New Insights into the Molecular Mechanisms Targeting Tubular Channels/Transporters in PKD Development

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Key Words

Polycystic kidney disease · Transporters · Channels · Molecular mechanism

Abstract

Background: Autosomal dominant polycystic kidney disease (PKD) or autosomal recessive PKD is caused by a mutation in the *PKD1*, *PKD2* or *PKHD1* gene, which encodes polycystin-1, polycystin-2 or fibrocystin, respectively. Embryonic and postnatal mutation studies show that transport or channel function is dysregulated before the initiation of cystogenesis, suggesting that the abnormality of transport or channel function plays a critical role in the pathology of PKD.

Summary: Polycystin-2 by itself is a calcium-permeable cation channel, and its channel function can be regulated by polycystin-1 or fibrocystin. In this paper, we reviewed the current knowledge about calcium transports and cyclic adenosine monophosphate (cAMP)-driven chloride transports in PKD. In addition, the function and the underlining mechanism of glucose transporters, phosphate transporters and water channels in PKD are also discussed. **Key Messages:** Abnormalities in calcium handling and exuberant cAMP-dependent cystic fibrosis transmembrane conductance regulator-mediated fluid secretion in the collecting duct are the most important issues in the pathogenesis of PKD.

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Introduction

Polycystic kidney disease (PKD) is one of the most common hereditary kidney diseases in the world [1, 2]. The incidence of autosomal dominant PKD (ADPKD) is 1 in 500–1,000, whereas the autosomal recessive PKD (ARPKD) only affects 1 in 20,000 individuals [1]. ADPKD is caused by mutations in either the *PKD1* or the *PKD2* gene, which encodes polycystin-1 or polycystin-2, respectively. In ARPKD, mutations in *PKHD1* lead to dysfunction of fibrocystin and subsequently form renal cysts [2].

Exuberant fluid secretion and cell proliferation drive the expansion of innumerable fluid-filled cysts in both kidneys of PKD patients [1, 2]. In ADPKD, renal cysts develop in all segments of the nephron but predominantly originate from the collecting duct [3]. Renal cysts detach from their parental tubule when their diameter reaches 2 mm [4]. In ARPKD, the fusiform dilated collecting ducts form cysts and will not be disconnected from their parental tubules. The pathophysiology of PKD is not completely understood, and currently there is no approved pharmaceutical drug in the USA or China to treat PKD patients.

Inactivation of the *Pkd1* gene before postnatal day 13 (P13) resulted in severe cystic kidneys within 3 weeks; however, *Pkd1* gene inactivation after P13 did not induce

Table 1. Summary of channels and transports related to PKD

Transporter/channel	Subcellular location	Transport/channel activity and its relation with PKD
Polycystin-2	ER, plasma membrane, primary cilium	Cation channel in plasma membrane; calcium channel in ER and primary cilium; mutation or dysfunction of polycystin-2 lowers cytosolic calcium level and intracellular calcium store in PKD
RyRs	ER	Calcium channel in ER; constitutive activation of RyRs may cause calcium leak from ER in PKD
IP3R	ER	Calcium channel in ER; constitutive activation of IP3R may cause calcium leak from ER in PKD
TRPC1	Primary cilium and plasma membrane	Interacts with polycystin-2 and behaves as a calcium channel; TRPC1 competes functionally with polycystin-1 for polycystin-2 binding
TRPC4	Plasma membrane	Interacts with polycystin-2 and behaves as a calcium channel; polycystin-2/TRPC4 interaction was enhanced and polycystin-2 plasma membrane localization was increased in response to angiotensin II treatment
TRPV4	Primary cilium	Interacts with polycystin-2 and changes polycystin-2 subcellular location; depletion of TRPV4 abolishes flow-induced calcium transient but does not result in cystic phenotype
CaV1.2	Primary cilium	Dopamine receptor-type 5 activation induces calcium signaling in primary cilium through CaV1.2; deletion of CaV1.2 results in a cystic phenotype in zebrafish
CFTR	Plasma membrane	CFTR-mediated chloride secretion is enhanced in PKD; the gating activity of CFTR is activated by PKA which is downstream of cAMP; in PKD, cAMP-dependent CFTR-mediated chloride secretion is the major force driving fluid secretion by cysts
CaCC	Plasma membrane	CaCC is overexpressed in cystic kidneys, and inhibition or deletion of CaCC prevents chloride secretion and renal cyst growth
SGLTs	Plasma membrane	Functions as transporters in renal epithelial cells to reabsorb glucose; inhibition of SGLTs with phlorizin retards disease progression in the rat model of ADPKD and increases osmolar, sodium and chloride excretion; however, specific inhibition of SGLT2 does not show a beneficial effect in the ARPKD rat model
Phosphate transporter regulator FGF23	Plasma membrane	FGF23 is upregulated and the FGF receptor is activated in cystic kidneys; however, the expression and activity of renal phosphate transporters are normal in PKD
Aquaporin 1	Plasma membrane	Expression of water channel aquaporin 1 is inversely correlated with cyst size in ADPKD; deletion of aquaporin 1 promotes cyst growth

renal cyst within 3 months [5]. Microarray analysis revealed that differentially expressed genes between P11 and P12 are categorized into transporters or catalytic enzymes as compared with genes expressed between P14 and P15. Through amniotic fluid analysis, a massive loss of solutes and glucose was found in *Pkd1*-null embryos at E13.5 preceding cystogenesis [6]. These data suggest that tubular channels or transporters may play an important role in cystogenesis or development [5, 6]. Channels or transporters which are related to PKD are summarized in table 1.

Calcium Transporters in PKD

Intracellular calcium homeostasis is disrupted in ADPKD, as demonstrated by reduced cytosolic calcium content, intracellular calcium store and dysregulated intracellular calcium channel activities [7, 8]. Dysregulated calcium homeostasis was also observed in ARPKD [9, 10]. A lower intracellular calcium level was found in ARPKD cells or *PKHD1*-silenced cells. Polycystin-2 is a calcium-permeable cation channel, and its channel selectivity is determined by its subcellular localization [11]. On plasma

membrane, polycystin-2 is a receptor-operated nonselective cation channel, while it functions as a calcium channel when localized to the primary cilium or endoplasmic reticulum (ER) [11]. In general, the transmembrane protein polycystin-1 and polycystin-2 form a complex that functions as a mechanosensor when it is localized in the primary cilium [1]. In normal physiological condition, polycystin-1 transduces extracellular mechanical signals by enhancing calcium influx through the calcium channel polycystin-2 in the primary cilium. In ADPKD, the dysfunction of polycystin-1 or polycystin-2 reduces intracellular calcium level and results in cystogenesis.

The channel activity of polycystin-2 can be activated by intracellular calcium, epidermal growth factor or binding to polycystin-1 [11]. Recently, it has been shown that phosphorylation of polycystin-2 at Ser829 increased adenosine 5'-triphosphate (ATP)-induced calcium transients [12]. The function of polycystin-2 can also be modulated through changing its subcellular localization [11, 13]. It has been shown that the phosphorylation status of polycystin-2 determines its subcellular distribution [11]. Phosphorylation of polycystin-2 on serine 812 by casein kinase 2 traps it in the ER [14]. Constitutive phosphorylation of polycystin-2 on serine 76 by glycogen synthase kinase 3 leads to plasma membrane localization of polycystin-2 [15]. The study by Streets et al. [12] firstly showed that polycystin-1 is also engaged in polycystin-2 phosphorylation. Dysfunction of polycystin-1 increased polycystin-2 phosphorylation at Ser829 through activating the cyclic adenosine monophosphate (cAMP)/protein kinase A (PKA) pathway or releasing PP1-mediated dephosphorylation on Ser829 by disrupting polycystin-1 and polycystin-2 interaction [12]. Following cAMP stimulation, basolateral expression of pSer829 increased to a greater extent than its ER expression [12].

Mutation of the *PKHD1* gene results in the dysfunction of fibrocystin/polyductin, a large transmembrane receptor-like protein [16]. Fibrocystin physically interacts with the intracellular N-terminal tail of polycystin-2 through its intracellular C-terminus [17]. The binding of fibrocystin and polycystin-2 requires the presence of ciliary motor protein kinesin-2 [18]. Several lines of evidence revealed that downregulation of fibrocystin reduces intracellular calcium level. Fibrocystin regulates intracellular calcium homeostasis probably through modulating the channel activity of polycystin-2 and preventing degradation of polycystin-2 [10, 18, 19]. Overexpression of fibrocystin C-terminus activated polycystin-2 channel activity and also prevented its downregulation [17, 18].

Fig. 1. Putative mechanism of calcium leak from ER in PKD. In PKD, a high level of intracellular cAMP activates PKA which in turn phosphorylates and enhances the gating activity of polycystin-2 (PC2), RyRs and IP3R in the ER. Constitutive activation of PC2, RyRs and IP3R results in calcium leak and depletion of the intracellular calcium store in PKD. Moreover, PC2 behaves as a brake to inhibit RyR activity, and dysfunction of PC2 may also cause calcium leak through RyRs. Polycystin-1 (PC1) inhibits PC2 activity through PP1-mediated dephosphorylation of PC2. PP1 interacts with PC1, and dysfunction of PC1 releases PP1-mediated PC2 dephosphorylation, thus promoting calcium leak through PC2.

In addition to acting as a calcium channel by itself, polycystin-2 modulates intracellular calcium homeostasis by physically interacting and regulating multiple calcium channels [7, 20]. Polycystin-2 colocalizes with ryanodine receptors (RyRs) in ER and inhibits its calcium release as a brake [8, 21, 22]. Knockout of *PKD2* results in depletion of intracellular calcium store and alteration of RyRs-dependent calcium signaling [21]. There is another mechanism that may account for the reduced internal calcium store in ADPKD. It is postulated that constitutive activation of polycystin-2 and RyRs by a high level of cAMP/PKA results in calcium leak and depletion of intracellular calcium store in *PKD1*- or *PKD2*-mutated renal epithelial cells (fig. 1) [20]. Moreover, polycystin-2 also interacts and activates calcium release through inositol 1,4,5-trisphosphate receptor (IP3R) in ER [23]. Mutation or downregulation of polycystin-2 decreases IP3-evoked calcium response [8, 23]. In addition, IP3R could

be phosphorylated and functionally regulated by PKA [24, 25]. Therefore, we cannot exclude that a constitutive activation of IP3R by PKA in PKD leads to calcium leak from the internal calcium store (fig. 1). A recent study shows that aging disrupts the functional interaction between polycystin-2 and RyRs or IP3, which might contribute to the accelerated disease progression in elderly ADPKD patients [8]. The putative molecular mechanism for the observed aging-related calcium handling abnormality could be related to the phosphorylation status of polycystin-2 and/or its binding partners [8].

Polycystin-2 belongs to the transient receptor potential (TRP) family of ion channels and is named as TRPP2 [11, 26]. Several studies showed that polycystin-2 complexes and other TRP family members such as TRPC1, TRPC4 and TRPV4 function as molecular sensors by regulating calcium influx [26–29]. Interestingly, deletion of TRPV4 did not result in the cystic phenotype in kidneys, although TRPV4 depletion completely abolished flow-induced calcium signaling [29]. These studies also found that TRP channels can regulate polycystin-2 subcellular distribution and compete with polycystin-1 for polycystin-2 binding; however, the impact of these channels on polycystin-1- or polycystin-2-mediated calcium signaling and intracellular calcium store in ADPKD is currently unknown.

Polycystin-1 may also participate in the regulation of intracellular calcium content, since it was found to physically interact with ER-localized calcium release channel IP3R [30]. However, experimental findings regarding the effect of polycystin-1 on IP3-evoked calcium release were inconsistent [30, 31]. A recent study in a more restricted experimental condition with stable resting calcium level and no reuptake of calcium, showed that endogenous polycystin-1 stimulated the activity of IP3R in the presence of polycystin-2 [32].

The L-type Ca^{2+} channel $\alpha 1\text{C}$ (CaV1.2) was found localized in the primary cilium of renal epithelial cells [33]. A recent study showed that zebrafish with CaV1.2 deletion presents a cystic phenotype [34]. Interestingly, CaV1.2 is upregulated in PKD-2 knockout cystic zebrafish and cilia-deficient cells. Further study needs to explain the effect of CaV1.2 overexpression in PKD.

Chloride Transporters in PKD

An early study with tritium showed that daily fluid turnover within a renal cyst is 10 times more than the cyst volume [35]. The transepithelial membrane fluid secre-

tion across the polarized monolayer of several epithelial cell lines was observed, and the fluid secretion was stimulated by a cAMP agonist [36, 37]. Moreover, forskolin, a nonspecific stimulator of adenylate cyclase, enhanced fluid secretion by excised human renal cysts [37]. Cystic fibrosis transmembrane conductance regulator (CFTR)-mediated chloride transport has been identified as the major force for cAMP-induced transmembrane fluid secretion in ADPKD [38, 39]. Several in vitro and in vivo studies indicated that CFTR is required for cAMP-stimulated cyst formation and expansion [40–42]. It is well known that the gating activity of CFTR is regulated by cAMP-dependent phosphorylation on the CFTR regulatory domain through PKA [43]. Therefore, it is believed that fluid accumulation in renal cysts is caused by active chloride secretion through CFTR which is stimulated by the cAMP/PKA pathway.

The energy of active chloride excretion in PKD is powered by the basolateral sodium-potassium ATPase which generates a chemical gradient driving the entry of potassium and chloride through basolateral sodium, potassium and chloride ($\text{Na}^+ - \text{K}^+ - 2\text{Cl}^-$) co-transporters [44, 45]. In PKD, chloride is excreted to lumen following the chemical gradient through cAMP-stimulated CFTR and creates a lumen-negative transepithelial electrical potential [44]. Potassium moves back through basolateral potassium channels [46, 47]. The lumen-negative potential generated by the chloride and potassium conductance drives sodium passively transported into lumen through a paracellular pathway [44]. The net increase in sodium and chloride in lumen leads water secretion by osmotic forces [44]. Inhibition Na^+ , K^+ -ATPase, and Na^+ - and $\text{K}^+ - 2\text{Cl}^-$ co-transporters and potassium transporters in the basolateral membrane inhibits chloride secretion and cyst expansion [42, 45–47].

In parallel to the predominant presentation of cysts in the collecting duct in PKD, the inner medullary collecting duct, especially the initial part has a robust capacity to secrete chloride in response to cAMP treatment [48]. The V2 receptor of arginine vasopressin (AVP) is predominantly located in the collecting duct and also expressed in the medullary thick ascending limb [49]. AVP interacts with the V2 receptor on the basolateral membrane to promote cAMP production through adenylyl cyclase 6 which is abundantly expressed in the inner medulla, and subsequently stimulates CFTR-mediated chloride secretion in ADPKD cells [50–53]. Numerous studies in cells, animals and recently in human settings have proven that AVP-stimulated cAMP production is a major factor for chloride-driven fluid secretion in PKD. Blocking the V2 re-

ceptor with different antagonists or lowering the plasma level of AVP by increasing water intake reduced renal cAMP levels and delayed cyst growth in animal models of PKD and ADPKD [53–57].

AVP promotes chloride secretion also indirectly by producing ATP or prostaglandin E₂ (PGE₂) [58, 59]. ATP was found accumulated in renal cysts in ADPKD patients [60]. It has recently been shown that ATP stimulation is required for the cAMP-dependent fluid secretion in the 3D cyst culture model; however, the current theory of ATP-stimulated chloride secretion involves increasing intracellular calcium concentration and activation of calcium-activated chloride channels (CaCC) [61–63]. CaCC is indeed strongly expressed in animal and human cystic kidneys, and inhibition or deletion of CaCC prevents chloride secretion and renal cyst growth [64]. However, the mechanism of ATP-driven chloride transport still needs to be further explored in cells with low intracellular calcium levels. PGE₂ is accumulated in renal cysts and interacts with EP₂ or EP₄ receptors to promote chloride secretion and cyst expansion [65, 66]. A mechanistic study has shown that PGE₂ induced chloride secretion by collecting duct cells involving cAMP-CFTR- and/or calcium-CaCC-dependent channel functions [25].

The function of CFTR in ADPKD is also regulated by other paracrine factors. A recent study has shown that endogenous concentrations of ouabain stimulate chloride secretion by ADPKD cells via epidermal growth factor [67]. The adrenergic system stimulates cAMP-dependent chloride secretion in the collecting duct through activation of the β -adrenergic receptor, which involves cAMP production by ADPKD cells [68, 69].

Besides PKA, CFTR is controlled by numerous kinases [64]. Adenosine monophosphate-activated protein kinase (AMPK) is a negative regulator for CFTR activity. Activation of AMPK by metformin inhibited CFTR-dependent fluid secretion and slowed cyst growth [70]. A recent study showed that Src and mitogen-activated protein kinase (MEK/MAPK) were involved in ouabain-stimulated and CFTR-mediated chloride secretion in ADPKD cells [67].

Sodium-Glucose Cotransporters in PKD

The sodium-glucose linked transporters (SGLTs) expressed in the proximal tubule are responsible for glucose homeostasis which is regulated by the kidney [71]. Inhibition of SGLTs with phlorizin retarded PKD progression in the Han:SPRD rat model accompanied by increased

osmolar, sodium and chloride excretion [72]. Phlorizin inhibited the growth of cysts which are predominantly expressed in the proximal tubule in this rat model by inhibition of cell proliferation which might be linked to the MAPK signaling pathway. In addition, the authors postulated that increased osmotic diuresis induced by phlorizin may impair the transepithelial fluid secretion and thus inhibit cyst expansion. However, a later study from the same group showed that inhibition of SGLT2 with dapagliflozin worsened renal function and increased cystic volume in a PCK rat model of ARPKD where cysts are predominantly presented in collecting ducts and are not closed off from their parental tubules [73]. They explained that the increased intratubular osmotic pressure induced dilation of distal tubular segments and cysts, since the cell proliferation and cAMP levels in cystic kidneys were not changed by SGLT2 inhibition. Therefore, ADPKD patients with diabetes should be cautious to use SGLT inhibitors to control hyperglycemia until the effect and underlying molecular mechanism of SGLTs inhibitors are confirmed in orthologous animal models of ADPKD.

Phosphate Transporter Regulation by Fibroblast Growth Factor 23 in PKD

Fibroblast growth factor 23 (FGF23) is a hormone regulating phosphate reabsorbance in proximal tubules by downregulation of sodium phosphate co-transporters [74]. In animal and human PKD, the plasma level of FGF23 is upregulated; however, the plasma level of phosphate is not reduced in animals and humans with ADPKD [75, 76]. In addition, animal studies showed that the expression and activity of renal phosphate transporters and the FGF23 co-ligand klotho are also normal in PKD as compared with wild-type rats, indicating PKD is resistant to overexpression of FGF23. Interestingly, FGF23 is strongly stained on cystic epithelial cells, and the FGF receptor is activated in cystic kidneys, suggesting that FGF23 may have an effect on cyst growth [75].

Aquaporin Water Channels in PKD

Water channel aquaporin 1 and aquaporin 2 are expressed in renal cysts in ADPKD [77, 78]. The expression of aquaporin 1 is inversely correlated with cyst size; however, the expression of aquaporin 2 is not changed in small and large renal cysts [77]. Aquaporin 1 is located in the proximal tubules, the descending thin limbs of Hen-

le's loop and the outer medullary descending vasa recta [79]. Overexpression of aquaporin 1 inhibited cell proliferation and impaired cystogenesis in a 3-D culture model [80]. Conversely, aquaporin 1 deletion promoted cyst growth, particularly in proximal tubule cysts, in animal models [80]. Aquaporin 1 interacts with β -catenin and promotes its degradation, and deletion of aquaporin 1 increases β -catenin expression and activates downstream Wnt signaling pathway leading to cystogenesis [80].

Conclusions

The deficient calcium handling, low levels of intracellular calcium and reduced calcium storage are fundamental defects in PKD. Current studies in the field of PKD focus on understanding the abnormal calcium signaling conducted by polycystin-2. Regulation of calcium storage by RyRs and IP3R in PKD is another hot research topic, since these two calcium channels can be tightly regulated by polycystin-1, polycystin-2 and cAMP. CFTR-dependent cAMP-driven fluid secretion is an important issue in this field. The concept of inhibition of cAMP-driven fluid secretion is encouraged by the positive result obtained from a clinical trial using the V2 receptor antago-

nist tolvaptan in ADPKD patients. The activity of CFTR is regulated by an array of kinases, which could be designed as targets to treat PKD patients in the future. CaCC is also involved in fluid secretion in PKD; however, more studies need to be performed to understand its working mechanism in the low calcium environment in PKD. Many studies regarding the transporters or channels located in the proximal tubules in PKD are also encouraging. However, cysts in ADPKD and ARPKD are predominantly found in the collecting duct. More importantly, cysts located in the distal part of the nephron have more potential effects than the proximal tubule cysts to affect renal function [4].

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Conflict of Interest Statement

The authors declare that there is no conflict of interest.

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