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Review Article

Cyst growth, polycystins, and primary cilia in autosomal dominant polycystic kidney disease

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Abstract

The primary cilium of renal epithelia acts as a transducer of extracellular stimuli. Polycystin (PC)1 is the protein encoded by the *PKD1* gene that is responsible for the most common and severe form of autosomal dominant polycystic kidney disease (ADPKD). PC1 forms a complex with PC2 via their respective carboxy-terminal tails. Both proteins are expressed in the primary cilia. Mutations in either gene affect the normal architecture of renal tubules, giving rise to ADPKD. PC1 has been proposed as a receptor that modulates calcium signals via the PC2 channel protein. The effect of PC1 dosage has been described as the rate-limiting modulator of cystic disease. Reduced levels of PC1 or disruption of the balance in PC1/PC2 level can lead to the clinical features of ADPKD, without complete inactivation. Recent data show that ADPKD resulting from inactivation of polycystins can be markedly slowed if structurally intact cilia are also disrupted at the same time. Despite the fact that no single model or mechanism from these has been able to describe exclusively the pathogenesis of cystic kidney disease, these findings suggest the existence of a novel cilia-dependent, cyst-promoting pathway that is normally repressed by polycystin function. The results enable us to rethink our current understanding of genetics and cilia signaling pathways of ADPKD.

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Introduction

It has been reported that abnormalities in the structure or function of primary cilia result in kidney cyst growth in animal models and human genetic diseases collectively known as ciliopathies [1–3]. Polycystin (PC)1 has been hypothesized to form a mechanosensitive cation channel complex with PC2 in the primary cilia [4–6]. Functional defects in this complex caused by mutation of *PKD1* or *PKD2* result in autosomal dominant polycystic kidney disease (ADPKD) [7,8]. Primary cilia are membrane-enclosed hair-like projections from the apical surface of renal epithelial cells, facing into the tubule lumen (Fig. 1). Primary cilia are microtubule-based organelles that are ideally positioned to detect extracellular stimuli and to transduce these signals into the cell to elicit physiological responses [6,9]. Experimental evidence that flow-mediated deflection of the cilia induces an increase in intracellular calcium has fostered the hypothesis that cilia may sense flow in the kidney tubule lumens [10–12]. Intraflagellar transport (IFT) is a general ciliary component transport mechanism required for assembly and steady-state maintenance of cilia [13,14]. IFT plays an additional role in regulating cell-cycle progression independent of its function in cilia formation [1]. Despite these data, there remains some lack of clarity on the relationship between PCs and cilia function. Recent studies have generated novel information regarding the genetic and molecular implications of ADPKD, its pathogenesis, and new potential strategies for targeted treatment. In this commentary, new signaling activity of interconnectedness between primary cilia and PCs, and dosage effects of PC are highlighted.

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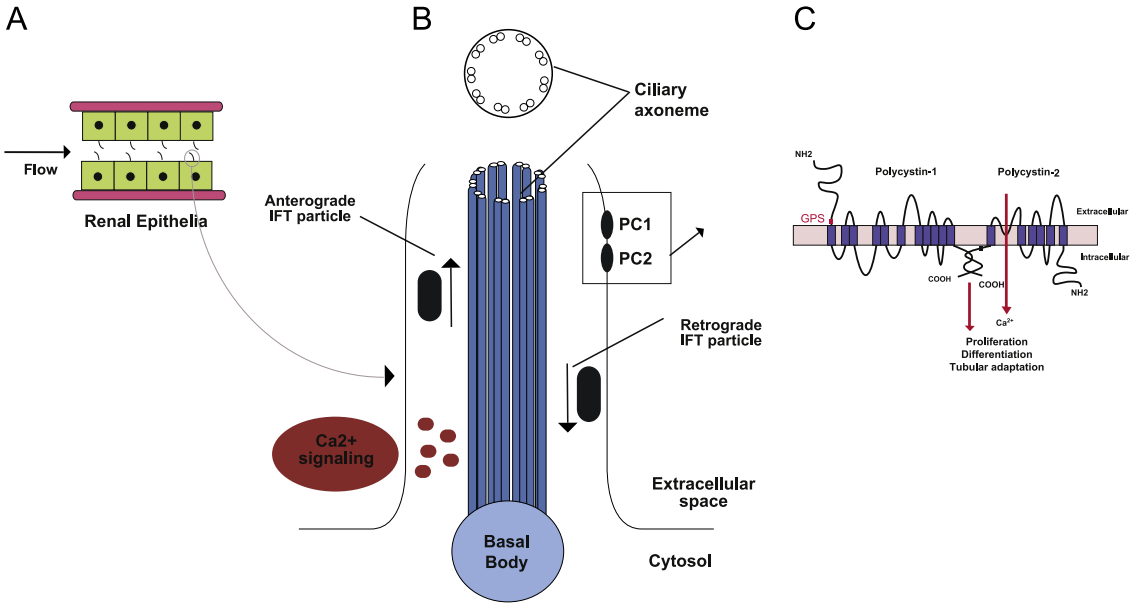

Figure 1. PCs and cilia. (A) Renal tubular epithelium depicted as cuboidal cells with apical primary cilia projections subjected to luminal flow. (B) Schematic of 9 + 0 primary cilia. As a microtubule-based structure that imports proteins via anterograde IFT and returns proteins to the cell body via retrograde IFT transport. PC1 and PC2 reside in the cilium membrane and are hypothesized to subsume a local calcium signaling process that may be modulated by mechanical or ligand stimuli. (C) Schematic representations of PC1 and PC2 showing their respective topologies and interaction via coiled coil domains in their carboxy termini. PC1 has the properties of a receptor and undergoes cleavage at the indicated (red letters) GPS sites. GPS site, whereas PC2 is a calcium channel of the TRP family. GPS, G protein coupled receptor proteolytic site; IFT, intraflagellar transport; PC, polycystin; TRP, transient receptor potential. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

ADPKD

ADPKD is a common, single-gene multi-system disorder. Its prevalence at birth is between 1:400 and 1:1,000 and it affects >12 million people worldwide, without regard to sex or ethnicity [5,7,8,15]. The disease is characterized by the development of renal cysts and various extrarenal manifestations. ADPKD results from excessive proliferation of renal tubular epithelial cells and remodeling of surrounding structures, giving rise to growth of epithelial-lined cysts accompanied by fibrosis and accumulation of extracellular matrix. As the disease progresses, this leads to destruction of the normal renal parenchyma, massive renal enlargement, deterioration of renal function, and eventually renal failure in $>50\%$ of affected individuals by late adulthood [5,15]. A two-hit model has been proposed to explain the focal nature of renal cysts and the variability in cyst size in both orthologous mouse models and in humans [16–18]. In this model, a germline and a somatic mutation inactivate the PKD alleles separately. The first hit, a germline mutation inherited from the affected parent, is predisposing but not sufficient for cyst formation. The first hit exists in all cells in the body. The second hit, a somatic mutation in an individual cell, inactivates the normal *PKD1* or *PKD2* allele, and when it occurs in kidney tubule cells, is thought to cause abnormal focal proliferation and tissue remodeling, giving rise to cyst formation [19,20]. Conditional knockout of *Pkd1* in mice indicates that the timing of the somatic second hit mutation affects the severity of cyst progression. Inactivation of *Pkd1* in mice prior to a developmental switch occurring prior to postnatal Day 13 results in severely cystic kidneys within 3 weeks, whereas inactivation at Day 14 and later results in cysts only after 5 months [21]. Multiple genetic mechanisms that result in an imbalance in the expression of either PC1 or PC2 below a critical threshold without complete loss can also cause cyst formation [22,23]. The presence of somatic PKD2 mutations detected in cystic patients with PKD1 germline mutations and increased disease severity in patients with heterozygous germline mutation in both PKD1 and PKD2 offer further support for the two-hit model and the dosage effect hypothesis for cyst formation [24,25].

ADPKD genes and proteins – *PKD1*, *PKD2*, and PC1 and PC2

PKD1 mutations account for ~85% of the clinically ascertained disease burden. The *PKD1* gene product, PC1, is a large protein consisting of 4,302 amino acids with a large extracellular amino terminus, 11 transmembrane domains, and a short cytosolic carboxy terminus (Fig. 1C) [5,15]. PC1 is possibly expressed in most nephron segments, despite expression at the tissue level being difficult to determine due to its low protein levels. At the subcellular level, it has been reported that PCs are expressed in several subcellular compartments of renal epithelial cells, remarkably primary cilia, lateral cell to cell junctions, and the endoplasmic reticulum. Investigations in other experiments have demonstrated that the localization of PC fractions to primary cilia is thought to be required for the function of cilia as mechanosensors [2,3,6,20,26].

PKD2, the gene mutated in 15% of polycystic kidney disease cases, encodes PC2 [5,27]. PC2 has been described as a nonselective, calcium-permeable cation channel belonging to the transient receptor potential (TRP) polycystic subfamily of TRP channels [28,29]. PC2 is an integral membrane protein with six transmembrane segments, and both the amino and carboxy termini face the cytoplasmic compartment [30,31]. PC2 contains several trafficking motifs: the RVxP motif at the

amino terminus of PC2 is necessary and sufficient for the ciliary location of the protein [30], whereas a region at the carboxy terminus may restrict the membrane location of PC2 to the endoplasmic reticulum and cilia. In the kidney, PC2 is found in all nephron segments with the exception of the glomeruli. PC1 is thought to function as a mechanosensor or chemosensor at the primary cilia that coordinates the activity of the PC2 calcium channel [15,32,33].

Disruption of primary cilia or mutations in cilia-associated proteins leads to renal epithelial proliferation and cyst growth in animal models and many human genetic diseases that are classified as ciliopathies [2,3,34,35]. Primary cilia have an axonemal structure consisting of a circular array of nine pairs of microtubules (9+0 arrangement; Fig. 1B) [3]. Primary cilia are single nonmotile projections on the apical surface of renal epithelial cells toward the lumen [9]. They have a plasma membrane but do not have subcellular organelles and protein-synthetic capacity. Primary cilia, which are usually nonmotile, are thought to serve either a chemosensory or mechanosensory function [4,6]. The phenotypes of ciliopathies in mammals include cystic kidneys, abnormal formation of the left–right body axis, abnormalities in neural tube closure and patterning, skeletal defects such as polydactyly, liver, and pancreatic cysts and fibrosis, and obesity [34,35]. In addition to their potential role as mechanosensors, primary cilia have important functions in sensing the external environment and are essential to processes as diverse as photoreception, olfaction, and developmental signaling [35].

Many cellular pathways have been proposed as effector pathways in ciliary and cystic diseases. They include planar cell polarity, Wnt, mammalian target of rapamycin, cyclic adenosine monophosphate (cAMP), G-protein-coupled receptor, cystic fibrosis transmembrane conductance regulator, epidermal growth factor receptor, mitogen-activated protein kinase (MAPK), cellular calcium, and the cell cycle [5]. Experimental evidence supports that cAMP stimulates MAPK/extracellular signal-regulated kinase (ERK) signaling [36,37], and promotes cyst growth. Functional loss of PC1 at the cilium results in reduced cellular calcium signaling [6]. This in turn results in increased adenylate cyclase and decreased phosphodiesterase activity, leading to increased levels of intracellular cAMP. Increased cAMP in cystic epithelium increases the activity of protein kinase A, which can result in increased cell proliferation and chloride-driven fluid secretion; features associated with cyst growth [5,36]. Lacking through all of these proposed functional pathway hypotheses is an understanding of the relationship of PCs with other ciliary component proteins and a clear definition of the direct role of PC signaling within cilia.

PC1 dosage in ADPKD

Studies on a possible gene-dosage effect for PCs have been described in *trans* heterozygous states in mice [16]. Mutations in *Pkd1* resulting in reduction of functional PC1 have suggested that reduced activity is sufficient to cause cyst formation in some situations [38–40]. Hence, PC1 dosage has been described as a modulator of cystic disease. These authors generated an intronic insertion of a neomycin cassette into *Pkd1*, which had a profound effect on splicing efficiency. Although the hypomorphic $Pkd1^{nl/nl}$ mice survive, they develop severe cystic kidneys from the collecting duct, thick ascending limb, and distal tubules. Another study [41] further supports that diminished expression of native PC1 is sufficient to induce renal cystic lesions of ADPKD. Studies have demonstrated that 50% reduction in PC1 levels, as occurs in true heterozygote ADPKD patients, does not lead to overt cyst formation, but 80% reduction may be sufficient to result in a polycystic phenotype [42]. A recent study demonstrating that the decrease in the steady-state levels of PC1 that can be partially rescued in a temperature-sensitive manner also points to the importance of PC1 dosage in the modulation of cyst burden [43]. Separately, high copy number *Pkd1* transgenic mouse lines have reported the presence of kidney cysts [44], suggesting that massively increased dosage of PC1 also leads to cyst formation. Although the mechanism for the latter is unknown, PC1 overexpression may generate cysts by disrupting the steady-state balance of the PC1/PC2 complex, thereby altering its signaling [44].

A comprehensive study of the mechanisms underlying isolated ADPKD has clearly demonstrated the central role of PC dosage in kidney cyst formation [23,45]. The two genes responsible for isolated polycystic liver disease, *PRKCSH* and *SEC63*, are both involved in modulating protein biogenesis and quality control for integral membrane and secreted proteins in the endoplasmic reticulum. Inactivation of either of these genes results in inefficient maturation of the PCs and cyst formation in both the liver and kidney in mouse models. This effect can be exacerbated by reduced gene dosage for *PKD1* or *PKD2* [23]. Overexpression of *Pkd1*, but not *Pkd2*, can overcome the tendency toward cyst formation in *Prkcsh* and *Sec63* mutants, thereby establishing PC1 as the rate-limiting component whose dosage determines both the rate and extent of cyst formation in ADPKD [23,45]. In a proof of principle experiment, inhibition of proteasomal degradation using the proteasome inhibitor carfilzomib was able to ameliorate polycystic disease progression in a manner analogous to increased PC1 expression [23,45]. Based on these data, PC1 dosage is one of the effector mechanisms of cyst formation in both mouse and human ADPKD.

Novel inter-relationship between primary cilia and PCs

Defects in primary cilia are associated with increased proliferation of cells characteristic of polycystic kidney disease [34,46]. IFT machinery is required to traffic component proteins into and out of cilia (Fig. 1B) [3,13,47]. Among the many proteins delivered to cilia by IFT are PC1 and PC2, loss of either of which results in ADPKD [4,48,49]. Kidney cysts also arise in mice following the disruption of cilia by targeted inactivation of genes encoding IFT components, such as the heterotrimeric kinesin component Kif3a [50] and IFT proteins, Ift20 [47] and Ift88 [11,51,52]. It is generally hypothesized that the primary cilium of kidney epithelial cells acts as a sensory organelle and that PC1 and PC2 form a receptor–channel sensory complex in the cilium.

A recent study from our laboratory based on genetic manipulation of polycystins and cilia in mutant mice has the potential to recalibrate significantly the concepts about the inter-relationship between PC and cilia function [53]. Inactivation of PCs or cilia alone can lead to the formation of kidney cysts. However, it has not been clear whether it is the loss of PCs or persistence of intact cilia under conditions of loss of PCs that is responsible for initiation of the signaling involved in cyst growth. We evaluated these possible mechanisms by using tissue-specific and inducible knockout of *Pkd1* and *Pkd2* alone or in combination with knockout of *Kif3a* and *Ift20* in mouse models. We found that disruption of cilia markedly delayed cyst growth caused by the loss of PCs.

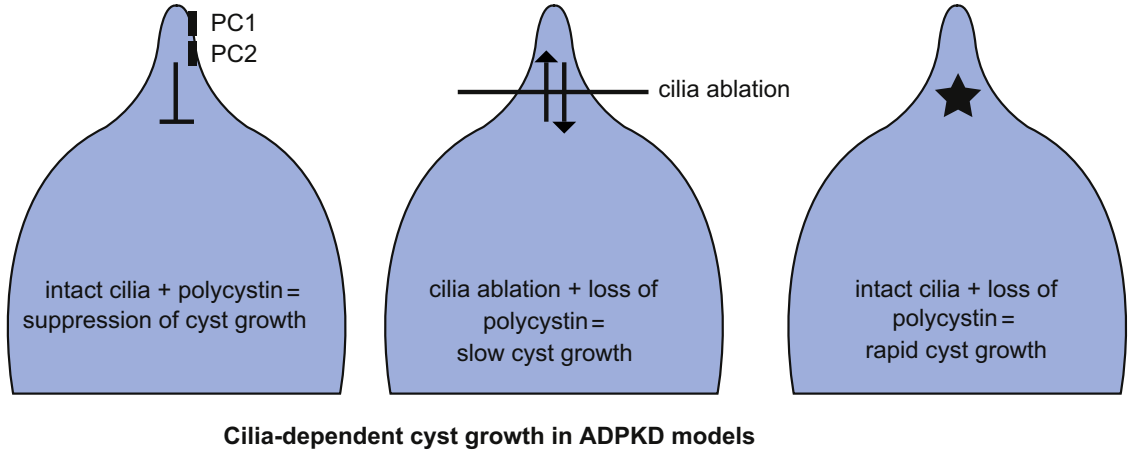

Figure 2. Schematic representation of the cilia dependent cyst promoting pathways in ADPKD. When PCs are normally expressed in cilia, renal tubular architecture is preserved (left panel). When PCs are lost, intact cilia are responsible for signal (depicted by the star) that promotes rapid cyst growth (right panel). When both PCs and cilia on non-functioning (center panel), indolent cyst growth results.
ADPKD, autosomal dominant polycystic kidney disease; PC, polycystin

Concomitant loss of *Pkd1* or *Pkd2* along with either *Kif3a* or *Ift20* resulted in much milder progression of polycystic disease than was seen with inactivation of either PC alone (Fig. 2).

We further tested whether disease severity was affected by a time-dependent persistence of cilia following PC loss. We found that the shorter the time interval until cilia disappearance following initial loss of PCs, the greater the protection conferred against cyst progression.

Therefore, the severity of ADPKD is directly affected by the duration of the persistence of cilia in the absence of PCs. These findings suggest that PCs provide baseline tonic inhibition of a cilium-dependent signal that promotes cyst growth when unchecked following inactivation of PCs. As part of normal physiology, this inhibitory function of PCs is likely altered based on mechanical flow or ligand-mediated regulation of PC1 that is currently not understood. Neither reduced *Pkd1* nor increased *Pkd1* gene dosage affects cyst formation following cilia ablation, suggesting albeit not proving, that polycystic disease following cilia inactivation occurs by PC-independent mechanisms [53]. Taken together, the data show that loss of PCs causes kidney cyst formation in a cilium-dependent manner, but loss of cilia causes cyst formation independent of PC function (Fig. 2).

The data suggest that the molecular mechanisms of the cilium-dependent cyst promoting activity following loss of polycystins are likely to be novel. We examined the activities of the MAPK/ERK, mammalian target of rapamycin, and cAMP pathways, all of which have been implicated in cystic mouse models and therapeutic targets in human clinical trials, as candidate effectors for this novel cilium-dependent process. Although there was increased activity of both the MAPK/ERK and cAMP pathways in cystic mice, both activities were primarily confined to distal nephron. These pathways were therefore unable to account for the ubiquitous inhibition of cyst growth observed following cilia ablation in all nephron segments, including proximal tubule. Our data strongly suggest the presence of a new, cilium-dependent cyst-promoting pathway, which is suppressed by functional PCs. Our *in vivo* studies prove the principle that targeting of the cilium-dependent, PC-inhibited pathway will be effective in slowing the progression of ADPKD. This novel cilium-dependent pathway is now proposed as the central homeostatic mechanism of polycystins and cilium signaling in ADPKD. Defining components of this pathway will lead to improved targets for potential therapies in ADPKD.

Summary

The polycystin proteins are thought to be part of the signaling system of the cilium required to maintain normal renal structural and functional homeostasis. Both of the functioning polycystins and intact cilia seem to be required for tubular adaptation and maintaining the architecture of renal epithelial tubules. The steady state of the physiological roles of PC1 and PC2 regulates this cilium-dependent pathway and elicits responses of tubule adaptation to either chemical or mechanical signals. The cyst-suppressive role of polycystins requires either mechanical or ligand-mediated activation to affect calcium-dependent inhibition of cyst growth [46,54,55], but the precise nature of these signals remains unknown. Our findings strongly suggest that the components of cilium-dependent cyst growth signals reside in cilia and require intact cilia for activity. PC1 dosage can regulate cyst progression in ADPKD by having inadequate levels to suppress fully the cilium-dependent cyst-promoting pathway. This would explain the hypothesis that cyst growth lies along a continuum from physiological changes in tubule luminal diameter in response to discrete stimuli, to slow cyst grow in the setting of reduced PC1 function, and to more rapid cyst growth in the setting of completely absent PC1 function. Altering PC1 levels by chemical chaperone therapy may slow down cyst progression in a subset of patients in whom reduced, rather than absent function is the cause of ADPKD. Our understanding of ADPKD has evolved to include reduced PC dosage or PC loss as a cause of ADPKD and have provided genetic evidence of the existence of yet to be discovered novel targets for ADPKD therapy that hold promise for the future wellbeing of these patients with this complex disease.

Conflicts of interest

None.

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