Transepithelial chloride secretion and cystogenesis in autosomal dominant polycystic kidney disease

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Introduction

Autosomal dominant polycystic kidney disease (ADPKD) is one of the most common monogenic hereditary diseases (prevalence 1:400–1:1000). It is characterized by the development of multiple cysts in both kidneys. These cysts grow slowly but steadily, inducing a progressive renal insufficiency which leads, in about half of the patients, to end-stage renal failure at a mean of 55 years. In Western Europe and North America, ADPKD is responsible for 4–10% of the patients requiring renal replacement therapy. Several extrarenal manifestations, including extrarenal cysts, cardiac valvular abnormalities, and arterial aneurysms, contribute to the morbidity and mortality of the disease. Treatment of ADPKD is currently restricted to the control of associated complications such as hypertension or infection.

Linkage to two loci named PKD1 and PKD2 was found in about 85 and 15% of the families affected with ADPKD respectively. A third locus is probably involved in a small number of cases. The PKD1 and PKD2 genes have been recently cloned [2,3], and the encoded proteins actively investigated [4]. The product of PKD1 (‘polycystin-1’) is a large (~460 kDa) protein including several transmembrane segments, and arterial aneurysms, contribute to the morbidity and mortality of the disease. Treatment of ADPKD is currently restricted to the control of associated complications such as hypertension or infection [1].

While the genetics of ADPKD was under deep scrutiny, another line of research focused on the mechanisms involved in intracystic fluid accumulation (for review, see [9,10]). That interest was triggered by two simple observations. First, careful morphological examination of ADPKD kidneys had revealed that most cysts are disconnected from their tubule of origin. Second, after drainage, ADPKD cysts quickly fill again with liquid, and this process is stimulated by adenylyl cyclase agonists. It was thus clear that intracystic fluid does not originate from glomerular filtrate but rather from a net, transepithelial fluid secretion. That is in striking contrast with the vast majority of fluid movements through the nephron, which normally end with reabsorption of more than 99% of the glomerular filtrate.

In most secretory epithelia, such as the trachea or intestine, fluid secretion primarily depends of a transcellular, cAMP-stimulated Cl− secretion. Several lines of evidence, chiefly generated by the Grantham group, have actually shown that this is also the case for ADPKD cyst-lining epithelia [10]. The key role of Cl− was demonstrated by monitoring in parallel currents and fluid secretion across intact cyst walls and monolayers of cultured ADPKD cyst cells. It was shown that fluid secretion induced by cAMP-stimulating agonists (such as forskolin) (i) was accompanied by an increase in luminal electronegativity, (ii) was abolished after isotonic replacement of Cl− in the basolateral medium, and (iii) was inhibited by the addition of bumetanide (an inhibitor of the Na-K-2Cl

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cotransport) in the basolateral medium, or alternatively by Cl⁻ channel inhibitors on the apical side of the preparation. Taken together, these results suggested that fluid secretion into ADPKD cysts is driven by a transepithelial Cl⁻ secretion involving a basolateral cotransporter and an apical channel.

**Molecular identification of chloride transporters**

The analogy between the properties of ADPKD cystic epithelia and secretory epithelia further led to suggest that CFTR (‘cystic fibrosis transmembrane conductance regulator’), the protein encoded by the CF gene [11], might be instrumental in the cAMP-stimulated Cl⁻ secretion that occurs in ADPKD. Mutations in CF are responsible for cystic fibrosis, a frequent autosomal recessive disease characterized by defective hydration of exocrine secretions, the major clinical consequences of which are pancreatic insufficiency and recurrent airway infections [11]. Although highly expressed in the human fetal kidney, CFTR is down-regulated in the adult kidney and there is no obvious renal phenotype in cystic fibrosis [12]. However, in ADPKD kidneys, CFTR is strongly expressed in the apical membrane area of epithelial cells lining about half of the cysts. Whole cell patch-clamp recording confirmed the occurrence of Cl⁻ currents with the pharmacological profile of CFTR in ADPKD cyst cells [13]. The role of CFTR in ADPKD cells was also substantiated by the demonstration of a significant reduction of fluid secretion after long-term incubation of the monolayers with an antisense oligonucleotide against human CFTR [14].

If the existence of an apical chloride channel accounted for Cl⁻ extrusion, the mechanism of its entry at the basolateral side of ADPKD cyst cell had then to be clarified. The reduction of both luminal electronegativity and fluid secretion after the addition of bumetanide on the basolateral side of ADPKD monolayers [10] suggested the implication of a transporter belonging to the bumetanide-sensitive Na⁺-K⁺-2Cl⁻ cotransporter (BSC, or NKCC) family [15]. This hypothesis was recently confirmed by identification of the BSC2 isoform at the basolateral pole of CFTR-positive ADPKD cysts [16].

**A model of intracystic fluid secretion**

With the molecular identification of transporters and channels in ADPKD kidneys, a tentative model of intracystic fluid secretion has been proposed (Figure 1A and B). Transepithelial Cl⁻ secretion is thus mediated by the basolateral BSC2 cotransporter and the apical CFTR channel operating in series. In parallel with this movement of Cl⁻, Na⁺ accumulates within the cyst lumen by electrical coupling. The nature of the transepithelial Na⁺ pathway remains debated. Most data indicate that Na⁺ transfer is mainly paracellular; experimental evidence suggests that a Na⁺-K⁺-ATPase

![Fig. 1](Figure 1A and B). Transepithelial Cl⁻ secretion is primarily driven by a transcellular Cl⁻ secretion into the cyst lumen. The luminal electronegativity drives Na⁺ and water movement by electric and osmotic coupling respectively. The pathways for Na⁺ ions and water molecules can be transcellular or paracellular. Note that the polarity of the cells is maintained by tight junctions. (B) Molecular mechanisms involved in transepolar Cl⁻ secretion in ADPKD cells. The process is mediated by two transporters operating in series at the two cellular poles. A basolateral sodium-potassium-chloride cotransporter identified as BSC2 is responsible for Cl⁻ entry into the cell, whereas the apical CFTR channel allows Cl⁻ accumulation into the lumen. The activation of the two transporters, i.e. the entire secretory process, is stimulated by cAMP and PKA-mediated phosphorylation. The adenylyl cyclase enzyme (AC) is a transmembrane protein located at the basolateral pole of the cell. (C) Extracellular ATP signalling and Cl⁻ secretion in ADPKD cells. The apical release of ATP into the cyst fluid might result in the stimulation of protein G-coupled P2Y purinergic receptors, which in turn activate outwardly rectifying chloride channels (ORCC). The hypothesized result of these events is stimulation of Cl⁻ secretion and exacerbation of fluid accumulation. The molecular identity of the channel involved in apical ATP release is debated; it has been suggested that CFTR, in addition to Cl⁻, might also conduct ATP. (Modified from references [9,18])
ATPase located in the apical membrane may play a role; whether the expression of the sodium channel ENaC is preserved in ADPKD cyst-lining cells is still unknown. Driven by the osmotic gradient, water crosses the epithelium and accumulates into the lumen, either by a transcellular pathway involving aquaporins or by a paracellular pathway [17]. This model may not be unique, given the diversity of tubule epithelia segments involved in cyst formation in ADPKD, as illustrated by the heterogeneity of the expression of CFTR and other transporters in ADPKD cysts [9,13].

Regulation of cyst fluid accumulation

Not unexpectedly for a secretory epithelium, Cl − secretion in ADPKD cyst cells appears to be regulated by cAMP, which stimulates protein kinase A-mediated phosphorylation and membrane expression of both CFTR and BSC2 [11,15]. Since the latter is the rate-limiting step of the transepithelial secretion, it is tempting to consider that abnormal stimulation of BSC2—induced for instance by modifications of the extracellular matrix—might promote fluid secretion (Figure 1B).

Cyst fluid accumulation in ADPKD might also be regulated by autocrine and paracrine agonists. ATP (a small, negatively charged molecule) has been shown to be released in the apical medium of cultured ADPKD cyst cells and has been detected in the micromolar range in a subset of cyst fluids harvested from ADPKD patients [18]. This ATP concentration is probably sufficient to stimulate purinergic receptors, a stimulation that exerts a potent secretagogue effect for fluid and Cl − in different epithelial cell types. Thus, intraluminal liberation of ATP (or its metabolites such as ADP, 5′AMP or adenosine) in ADPKD renal cysts might enhance fluid secretion and therefore cyst expansion (Figure 1C). One potential mechanism is the activation of outwardly rectifying chloride channels (ORCC) by ATP, mediated by the G protein-coupled P2Y purinergic receptor. Of note, apical channels permeable to ATP remain to be identified, keeping in mind that cAMP-activated CFTR might itself be implicated [19].

Towards modifier genes

The wide variability in the ADPKD phenotype has been emphasized [1]. Whereas interfamilial differences may be accounted for by the nature of the mutation itself, as recently shown in PKD1 disease [20], the intrafamilial variability may be explained by the random occurrence of a second hit [5]. An alternative possibility is that molecular variants of the different transporters involved in intracystic fluid accumulation exert a disease-modifying effect. For instance, variants associated with a decreased Cl − secretion might reduce intracystic fluid secretion, and thus slow cyst growth and delay the progression of renal failure.

Preliminary data suggest the existence of modifier genes in polycystic kidney disease in animals and man. A total genome scanning in the DBA/2- pcy/pcy mouse model of recessive PKD has led to the identification of two modifying loci named MOP1 and MOP2 [21]. Three loci affecting the severity of PKD have also been described in another (kat+/kat+) mouse model of recessive PKD [22]. In man, the simultaneous occurrence in the same family of PKD1 disease and cystic fibrosis provided the opportunity to look for a possible modifying effect of the CF gene. Renal involvement, as assessed by renal size and creatinine clearance was actually found to be less severe in two patients affected by both ADPKD and cystic fibrosis (CF mutations: ΔF508/E60X and ΔF508/3849 + 10 kb C → T) than in relatives affected with ADPKD but heterozygous (AF508/N) or unaffected (N/N) by CF [23], an observation which is in agreement with a protective effect of CF mutations in ADPKD. This finding should be confirmed in a larger population of patients affected by the two diseases and harbouring different CF mutations. It must be noted that the possibility of a modifier role of CF gene in ADPKD is also suggested by the lack of cystic phenotype in collecting duct cell lines derived from CF knock-out mice, as compared to numerous cysts formation observed with cells derived from wild-type mice [24].

Variants of other transporters involved in cyst expansion could also modulate ADPKD phenotype. For example, Na + transporters present in cystic epithelium and remaining normal tubular epithelium could regulate cyst fluid accumulation and plasma volume expansion respectively. A modification of the latter might play a role in the development of hypertension, a probable determinant of progression of renal failure in ADPKD patients [1]. Other candidate genes are those coding for molecules involved in the regulation of fluid secretion (e.g. activation and polarization of the transporters, components of the phosphorylation pathway), growth factors, and binding partners of polycystins.

Conclusion, questions, and perspectives

Whereas the sequence of events leading from mutations in PKD1 or PKD2 to cyst fluid accumulation remains largely speculative, some of the transporters involved in that process are now well characterized. These data support the hypothesis that Cl − secretion is involved in the abnormal cyst fluid accumulation that characterizes ADPKD. On the other hand, important questions—in particular the putative link between the genetic defect in ADPKD and hypersecretion—remain unsolved. Recent studies performed in C. elegans provided evidence that worm homologues of human PKD1 and PKD2 act together in a signal transduction pathway in sensory neurons [25]. Expression studies in Xenopus oocytes demonstrated that the polycystin-like protein (PCL), a human homologue of polycystin-2 that also contains an EF hand domain, is a cation channel regulated by Ca 2+ [26]. Thus, PKD-related
proteins might belong to a conserved signalling pathway involving Ca$^{2+}$ as a secondary messenger. Alterations in this pathway might impair cell maturation and, in given tissues, modify the expression and/or activity of transporters with the resulting hyper-secretory phenotype.

Finally, one might speculate that activating or inactivating variants of molecules involved in cyst fluid accumulation might provide an additional explanation for the intra-familial variability in ADPKD phenotype. Unravelling these modifier effects might pave the way towards therapies targeting cyst fluid secretion.

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