of CFTR by antisense oligo-nucleotides inhibited cAMP-activated Cl^- currents in cyst cells [130]; 8807590). CFTRinh-172 or Ph-GlyH-101 reduced cyst growth of renal MDCK cells, in a metanephric mouse kidney model and a rapidly progressive neonatal Pkd1 knockout mouse model [119,120]. In three CF patients with concomitant ADPKD, diseases progress was delayed when compared to their siblings without CF [131,132]. However, the ADPKD-protective effect by CF was not confirmed in a subsequent report [133] and CFTR expression in isolated ADPKD cyst cells was shown to be very heterogeneous [118,130,134]. It is therefore not entirely conclusive to inhibit CFTR to slow down cyst progression. Moreover, both CFTRinh-172 or Ph-GlyH-101 have pronounced off-target effects and affect intracellular Ca^{2+} signals which actually inhibits ANO1 [46].

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Initial studies showed that Ca^{2+}-activated ANO1 Cl^- currents contribute to cyst growth [135,136]. ATP is released by cyst cells, accumulates in the cyst lumen and activates ANO1 via stimulation of purinergic receptors [115,135,137]. By contrast, scavenging of ATP by apyrase, the P2Y2 receptor antagonist suramin, and knockdown of P2Y2 inhibited cyst growth [115,138]. These studies and the subsequent work outlined above [121,122] suggested ANO1 as the relevant pharmacological target to inhibit in ADPKD.

Taken together, in mouse studies ANO1 is a dominant driver of secretion-dependent cyst enlargement, while we also found that knockout of CFTR had no significant impact on cyst growth [139] (Figure 4). Nevertheless, it is important to keep in mind that the physiological contribution of ANO1 in the mouse is probably greater while the contribution of CFTR is lower than in humans. In mouse, CFTR shows lower activity in the airways but has a more pronounced contribution to intestinal transport [140]. In the kidneys of healthy mice, CFTR is only clearly expressed in ß-intercalated cells, where it controls HCO_3^- secretion [110]. Interestingly, a recent report shows that application of VX-809 (Lumacaftor) in a Pkd1 knockout and the $Pkd1^{RC/RC}$ mouse model reduced cyst growth [141,142]. These findings were explained by a cellular translocalization of CFTR and the Na^+/H^+ exchanger 3. A clinical phase 2, placebo-controlled, randomized trial investigates the efficacy and safety of the CFTR corrector GLPG2737 in ADPKD patients (NCT04578548) [143]. More studies are required to analyse the contribution of ANO1 to cyst formation in human tissue. Central aspects of ANO1 are its obvious pro-proliferative and de-differentiating properties [98], which after all may have a larger impact on cyst progression than fluid secretion.

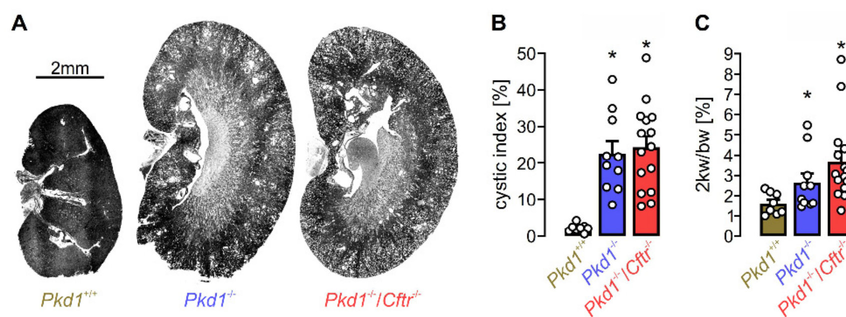

Figure 4. *Knockout of Cftr does not affect cyst growth in an ADPKD mouse model.* KspCreER^{T2}; $Pkd1^{lox;lox}$ mice ($Pkd1^{-/-}$; n=10) and KspCreER^{T2}; $Pkd1^{lox;lox}$/$Cftr^{lox;lox}$ mice ($Pkd1^{-/-}$/$Cftr^{-/-}$; n=15) received daily intraperitoneal injection of tamoxifen (2 mg/kg body weight dissolved in 5% ethanol and 95% neutral oil at postnatal days 20-22) to induce tubule-specific deletion of Pkd1 or co-deletion of Pkd1 and Cftr. Non-induced KspCreER^{T2}; $Pkd1^{lox;lox}$ mice ($Pkd1^{+/+}$; n=8) served as controls. Analyses were performed 10 weeks after induction with tamoxifen. **A**) Representative kidney sections at the end of the experiment. For methods see [121]. **B**) Analysis of the cystic indices defined as the ratio of cortical cystic area divided by the whole cortex area. **C**) Two-kidney weight per body weight ratio. No significant effect of CFTR-knockout was found [139]. Bars show means ± SEM, dots indicate individual values. *significant increase compared to $Pkd1^{+/+}$ ($p<0.05$; one-way ANOVA, Posthoc test: Tukey); n.s. means no statistical difference (one-way ANOVA, Posthoc test: Tukey and unpaired t-test). For methods see [139].

Inhibitors of ANO1

Given the promising results obtained by genetic and pharmacological inhibition of ANO1, ANO1 has qualified as a potential target for the treatment of ADPKD. ANO1 function can be addressed by drugs that have already been approved for other indications like niclosamide or benzbromarone [121]. Niclosamide is an essential oral anthelminthic drug used for decades to treat parasitic infections, but it is meant for short-term use [144]. Benzbromarone is a uricosuric drug that has been used in the treatment of gout over the last 30 years. Although withdrawn by Sanofi for safety reasons after reports of hepatotoxicity, it is still marketed in several countries by other drug companies (drugs.com/international/benzbromarone.html). Hepatotoxicity is rare and occurs in

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1:17000 patients [145]. For comparison, the only drug approved for treatment of ADPKD, the vasopressin-2-receptor antagonist tolvaptan, has a hepatotoxic risk of 1:3000. Therefore, many experts in the field have questioned withdrawal of benzbromarone [145].

Conclusion

The present review summarizes recent findings for CFTR, SLC26A9 and the anoctamins 1 and 6 in airways and kidney. It becomes clear that these ion channels and transports cannot only be examined individually but should be analyzed in the context of their molecular and functional interaction. This is particularly important in pharmacotherapy and the choice of the choice of the right pharmacological target.

Authors contributions: Conceptualization, K.K., J.O., A.K., R.S., B.B.; methodology., J.O., A.K., J.H.P., T.M., R.S., B.B.; formal analysis, J.O., A.K., B.B.; writing—original draft preparation, K.K., R.S., B.B.; writing—review and editing, ., J.O., A.K., J.H.P., T.M., R.S., B.B. All authors have read and agreed to the published version of the manuscript.

Funding: Supported by DFG Transregio-SFB, Project-ID 509149993, TRR 374 (Project A3).

Institutional Review Board Statement: All animal experiments complied with the general guidelines for animal research, in accordance with the United Kingdom Animals Act, 1986, and associated guidelines, and EU Directive 2010/ 63/EU for animal experiments. All animal experiments were approved by the local Ethics Committee of the Government of Unterfranken/Wurzburg/Germany (AZ: 55.2-2532-2-677) and were conducted according to the guidelines of the American Physiologic Society and German Law for the Welfare of Animals.

Data Availability Statement: MDPI Research Data Policies at https://www.mdpi.com/ethics.

Conflicts of Interest: The authors declare no conflict of interest.

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