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Review

Fluid transport and cystogenesis in autosomal dominant polycystic kidney disease ☆

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ABSTRACT

Autosomal dominant polycystic kidney disease (ADPKD) is the most frequent inherited nephropathy. The development and enlargement of cysts in ADPKD requires tubular cell proliferation, abnormalities in the extracellular matrix and transepithelial fluid secretion. Multiple studies have suggested that fluid secretion across ADPKD cyst-lining cells is driven by the transepithelial secretion of chloride, mediated by the apical CFTR channel and specific basolateral transporters. The whole secretory process is stimulated by increased levels of cAMP in the cells, probably reflecting modifications in the intracellular calcium homeostasis and abnormal stimulation of the vasopressin V2 receptor. This review will focus on the pathophysiology of fluid secretion in ADPKD cysts, starting with classic, morphological and physiological studies that were followed by investigations of the molecular mechanisms involved and therapeutic trials targeting these pathways in cellular and animal models and ADPKD patients. This article is part of a Special Issue entitled: Polycystic Kidney Disease.

1. Introduction

Autosomal dominant polycystic kidney disease (ADPKD) is the most frequent inherited nephropathy, with a prevalence ranging from 1:400 to 1:1000. Mutations in two genes, *PKD1* and *PKD2*, have been associated with ADPKD. Mutations in *PKD1* account for ~85% of the affected families, and they cause a renal disease that progresses more rapidly than in *PKD2* families. *PKD1* and *PKD2* encode integral membrane proteins, polycystin-1 (PC-1) and polycystin-2 (PC-2) respectively [1].

The polycystins 1 and 2 constitute a subfamily of transient receptor potential channels, namely TRPP [2]. Polycystin-1 has 11 transmembrane domains, a short cytoplasmic tail and a large extracellular region and probably functions as a receptor and/or and adhesion molecule. The C-terminal portion interacts through a coil-coiled domain with PC-2, G-proteins and cytoskeletal proteins. Polycystin-1 localizes to the primary cilium that projects into the lumen, and to structures involved in cell-cell contacts such as tight junctions, adherens junctions, desmosomes and focal adhesions [3, for review]. PC-1 forms a complex with E-cadherin and α-, β- and γ-catenins, which could regulate the mechanical strength of the adhesion and act as a matrix sensor [4–6]. Polycystin-2 is a non-selective cation channel capable of transporting calcium ions, predominantly localized to the endoplasmic reticulum (ER) but also to the plasma membrane, primary cilium and mitotic spindles in dividing cells [7]. Interactions with PC-1 and adaptor proteins control the trafficking and localization of PC-2 to the plasma membrane [8].

The best-characterized function of polycystins is the regulation of calcium homeostasis and signalling. The polycystin complex is thought to act as a mechanosensory calcium channel in the primary cilium, a protrusion on the apical surface of kidney epithelial cells involved in flow-sensing and other critical processes such as signalling, polarity, cell proliferation and differentiation [9]. Mechanical or flow-induced bending of the primary cilium leads to a Ca^{2+}-influx through PC-2 which in turn activates a calcium-induced Ca^{2+}-release via ryanodine receptors (RyR) in the ER, overall increasing intracellular Ca^{2+}-concentration ($[Ca^{2+}]_i$) [10]. Intracellular Ca^{2+} homeostasis and signalling is important for regulation of a variety of cellular functions, including cell volume regulation, ion and fluid transport, differentiation and cell proliferation [3,11]. Functional loss of either PC-1 or PC-2 disrupts intracellular Ca^{2+} regulation by decreased Ca^{2+}-influx, reduction of ER stores and store-operated Ca^{2+} entry, leading to a decrease in $[Ca^{2+}]_i$ and an abnormal cell proliferation phenotype [3].

In addition to epithelial cells, both PC-1 and PC-2 are expressed in vascular smooth muscle cells and endothelial cells of most blood vessels, which is relevant when knowing that ADPKD patients often present

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with hypertension and endothelial dysfunction and that some of them develop various types of vascular lesions including intracranial aneurysms [1]. Recent studies [reviewed in Ref. 12] suggest that flow stimulation activates the polycystin complex in the primary cilium of endothelial cells, leading to increased intracellular Ca^{2+} concentration, release of nitric oxide and vasodilation. Furthermore, the group of Honoré recently demonstrated that the polycystins (and more exactly, the PC-1/PC-2 ratio) regulate the opening of non-selective stretch-activated ion channels (SACs) that mediate the myogenic response of blood vessels, i.e. the pressure sensing by arterial myocytes [13]. The potential link between a modified SAC activity resulting from mutations in *PKD1* or *PKD2* and the propensity for arterial lesions in patients with ADPKD remains to be demonstrated.

The ADPKD cysts derive from 1% to 3% of the nephrons. They potentially involve all nephron segments, although an important fraction of the cysts is derived from the collecting ducts [14,15]. Although the cysts already exist *in utero*, they usually become clinically detectable only in the young adult [16]. The prospective follow-up of ADPKD patients with yearly magnetic resonance imaging examinations has established that cysts increase at a stable rate of ~5% per year [17]. Total kidney volume and cyst volume progression are the strongest predictors of renal function decline in ADPKD [18], with slow progression to end-stage renal failure in about 50% of patients. ADPKD is responsible for 4% to 10% of the patients requiring a renal replacement therapy.

A large body of evidence suggests that cyst development and enlargement in ADPKD requires tubular cell proliferation, abnormalities in the extracellular matrix and a net, transepithelial fluid secretion (i.e. directed towards the cyst lumen). Since the cysts are anatomically separated from the tubule from which they derive [19], the intracystic fluid does not originate from the glomerular filtrate but rather from a transepithelial fluid secretion. The existence of such a fluid secretion is in itself a major anomaly as the normal function of the different tubular segments is to reabsorb more than 99% of the glomerular filtrate. In this article, we will review the data substantiating the pathophysiology of fluid secretion in ADPKD cysts, starting with macroscopic studies (cyst fluid composition; electrical properties of cystic epithelia) that were followed by the molecular characterization of the transport mechanisms involved and therapeutic trials targeting these pathways in animal models and ADPKD patients.

2. Cyst fluid composition

The pioneering work of P.P. Lambert in the 1940s has established that cysts in ADPKD arise as saccular expansions of a tubule from which they later separate—to become totally independent as they expand in volume [20]. Initially, intracystic tubular fluid can thus be considered as a plasma ultrafiltrate. However, as the cysts lose their connection with their parent tubular segment, intracystic fluid accumulation necessarily reflects water and solutes secretion by the cystic epithelium itself. As cysts may arise from any tubular segment of the nephron, cyst fluid composition should somehow reflect its tubular origin. Gardner classified ADPKD cysts into two categories on the basis of a Na^+ concentration above or below 100 mmol/l [21]. As shown in Table 1, both types of cysts also differ in the length of their junctional complexes [22]. Cysts with a low Na^+ concentration ("gradient cysts") are capable of maintaining a transepithelial ionic gradient, suggesting that they derive from the tight, distal nephron segments. Conversely, cysts exhibiting a high Na^+ concentration nearly equal to plasma ("no gradient cysts") most probably derive from the leaky, proximal tubule [23]. Although osmolality was found to be similar in both types of cysts, the gradient cysts were characterized by a significant osmolal gap which could correspond to unmeasured organic anions (i.e. organic phosphates) or to osmoles normally made by the cells of the ascending limb of Henle (i.e. sorbitol, betaine) [24]. The presence of proteins within the cystic fluid could result from epithelial cells desquamation associated with ischemia, partial necrosis or proliferation [24].

3. Electrical properties of cystic epithelia

Epithelia can be classified on the basis of the permeability of their junctional complexes, a parameter that determines the transepithelial ohmic resistance. Only epithelia possessing true "tight" junctions are able to maintain a sizeable transepithelial potential difference and ionic gradient. Perrone [25] was the first to measure the electrical parameters of ADPKD cystic epithelia mounted in Ussing chambers (Table 1). "No gradient" cysts showed a low transepithelial resistance and an almost null transepithelial potential difference. By contrast, "gradient" cysts exhibit a transepithelial resistance similar to tight epithelia and a sizeable transepithelial potential difference that could be short-circuited. The short-circuit current (15–20 μA/cm^2) and the

Table 1
Fluid composition, ultrastructural features and electrical parameters in ADPKD cysts.

	Type of cyst		Plasma
	No-gradient	Gradient	(Ref. values)
Composition			
Na^+ (mmol/L)	138 (129–150)	4.8 (3–75)	135–145
K^+ (mmol/L)	5.1 (4.6–8.6)	25.3 (5.1–58)	3.5–5.0
Ammonia (μmol/L)	0	9.4 (3.2–15.5)	10–35
Total Ca^{2+} (mmol/L)	2.0 (1.4–3.7)	1.0 (0.25–1.95)	2.1–2.5
Mg^{2+} (mmol/L)	1.5 (1.2–2.6)	7.4 (2.7–12.2)	0.6–0.8
Cl^- (mmol/L)	96 (88–104)	18.3 (8.7–27.9)	95–105
Glucose (mmol/L)	5.8 (2.6–8.8)	14.1 (7.3–32.9)	4.0–6.0[a]
pH	7.4	5.2	7.35–7.45[b]
Osmolality (mOsm/kg H_2O_2)	290 (264–336)	293 (193–313)	275–295
Osmolal gap	0	97	~0
Ultrastructural characteristics			
Length of junctions (μm)	<500	>500	
Electrical parameters			
R (Ω/cm^2)	31 (11–50)	208 (153–332)	
V (mV)	0.1 (−0.4 to +0.6)	5 (2.6–7.2)	
I (μA/cm^2)	7 (−6 to +11)	19 (16–22)	
I/V inhibition by amiloride	–	++	

Abbreviations: R, Transepithelial ohmic resistance; V, Transepithelial potential difference; I, short-circuit current intensity. Amiloride (10 μmol/L) is added on the apical side. Mean values (range) are shown (adapted from Refs. 21,23,25,26).
Plasma reference values are shown for comparison with the regular filtrate: [a], fasting; [b], arterial blood.

potential difference (−5 mV, lumen negative) were inhibited by apical amiloride, indicating that the current reflects Na^+ reabsorption. Radioactive flux measurements confirmed a net Na^+ reabsorption by "gradient" cysts epithelia [25,26].

In 1991, Wilson et al. studied bilateral ^{22}Na fluxes through monolayers of cystic cells grown on filters [27] and observed a net secretory flux of ^{22}Na into the cyst lumen that was inhibited by ouabain (a cardiotonic steroid inhibitor of Na^+-K^+-ATPase) from the apical side. Of note, these fluxes were obtained in the presence of a spontaneous transepithelial potential difference, so that the observed ^{22}Na secretion could be driven by a primary Cl^- secretion. A primary secretion of Na^+ should yield a transepithelial potential difference, lumen positive, but this parameter was not available [27].

Most fluid secretion processes are secondary to the secretion of Cl^-, as exemplified for the fluid secretion of the cryptic small intestine epithelium and the secretory diarrhea induced by cholera toxin [28]. Evaluating the determinants of cyst fluid secretion, the group of Grantham proposed a similar sequence of events in ADPKD, based on elegant studies performed on cysts *in situ* and on cystic cells cultured as confluent monolayers and in three dimensions on collagen matrix. Electrical data, recorded in Ussing chambers, showed (i) the existence, in the basal state, of a transepithelial potential difference lumen negative, implying a net secretion of anions or a net reabsorption of cations; (ii) an increase of luminal electronegativity after the addition of forskolin (an adenylate cyclase agonist stimulating the production of cAMP) [29]. Several secretagogues have been identified in human ADPKD cyst fluid, most of which stimulate adenylyl cyclase and the cAMP cascade, including a lipophilic, forskolin-like molecule [30,31].

4. Na^+-K^+-ATPase

The Na^+-K^+-ATPase (often referred to as the "sodium pump") is an ubiquitous enzyme discovered by J.C. Skou [32], who was awarded the 1997 Nobel Prize in Chemistry. The fundamental role of the Na^+-K^+-ATPase is to generate and maintain Na^+ and K^+ gradients which in turn control cell membrane potential difference and cell volume. The Na^+-K^+-ATPase is made of the α subunit which catalyzes Na^+ and K^+ translocation, hydrolyzes ATP and binds ouabain; the β subunit, which plays a role in the enzyme maturation and membrane targeting; and a γ subunit (FXYD2) which belongs to the family of FXYD proteins that modulate the Na^+-K^+-ATPase activity in a tissue-specific way [33]. Of interest, Zatti et al. demonstrated that the C-terminal tail of PC-1 interacts with the α-subunit of the Na^+-K^+-ATPase, and that this interaction could regulate the Na^+-K^+-ATPase activity in the kidney [34].

In most epithelia, with the exception of the retinal epithelium and the choroid plexus, Na^+-K^+-ATPase is localized within the basolateral plasma membrane domain. By contrast, immunolocalization studies performed by Wilson et al. showed that Na^+-K^+-ATPase was localized exclusively in the apical plasma membrane domain of cystic epithelial cells, potentially accounting for the ^{22}Na net flux as well as the action of ouabain from the apical side of the cyst-lining cells reported by the same authors [27]. The further demonstration of the β2 subunit within these apical complexes led to propose that the mispolarization of Na^+-K^+-ATPase was related to the formation of α1β2 complexes in ADPKD cells [35]. Since the β2 subunit is normally expressed in the fetal kidney, where it has been implicated in apical targeting of Na^+-K^+-ATPase in the ureteral bud, its abnormal expression in the ADPKD cystic epithelium could reflect cellular dedifferentiation and participate in the driving force for cyst fluid accumulation [35].

The view that the apical localization of Na^+-K^+-ATPase in the cystic epithelium plays a role in cyst fluid accumulation has been strongly debated. Such a distribution cannot explain the luminal electronegativity or the lack of effect of apical ouabain reported by others [25,36]. In a study designed to minimize ischemic damage, Carone et al. observed an apical Na^+-K^+-ATPase polarization in 25% of the ADPKD cystic cells, whereas most cystic cells exhibited a normal basolateral Na^+-K^+-ATPase and a normal capacity for apical endocytosis [37]. In contrast, Brill et al. localized Na^+-K^+-ATPase within the basolateral membrane of cultured cystic cells, although there was some heterogeneity in this expression [38]. The observations of Wilson et al. might thus reflect cell dedifferentiation, perhaps related to culture conditions, or a loss of polarity caused by ATP depletion or tissue ischemia [39]. Further studies in MDCK cells stably expressing the β1 and β2 subunits demonstrated a strictly basolateral targeting under normal growth conditions, whereas inclusion of butyrate in the growth medium led to the upregulation of β1- and β2-subunits and their appearance at the apical surface [40]. On the other hand, Nguyen et al. demonstrated that nanomolar concentrations of ouabain induced proliferation of ADPKD cells at a much higher rate than normal human kidney cells, via the activation of the MEK-ERK pathway. The ADPKD cells showed a higher affinity for ouabain, which was not due to differences in the α1/β1 subunits but could involve the γ subunit which was upregulated in these cells [41].

The possibility of apical mistargeting of the Na^+-K^+-ATPase has been investigated in different models of polycystic kidney disease. An increased activity of the Na^+-K^+-ATPase, together with a transient increase in the apical expression of α1 and β1 subunits have been demonstrated in the early stages of cyst development in the *cpk* mouse, a model of autosomal recessive polycystic kidney disease [42]. In the SBM transgenic mouse model of ADPKD, in which cyst development is related to a proliferation induced by the targeted expression of the *c-myc* proto-oncogene in the renal epithelium, apical labeling for Na^+-K^+-ATPase was observed in the early stages of cyst development of the young animal, to significantly decrease in the adult stage [43]. In contrast, a detailed histopathological analysis of renal cystic epithelia failed to show a mispolarization of the α1 subunit in the *Pkd2*$^{WS25/-}$ mouse model, which recapitulates cystogenesis in human ADPKD [44]. More recently, an increased expression of the β2 subunit in cystic kidneys was reported in a homozygous *Pkd1* mutant mouse model [45], but no apical misolocalization could be detected.

5. Chloride secretion and CFTR

Although the renal tubule epithelium reabsorbs more than 99% of the glomerular filtrate, early studies performed in the isolated rabbit proximal tubule [46] have shown that the renal tubule epithelium possesses a capacity for fluid secretion. In particular, an apical conductance for Cl^-, stimulated by cAMP, has been evidenced in several epithelia [47–49] and cell lines derived from the distal nephron [50,51].

Fluid secretion at the level of ADPKD cystic epithelium was first suggested by the fact that, after drainage, a renal cyst quickly fills again [52]. The demonstration of *in vivo* fluid secretion by ADPKD cysts following injection of secretin, an adenylyl cyclase agonist, was provided by Everson et al. [53]. Fluid secretion by cyst walls was confirmed *in vitro* and *ex vivo* by numerous investigations from Grantham and colleagues [29,54]. By analogy with other epithelia, Grantham then suggested that Cl^- secretion was the driving force for fluid secretion in ADPKD cysts [29]. The key role of Cl^- was demonstrated by studying in parallel currents and fluid secretion in monolayers of ADPKD cyst cells [36,55]. These studies confirmed that fluid secretion was stimulated by adenylyl cyclase agonists, and also by the phosphodiesterase inhibitor 3-isobutyl-1-methylxanthine (IBMX) or by 8-Br-cAMP, a form of cAMP insensitive to hydrolysis by phosphodiesterase. Furthermore, the fluid secretion induced by forskolin was accompanied by an increase in luminal electronegativity. These effects were inhibited by the addition of bumetanide or the isotonic replacement of Cl^- in the basolateral medium, or by the Cl^- channel inhibitor diphenylamine-2-carboxylate (DPC) on the apical side [36,55].

The analogy between the properties of the ADPKD cystic epithelium and those of other secretory epithelia suggested that the CFTR ("cystic fibrosis transmembrane conductance regulator") channel could be the molecular counterpart of the cAMP-stimulated Cl^- secretion in ADPKD cysts. Mutations in the *CFTR* (or *ABCC7*) gene CFTR cause cystic fibrosis,

the most common lethal autosomal recessive disease in Caucasians. The CFTR protein is a member of the ATP-binding cassette (ABC) superfamily of integral membrane transporters [56]. The protein is organized symmetrically in two transmembrane domains (TMD1 and TMD2) and two nucleotide binding domains (NBD1 and NBD2), separated by a large, polar, regulatory (R) domain unique within the ABC family. CFTR is regulated by cAMP-dependent phosphorylation of the R domain via PKA, followed by ATP-dependent gating events initiated by ATP binding to the cytoplasmic nucleotide-binding domains and resulting in transepithelial Cl^- transport [56]. Multiple interactions regulate CFTR-mediated Cl^- secretion by modulating both its channel activity and its intracellular trafficking [57]. CFTR interacts functionally with other channels, including the outwardly rectifying Cl^- channels (ORCC) and the Na^+ channel ENaC, and it participates in exocytosis and the formation of macromolecular complexes at the plasma membrane [58].

Several studies have demonstrated that CFTR is significantly expressed in the mammalian kidney [59–61]. In mouse kidney, CFTR is mainly expressed in the apical area of PT cells, with a subcellular distribution compatible with endosomes [61]. In human kidney, CFTR protein expression was detected in the PT, in addition to the thin limbs of Henle's loop, distal tubules and collecting ducts [59,60]. The expression of CFTR has been evidenced in primary cultures of ADPKD cells and ADPKD kidney extracts, with a staining pattern suggesting a localization in the apical membrane of cyst-lining cells [62]. Selective Cl^- currents were recorded in cultured ADPKD cyst cells, stimulated by forskolin and a permeant analogue of cAMP, and totally blocked by the CFTR blocker DPC [62]. A significant heterogeneity in CFTR expression has been observed [38,62,63], which could suggest that some cells are too dedifferentiated to express the protein and that a subset of cyst-lining cells with functional CFTR is sufficient to drive fluid secretion. Alternatively, other types of Cl^- channels or transporters might be implicated in fluid secretion.

The role of CFTR in mediating Cl^- and fluid secretion in ADPKD was first substantiated in vitro by showing that the forskolin-stimulated fluid secretion was dramatically reduced after incubation of monolayers of ADPKD cells with an antisense oligonucleotide against human CFTR [63]. The discovery of various types of specific CFTR inhibitors by the group of Verkman provided additional evidence for the essential role of CFTR in vitro and in vivo. The thiazolidinone inhibitor $CFTR_{inh}$-172, which stabilizes the channel closed state, inhibited cyst growth in MDCK cells [49] and in metanephric kidney organ culture [64]. A further screening allowed to show that two CFTR inhibitors (tetrazolo-$CFTR_{inh}$-172 and the phenyl-derived glycine hydrazide Ph-GlyH-101) suppressed cyst growth in MDCK cells without affecting cell proliferation; inhibited cyst number and growth in a cAMP-stimulated embryonic kidney cyst model; and, remarkably, slowed kidney enlargement and cyst growth and preserved renal function in a neonatal, kidney-specific *Pkd1* knock-out model (*Pkd1*$^{flox/-}$*:Ksp-Cre* mice) [65]. These data suggest a potential therapeutic role for CFTR inhibitors to slow cyst fluid accumulation in ADPKD (Fig. 1). Since these inhibitors concentrate in the kidney and show little accumulation in the lung, and because inactivation of >90% of CFTR is necessary to affect lung function, their use should not be complicated by a cystic-fibrosis like pulmonary disease [65]. Of note, a milder cystic phenotype has been reported in three patients with ADPKD coexisting with cystic fibrosis, as compared to siblings with ADPKD alone [66,67]. This protective effect has not been confirmed in a subsequent report, which evidenced the continuous expression of apical CFTR in a large fraction of ADPKD cysts [68]. The protective role of CFTR mutations in ADPKD may be related to the nature of the mutation (>1000 documented in cystic fibrosis) and the residual expression of the mutated CFTR protein in cyst-lining epithelial cells [68].

There is increasing evidence that the epithelial cells lining various nephron segments can release ATP (and adenosine) into the lumen under the influence of mechanical or chemical stimuli, followed by autocrine and paracrine signaling via various types of purinergic receptors expressed in the apical membrane of the cells. In turn, this "purinergic signaling microenvironment" plays an important role in the regulation of ion transport in various nephron segments [69]. Several investigators documented the presence of such purinergic signaling in the encapsulated cysts of ADPKD, where it could stimulate the secretion of Cl^-, and thus cyst growth, through Ca^{2+}-activated chloride channels [70,71].

6. Basolateral chloride transport

The search for the mechanism involved in the basolateral Cl^- uptake in ADPKD cyst-lining cells was guided by the effect of the loop diuretic bumetanide, which reduces rapidly the luminal electronegativity and decreases fluid secretion when applied at the basolateral pole of ADPKD monolayers [36]. Loop diuretics specifically inhibit a family of cotransporters (SLC12A1 and SLC12A2) that transport Na^+, K^+ and Cl^- in an electroneutral way [72]. In addition to their role in cell volume regulation, these cotransporters represent a pathway for Cl^- entrance in epithelia involved in the reabsorption (ascending limb of the loop of Henle) or secretion (salivary glands, respiratory tract) of chloride. The SLC12A1 isoform (also called NKCC2) is exclusively located in the apical membrane of the thick ascending limb of Henle's loop, whereas the SLC12A2 isoform (also named NKCC1) is ubiquitous expressed in both the basolateral pole of Cl^--secreting epithelia and non-epithelial cells. Lebeau et al. investigated the expression of NKCC1 and CFTR in ADPKD kidneys and cultured ADPKD cells [73]. Immunoblotting and immunoprecipitation detected NKCC1 in ADPKD cells and kidney extracts. Immunostaining located NKCC1 in one-third of ADPKD cysts, with a pattern of basolateral reactivity. Staining of serial sections showed that cysts positive for NKCC1 also stained for CFTR. The fact that most CFTR-positive ADPKD cysts also express NKCC1 suggests that transepithelial Cl^- secretion in ADPKD involves molecular mechanisms similar to secretory epithelia. This conclusion has been supported by the demonstration of NKCC1 in the basolateral membranes of advanced cystic lesions in the *Pkd2*$^{WS25/-}$ mouse model [44]. Since the activity of NKCC1 is critical for maintaining the intracellular concentration of Cl^-, it would be interesting to evaluate the state of the WNK (with no lysine) serine-threonine kinases and their key downstream substrates SPAK (Ste20/sps1-related proline/alanine-rich kinase) and OSR1 (oxidative-stress responsive kinase 1) in the ADPKD cyst-lining epithelia [74]. Targeting NKCC1 with loop diuretics could theoretically be envisaged, but the efficacy (remaining blood supply) and safety (risk of hypokalemia) of such an approach remains very uncertain. Of note, Lebeau et al. demonstrated that the Cl^-–HCO_3^- anion exchanger type I (AE1), which normally operates in the basolateral membrane of α-type intercalated cells in the collecting ducts, is also expressed at the basolateral pole of CFTR-positive ADPKD cysts that do not express NKCC1. Accordingly, AE1 might be an alternative basolateral pathway for Cl^- in a subpopulation of cysts [44,73].

The sustained activity of NKCC1 in the basolateral membrane of cyst epithelial cells requires basolateral recycling of K^+ and Na^+, as well as the maintenance of a hyperpolarized membrane potential (Fig. 1). In a series of elegant studies, Albaqumi and colleagues recently demonstrated the role of the Ca^{2+}-activated potassium channel KCa3.1 in mediating the efflux of K^+ and maintaining a relatively negative intracellular membrane potential that drives the apical Cl^- secretion by CFTR in monolayers of kidney cells derived from patients with ADPKD [75]. The clotrimazole analogue TRAM-34, which specifically inhibits KCa3.1, inhibited forskolin-stimulated Cl^- secretion across the monolayers (without affecting CFTR or other apical Cl^- channels), and inhibited cyst formation and enlargement in collagen matrix. It must be noted that such des-imidazolyl trityl derivatives are already used in phase 3 clinical trials to block KCa3.1 in sickle cell disease without side-effects, which opens the way for testing the effect of these compounds in animal models of ADPKD [75,76]. Both cAMP and PKA may activate KCa3.1 channels, which is relevant when considering the importance of cAMP in the pathogenesis of ADPKD (see below).

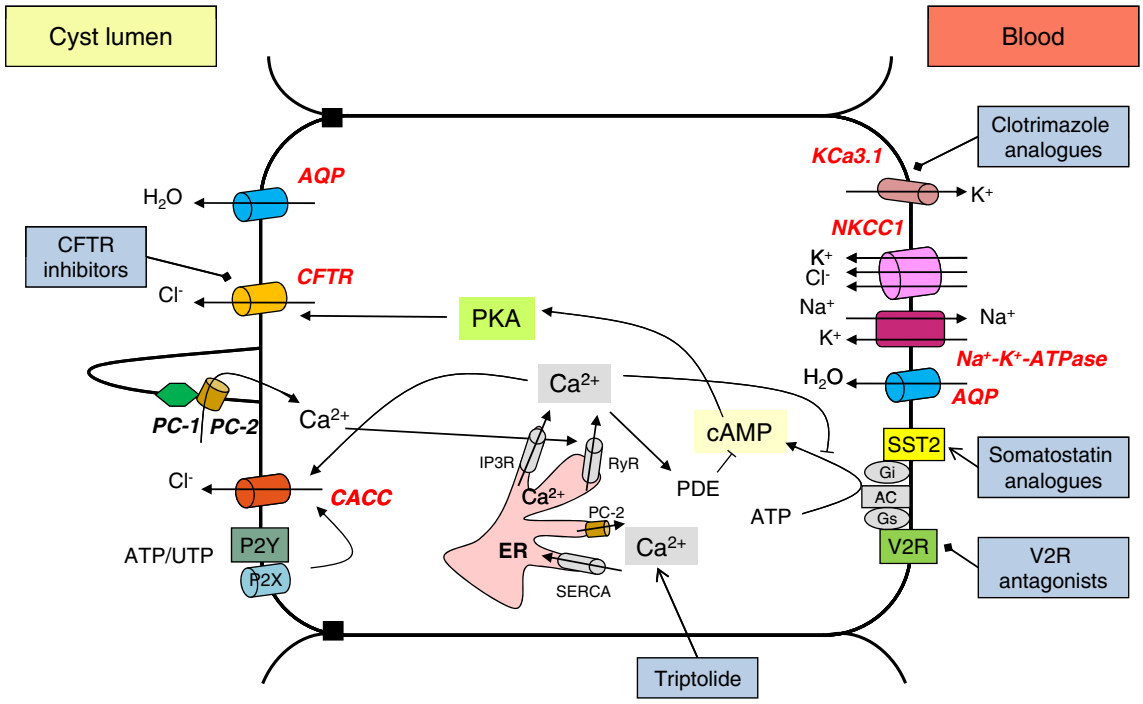

Fig. 1. Mechanisms of fluid secretion in ADPKD cyst-lining epithelial cells. Model of the transporters and channels involved in fluid accumulation in the lumen of ADPKD cysts. The apical and basolateral poles of the cell are delineated by tight junctions. The transepithelial secretion of Cl^- is mediated by the basolateral Na^+-K^+-$2Cl^-$ cotransporter NKCC1 and apical Cl^- channels including the protein kinase A (PKA)-stimulated CFTR and Ca^{2+}-activated Cl^- channels (CACC). This transepithelial pathway is stimulated by an increased concentration of cAMP, probably reflecting a reduction in intracellular calcium levels (which stimulates of Ca^{2+}-inhibitable adenylyl cyclase (AC) and/or inhibits the Ca^{2+}-dependent phosphodiesterase (PDE)) and stimulation of the vasopressin V2 receptor (V2R) pathway. The apical CACC are stimulated by an autocrine/paracrine purinergic signaling mediated by purinergic receptors (P2X and P2Y). The activity of NKCC1 requires the recycling of K^+ via the basolateral potassium channel KCa3.1 and that of Na^+ via the Na^+-K^+-ATPase. The net secretion of Cl^- induces a transepithelial movement of Na^+ (electric coupling) and water (osmotic coupling). The presence of water channels (AQPs) in some cysts may facilitate transcellular water transport. Disruption of the complex formed by polycystin-1 (PC-1) and polycystin-2 (PC-2) is probably involved in the alteration of intracellular Ca^{2+} levels. The drugs targeting these transport pathways that are currently investigated *in vivo* and/or *in vitro* are indicated in blue boxes. For details, see text. Additional abbreviations: IP3R, inositol trisphosphate receptor; RyR, ryanodine receptor; SERCA, sarco(endo)plasmic reticulum Ca^{2+}-ATPase; SST2, somatostatin receptor. (Adapted from Refs. 3,70,76,97).

7. Osmoregulation, vasopressin and cAMP in ADPKD

A defect in the urine concentration ability has long been established in ADPKD patients, including children, even before the onset of renal failure [77–80]. The severity of the urine concentrating defect has been correlated to renal and cyst volume [79,80]. In parallel, higher plasma levels of vasopressin were detected in hypertensive ADPKD patients as well as after hypertonic saline loading [81] and, in one cohort, at baseline [82]. Recently, Meijer et al. showed that plasma copeptin concentration, a surrogate marker of endogenous vasopressin levels, was associated with various markers of disease severity in a cross-sectional analysis of a cohort of 102 ADPKD patients [83]. The vasopressin V2 receptor (V2R) is the major regulator of adenylyl cyclase activity and source of cAMP production in the principal cells lining the collecting ducts. Increased levels of cAMP and cAMP-target genes have been observed in the cystic kidneys of various rodent models [3]. The increased cAMP levels may be the direct consequence of decreased intracellular Ca^{2+} levels caused by mutations in PC-1 and PC-2, via the downregulation of phosphodiesterase PDE and stimulation of the Ca^{2+}-inhibitable adenylyl cyclase 6 [3]. In turn, the increased production of cAMP stimulates the proliferation and growth of ADPKD cells and drives Cl^- and fluid secretion via PKA-stimulated CFTR and, potentially, the basolateral channel KCa3.1 (Fig. 1).

The importance of the V2R-cAMP pathway has been demonstrated by the spectacular effects of V2R antagonists on lowering renal cAMP levels and slowing renal cyst development in various models of PKD and nephronophthisis (*cpk* mouse, PCK rat, *Pkd2*$^{WS25/-}$ mouse, *pcy* mouse) [3, for review]. Nagao et al. demonstrated that vasopressin suppression by high water intake decreased cyst and renal volumes in PCK rats, with a reduced activity of cAMP-dependent B-Raf/MEK/ERK pathway [84]. Furthermore, the deletion of vasopressin in these PCK rats (by crossing them to Brattleboro rats) led to lower renal cAMP levels and an almost complete inhibition of cystogenesis, whereas administration of dDAVP recovered the cystic phenotype in the PCK x Brattleboro rats [85]. These results support the importance of cAMP in the pathogenesis of polycystic kidney disease and confirm that targeting the vasopressin-V2R axis effectively inhibits cystogenesis. Based on these animal studies, a phase III clinical trial investigating the effect of the highly-selective V2R antagonist Tolvaptan (OPC-41061) in ADPKD patients is ongoing. Of note, V2R antagonists have no effect on liver cysts because V2 receptors are not expressed in the liver.

A distinct approach to lower cAMP levels is to mimic the action of somatostatin on the Gi-coupled SST2 receptors which induce acute desensitization of adenylyl cyclase coupling [86]. Octreotide, a long-acting somatostatin analogue halted the cystic progression in the liver and kidney of PCK rats [87,88] and slowed renal cystic progression in a preliminary study performed in ADPKD patients [89]. These encouraging results have been confirmed in clinical trials showing that octreotide and lanreotide significantly inhibit the progressive increase in liver volume and total kidney volume, with an acceptable safety profile [88,90].

8. Aquaporins

The long quest for the molecular identity of the water pore ended with the discovery of aquaporin-1 (AQP1) by Agre, who obtained the 2003 Nobel Prize in Chemistry [91, for review]. Antibodies raised against the protein purified from red blood cells showed its localization in the proximal tubules and the descending thin limbs of the loop of Henle, i.e. the very tubular segments characterized by a high intrinsic water permeability. AQP1, the archetypal water channel, facilitates the selective transport of 3 billions of water

molecules per second [91]. To date, 13 members of the AQP family (AQP0 to AQP12) have been identified in mammals, with specific expression patterns and distinct roles in given tissues and cells. With the exception of AQP2, whose membrane expression is regulated by the antidiuretic hormone vasopressin, most AQPs are constitutively expressed in the plasma membrane. Aquaporins play specific roles in epithelial cells lining various segments of the kidney [92]. The apical and basolateral distribution of AQP1 in proximal tubule cells allows a massive, transcellular reabsorption of water driven by a minimal osmotic gradient. The AQP1 channels localized in the cells lining the thin descending limb of Henle's loop allow a rapid osmotic equilibrium with the interstitium, an essential step for the counter-current mechanism. In contrast, AQP2 is expressed only in the principal cells of the collecting ducts (apical membrane and intracellular vesicles). The increased water permeability induced by vasopressin in that segment corresponds to the phosphorylation and rapid insertion of AQP2-containing vesicles in the apical plasma membrane of the principal cells. Vasopressin exerts also a long-term effect on AQP2 expression via a cAMP-responsive element located in the 5′ region of the *AQP2* gene. Two other types of aquaporins have been identified in the basolateral membrane of principal cells of the collecting ducts: AQP3 (also permeable to glycerol), and AQP4 (the only aquaporin that is not inhibited by mercurials) [92,93].

In addition to the Cl^- secretion, it was assumed that water channels could also play a role in the abnormal fluid transport in ADPKD by facilitating water transport across cystic epithelia. Bachinski et al. located AQP1 in a majority (~70%) of cysts of proximal tubule origin (gp330 positive) in human ADPKD kidneys, whereas a minority of the cysts (8%), negative for AQP1 and gp330, expressed AQP2 [94]. Devuyst et al. confirmed the selective and mutually exclusive expression of AQP1 and AQP2 in various stages of ADPKD [15]. The distribution of AQP1 and AQP2 was maintained in the early stages of ADPKD, when normal tubules coexist with cysts. In end-stage ADPKD, two-thirds of the cysts expressed either AQP1 or AQP2, but the two water channels never colocalized in the same cyst. Of note, the proportion of AQP2-positive cysts significantly increased with cyst size, suggesting a role for vasopressin in cyst enlargement [15]. Based on the simultaneous expression of AQP2 and AQP3 in the collecting ducts, Hayashi et al. showed that approximately 30% of the cysts derived from collecting ducts [95]. The preservation of a specific AQP expression in cystic cells may suggests indeed that, next to Cl^- secretion, channel-mediated water permeability might be implicated in fluid secretion. The fact that one-third of the cysts do not express AQP1 or AQP2 [15] suggests that cysts originate from segments that do not express aquaporins, such as the thick ascending limb of the loop or the distal convoluted tubule. This hypothesis has been confirmed by subsequent studies performed in the *Pkd2*$^{WS25/-}$ mouse model [44] and, more recently, in *Pkd1*-null embryos [96].

Recently, Aharabi et al. showed that the complex chain of events mediating AQP2 trafficking in the collecting duct was modified by *Pkd1* haploinsufficiency in mouse [97]. Reduced *Pkd1* gene dosage resulted in inappropriate antidiuresis and positive water balance, reflecting decreased intracellular calcium levels with lowered RhoA activity and recruitment of AQP2 in the apical membrane of CD principal cells, and inappropriate expression of AVP in the brain. These data emphasize the potential role of PC-1 dosage in the regulation of vasopressin signaling and AQP2 trafficking in the collecting duct [97].

It must be noted that mice that lack AQP11, which is localized in the ER of proximal tubule cells, died shortly after birth from massive polycystic kidney disease derived from proximal tubules [98]. In that peculiar case, it is important to mention that AQP11 is not expressed in the plasma membrane and therefore does not contribute to transepithelial water transport. Instead, the cystic phenotype of these KO mice may be due to the dysfunction of PT cells potentially linked to ER stress and vacuolization [98].

9. Conclusions and perspectives

The multiple lines of evidence summarized above have delineated mechanisms involved in the accumulation of fluid in ADPKD cysts. As summarized in Fig. 1, fluid secretion is driven by the transepithelial secretion of Cl^-, mediated by systems operating in series at the level of the two cellular poles : the basolateral NKCC1 cotransporter is responsible for Cl^- entry into the cell, whereas apical Cl^- channels including CFTR allow the accumulation of Cl^- within the cyst lumen. The whole secretory process is stimulated by cAMP and by autocrine and paracrine purinergic signaling. The activity of NKCC1 requires the basolateral recycling of K^+ via the KCa3.1 and of Na^+ via the Na^+-K^+-ATPase. The former also plays an important role in maintaining a negative intracellular membrane potential that provides the electrochemical driving force for apical Cl^- secretion. The luminal accumulation of Cl^- drives Na^+ transport by electrical coupling. Driven by the osmotic gradient, water crosses the epithelium and accumulates into the cyst lumen. This movement of water is probably facilitated by aquaporins in a subset of cysts. This model is certainly not unique, since ADPKD typically involves different tubular segments and therefore a diversity of transport systems.

Since cyst expansion is a major factor for the progressive deterioration and loss of renal function in ADPKD [17,18], therapies targeting fluid secretion and, thereby, cyst enlargement are of major clinical interest. To date, the effect of vasopressin V2 receptor antagonists, somatostatin receptor agonists and inhibitors of CFTR have been successfully tested in animal models and/or in patients with ADPKD [1,3]. Additional targets related to transport mechanisms include the basolateral KCa3.1 [75,76], regulators of intracellular Ca^{2+} levels [99,100], molecules involved in the regulation of CFTR [58], NKCC1 [74] or AQPs [101], and regulators of intracellular levels of cAMP [102].

The long-term efficacy and safety of successful fluid transport inhibition in patients with ADPKD remain to be established. However, the increasing availability of selective drugs targeting transport systems offers an alternative – or a complement – to the anti-proliferative therapies in ADPKD.

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