The Pathogenesis of Autosomal Dominant Polycystic Kidney Disease

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Key Words

Polycystin 1 · Polycystin 2 · Autosomal dominant polycystic kidney disease · Cell calcium · Cell differentiation

Abstract

In individuals with autosomal dominant polycystic kidney disease (ADPKD), renal function deteriorates as the kidneys become replaced by multitudes of fluid-filled cysts. Although the PKD genes were identified a decade ago, the pathway(s) leading from mutation to disease remain the subject of intense investigation. As a result of this work, it has become apparent that the polycystins are multifunctional proteins that, in the broadest sense, appear to be involved in the transduction of a number of environmental cues into appropriate cellular responses. It is likely that the central pathogenetic pathway for cystogenesis stems from de-differentiation of tubular epithelial cells. Available evidence indicates that loss of polycystin activity leads to subtle derangements of cell calcium regulation through several possible pathways. Abnormal cell calcium homeostasis might then lead to altered differentiation in affected cells. The study of the polycystins has revealed some entirely novel insights into fundamental cell biology but these have not yet been satisfactorily integrated into a verified pathogenetic pathway for the development of ADPKD.

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Introduction

Autosomal dominant polycystic kidney disease (ADPKD) arises as a consequence of mutations in either one of two genes, named PKD1 and PKD2 [1, 2], which encode polycystins 1 and 2 (pc-1 and pc-2). The existence of a third locus for ADPKD has been suggested in ADPKD families lacking PKD1 or PKD2 linkage, but because of the relatively high prevalence of PKD mutations, this situation may arise from bilineal inheritance [3]. ADPKD arising from mutations in PKD1 (ADPKD type 1) has an identical phenotype to the disease related to mutations in the PKD2 gene (ADPKD type 2), with one notable exception – ADPKD type 1 patients exhibit more rapid progression of renal disease. The fact that the phenotype is so similar in both types of ADPKD suggests that the two polycystins almost certainly function in the same disease-related pathway(s). However, it appears that the polycystins might also function independently of each other, and, as such, are probably active in pathways that are not directly linked to ADPKD pathogenesis. In an attempt to focus on the molecular pathogenesis of ADPKD, this review will therefore concentrate on data from studies indicating that the activity of one polycystin requires interactions with the other. Because of this focus and the constraints of space, the literature review is selective. Therefore it is with regret that many other studies of undoubted relevance to the overall cell biology of the polycystins have been omitted from the bibliography.

Fig. 1. pc-1 and pc-2 are both transmembrane proteins that interact to form a functional complex whose disturbance results in ADPKD. The NH₂ terminus of pc-1 lies outside the cell and has a number of domains that are likely to bind extracellular proteins or carbohydrates. The extracellular domain of pc-1 is physiologically cleaved at the G-protein proteolytic site. The polycystin/lipoxygenase/ α -toxin (plat) domain in the first cytoplasmic loop indicates the potential for protein-protein or membrane interactions. The shorter COOH cytoplasmic tail of pc-1 contains a heterotrimeric G-protein binding and activation domain and a coiled-coil motif that is required for interaction with pc-2. Both the NH₂ and COOH termini of pc-2 are intracytoplasmic. The transmembrane spans of pc-2 share homology with voltage-activated α_1 channels that conduct Na⁺ and Ca²⁺ ions, and indicate that pc-2 might homodimerize to constitute a cation channel. The EF hand (configuration of E and F helices) is a Ca²⁺-sensing domain also found in α_1 channels.

The Polycystin Proteins (fig. 1)

Polycystin-1

Polycystin-1 (pc-1) is a 4302 amino acid protein with a large extracellular NH₂ terminal domain, 11 transmembrane domains and a shorter cytoplasmic COOH terminus. At the time of its identification, pc-1 appeared to be an entirely new type of protein, exhibiting no overall homology with any other known protein. However, within the next 2 years the receptor for egg jelly (REJ) protein was identified in the sea urchin sperm head and noted to possess a 700 residue module which resembled a portion of the pc-1 extracellular domain [4]. This part of pc-1 was subsequently named the REJ domain. In the sea urchin, the REJ protein is required for the triggering of transmembrane cation fluxes that activate the acrosome reaction in fertilization of the sea urchin egg. As depicted in figure 1, the extracellular NH₂ domain of pc-1 contains motifs likely to function as protein and carbohydrate binding sites and the PKD repeats may mediate homodimerization of pc-1. Between the REJ domain and the plasma membrane, there is a G-protein-coupled receptor proteolytic site (GPS). Physiologically important cleavage at this site explains why a majority of pc-1 exists as COOH and NH₂ terminal fragments [5], the former tethering the latter to the plasma membrane. The lipoxygenase homology (polycystin/lipoxygenase/ α -toxin or PLAT)

domain in the first cytoplasmic loop of pc-1 suggests that this site subtends interactions with other proteins or the cell membrane. Together, the REJ, GPS and PLAT domains are considered the defining features of an expanding family of pc-1-like proteins. The cytoplasmic COOH terminus contains a G protein binding and activation domain [6], several phosphorylation sites and a coiled-coil domain that is required for interaction with the COOH terminal of polycystin-2 [7].

Polycystin-2

Polycystin-2 (pc-2) is a 968 amino acid protein whose NH₂ and COOH termini are both cytoplasmic. This protein has six transmembrane domains (TMD) with homology to voltage-gated Ca²⁺ and Na⁺ channels. TMD 5 and 6 share homology with TRP (transient receptor potential) type channels. TRP channels might mediate the so-called capacitative calcium entry required for repletion of cellular calcium stores following a calcium release response. Other TRP-type proteins serve as plasma membrane mechano-, chemo- and thermoreceptors [8]. The TMD 1–6 of pc-2 have a moderate degree of homology to the last 6 TMD of pc-1. The COOH terminus of pc-2 has a coiled coil, an ER localization signal, a calcium-sensing region called an EF hand and four phosphorylation sites. Functional studies have demonstrated that pc-2 is a non-selective cation channel which transmits

calcium [9]. Importantly, one of the proven disease-causing mutations of pc-2 results in a single amino acid substitution in the pore-forming domain that abolishes its channel function whilst preserving interaction with pc-1 and normal subcellular localization [10]. This indicates that loss of the channel function of pc-2 might be sufficient to cause ADPKD.

Subcellular Localization

Polycystin-1 has been localized to the plasma membrane of renal epithelial cells in a basal distribution in relation to cell-cell contacts such as adherens junctions, areas of cell-matrix contact and also in the central apical cilia. There is some debate about the localization of pc-2. It appears that the majority of native pc-2 resides in the ER membrane, but significant signal is also seen to co-localize with pc-1 in the apical cilium of renal epithelial cells [11] and, as discussed below, there is some evidence that pc-2 can function as a channel in the plasma membrane [12]. Polycystin-2 is also localized to the basal body at the base of the cilia [13]. Since this structure is derived from the centriole, it may be that pc-2 is involved in the movement of chromosomes in mitosis. The localization of pc-1 might be dynamically regulated by pc-2 [14], and the distribution of pc-2 might be determined by trafficking proteins that shuttle pc-2 between the ER, Golgi and plasma membranes [15]. In view of the range of locations of pc-1 and pc-2, it may be that the distribution of these proteins is dependent upon cell type or culture conditions.

Molecular Genetics

The Second-Hit Hypothesis

From an analysis of human mutations and knockout (KO) mice, it is reasonably certain that ADPKD is caused by loss of function of one or other of the polycystins. However, although one would expect a generalized abnormality (in view of the ubiquitous germline mutation affecting one PKD allele), cysts appear to arise focally in otherwise normal appearing tubule segments. Furthermore, the type of dramatic cystic degeneration seen in human disease is only evident in mice in which both PKD alleles are disrupted (all these homozygous animals die in utero). By contrast, heterozygous PKD KO mice develop only a few cysts by adulthood. These observations were reconciled by the demonstration that 'two hits' are required for

development of the cystic phenotype [16] – the first being the germline mutation, followed by a somatic mutation in the remaining allele. Since the second hit would be predicted to be a rare and stochastic event, affected cells would probably be few and far between and cysts would be borne from individual cells rather than epithelial fields. Consistent with this scheme, epithelial cells harvested from at least some cysts were shown to be monoclonal [16]. However, more recent studies in chimeric mice suggest that part of the cyst wall can be formed by epithelial cells retaining the heterozygous state, raising the possibility that environmental cues elaborated by one group of cells may influence the phenotype of neighboring cells in the nephron [17]. The trans-heterozygous state (loss of one PKD1 allele and one PKD2 allele) represents another variation on the two-hit paradigm but is generally insufficient for cyst formation. However, there is evidence that the disease arising from homozygous loss of one PKD gene (due to the germline mutation compounded by somatic loss of the remaining allele) becomes more severe in the context of a co-existent germline deletion of one allele of the other PKD gene in a trans-heterozygous murine model [18]. Finally, a recent murine hypomorphic PKD1 model indicates that there might be a threshold level of pc-1 expression below which the polycystic phenotype develops, without the need for complete loss of functional protein [19].

The Two Polycystins Function in the Same Pathway

The similarity of the two ADPKD phenotypes and the molecular interactions between the two polycystins strongly suggest that the two proteins function in the same disease-related pathway. The first direct genetic evidence for this was derived from experiments in *Caenorhabditis elegans*, where loss of the worm pc-1-homologue *lov-1* resulted in a disturbance of mating behavior that was exactly replicated by loss of the worm pc-2 [20]. In the worm, both proteins are expressed in the cilia of sensory neurons. On the other hand, the functions of pc-1 and -2 are not so neatly superimposed in mice. Although pc-1 KO in mice gives rise to renal and pancreatic cysts just as seen in pc-2 KO mice, the two murine models differ in important ways. Polycystin-2 KO results in randomization of the left-right axis, perhaps due to loss of the capacity of embryonic nodal cilia to detect the flow of fluid that establishes lateralization. By contrast, left-right randomization is not seen in pc-1 KO mice. Conversely, pc-1 KO mice exhibit skeletal abnormalities that have not been described in pc-2 KO animals. Along these lines, pc-1 and pc-2 expression at the tissue level is not always concor-

Fig. 2. The polycystin complex probably responds to one or more extracellular signals by activating multiple intracellular signal transduction cascades. pc-1 and pc-2 have been shown to modulate a number of discrete signal transduction cascades including activation of the JAK/STAT pathway (Janus kinase/signal transducers and activators of transcription), AP-1 (a dimeric transcription factor composed of jun and one of a range of other subunits) and heterotrimeric G proteins. The polycystin complex might be responsive to soluble ligands (1), cell-cell contact (3) or cell-matrix interactions (5). The polycystins also signal through changes in cytoplasmic Ca^{2+} . Cilia deflection results in Ca^{2+} entry and subsequent Ca^{2+} -mediated Ca^{2+} release from the endoplasmic reticulum (ER) (2). The polycystin complex is also involved in ER Ca^{2+} turnover (4). Even if non-cilia pc-2 were to be restricted to the ER membrane, interactions between the polycystins might still be possible since the ER is a highly dynamic structure that could migrate into proximity with the plasma membrane. In addition to the sites listed above, pc-2 has also been localized to the basal body (6), a centriole-derived structure implicated in both ciliogenesis and, as the centrosome, the arrangement of the mitotic spindle.

dant in time or space. Although some subcellular expression patterns overlap (mutual localization to the apical cilia) some investigators hold the view that pc-2 is confined to the cilia and ER and is not represented at the other plasma membrane locations ascribed to pc-1. However, even if pc-2 is not distributed to all the plasma membrane domains inhabited by pc-1, the mobility of the ER

might still permit molecular interactions between the two proteins. Nonetheless, in addition to their concordant functions, the polycystins probably also function independently of each other in discrete pathways that might have nothing to do with ADPKD.

What Are the Functions of the Polycystins That Are Relevant to ADPKD? (fig. 2)

ADPKD cyst epithelial cells are flattened, lose tubule-specific markers and express a number of proteins associated with proliferation; all indicators of de-differentiation from the stable tubule epithelial phenotype [21]. These observations suggest that the polycystins might serve to maintain terminal differentiation in the renal tubular epithelium. The molecular characteristics and localization of the polycystins strongly suggest a role for the polycystin complex as a transducer of extracellular stimuli. Therefore it is likely that the fundamental defect in ADPKD is a profound disturbance of cellular responses to environmental cues, which leads to de-differentiation of renal tubular epithelia.

Polycystins and Cilia

In the same year that the worm polycystin homologues were identified as a functional complex residing in the cilia of sensory neurons, further examination of an established mouse model for PKD revealed that the protein product of the gene linked to the cystic phenotype, polaris, localized to the cilia and was required for the normal assembly of this organelle [22]. Since then, detailed examination of a number of other established murine models for PKD has revealed that most (but not all) arise from mutations in genes encoding cilia-associated proteins. Indeed, the autosomal recessive form of human polycystic kidney disease (ARPKD) is caused by loss of function of the PKHD1 gene product (a protein called fibrocystin or polyductin), which also localizes to the central cilia of renal and other epithelia [23]. Although the function of the central cilium of renal epithelial cells remains a mystery, both pc-1 and pc-2 appear to be required for the cell calcium response to flow across the apical membrane of ciliated cells, a response thought to be mediated by cilia deflection [24]. Since pc-2 belongs to the TRP family, which includes mechanosensors, the pc-1/pc-2 complex might detect deformation of the cilia membrane and mediate calcium influx with resultant calcium-mediated calcium release from the ER. However, regarding the cilia as solely a flow sensor is probably an oversimplification.

The *Chlamydomonas* flagellum (a cilium) contains well over 500 proteins indicating that cilia are actually rather complex organelles [25]. Cilia might be crucial regulators of cellular differentiation through a role in signal transduction, as chemosensors or as regulators of the cell cycle. The ciliary role of the polycystins, and how cilia relate to the ADPKD pathway, will likely remain uncertain until we more fully understand the broader cell biology of cilia and are able to develop more physiological functional readouts.

Although attractive, the hypothesis that it is loss of the ciliary responsiveness to luminal flow that serves as the trigger for the chain of events leading to cyst formation remains unproven. Cystogenesis could just as easily be initiated by cellular de-differentiation arising from disturbances in a wide range of alternative pathways. In addition, given the widespread subcellular distribution of the polycystins, these alternative pathways might be related to the cilia, or arise in other cellular compartments. Even if cilia are dysfunctional in ADPKD, the ciliary abnormality might be an indicator of a more central cell biological abnormality that is the actual driving mechanism for the disease process. This possibility is suggested by the fact that one organelle, the centriole, is dually responsible for the organization of cilia (basal body) and mitotic spindles (centrosome). Since pc-2 localizes to the basal body, polycystin signaling might be implicated in both the organization of mitosis and cilia function, with the former being in the disease-specific pathway. Indeed, it has recently been demonstrated that the normally longitudinal axis of mitosis in the developing nephron becomes disorganized in two animal models for cystic disease, the *Pck* rat and HNF1 β kidney-specific KO mouse [M. Pontoglio, pers. commun.].

Polycystins and Other Pathways

The polycystins have been identified in a number of non-ciliary locations, and implicated in a variety of signal transduction pathways not (yet) associated with ciliary function. Perturbation of any one of these pathways could lead to the kind of profound alterations in cellular differentiation and proliferation that might culminate in cyst formation. Many of these pathways also appear to require interactions between pc-1 and pc-2, which would substantiate their relevance to the ADPKD phenotype. As depicted in figure 1, pc-1 has an NH₂ terminal domain that looks like a receptor and has been shown

to interact with matrix proteins and carbohydrates. Perhaps the ciliary localization of the polycystins serves to ensure that pc-1 (and likely many other cell surface receptors [26]) gains access to extracellular ligands, unimpeded by the unstirred layer immediately adjacent to the external leaflet of the cell membrane. Hence, loss of ciliary integrity from any number of divergent genetic defects might not only interfere with flow sensing, but also lead to displacement of the components of other signal transduction cascades required for maintenance of cellular differentiation.

Polycystins and Ca²⁺ Signaling

The physical interaction between pc-1 and pc-2 raises the possibility that the binding of pc-1 to some extracellular ligand leads to modulation of the channel function of pc-2, a situation curiously analogous to the sea urchin fertilization response. The idea that pc-2 is a membrane channel regulated by pc-1 is supported by the observations that pc-2 can function as a plasma membrane calcium channel that requires pc-1 for stabilization at the cell surface in intact cells [12] and that the isolated COOH terminus of pc-1 attenuates pc-2 channel rundown in lipid bilayer experiments [27]. Even if pc-2 is indeed confined to the ER, interaction with plasma membrane pc-1 might still be permitted by way of apposition of the two membrane systems, depending upon the configuration of the cytoskeleton. In this way the polycystin complex might regulate calcium turnover across the ER membrane. Since pc-1 does not appear to be a channel [28], it is quite likely that the effects of pc-1 on the regulation of intracellular calcium seen in numerous studies are actually mediated by interactions with pc-2, and therefore fulfill the 'common pathway' condition to be considered as relevant to ADPKD pathogenesis. Loss of functional pc-1 prolongs ligand-activated cell calcium responses [24]. Conversely, heterologous expression of pc-1 appears to abbreviate the cell calcium response through acceleration of ER calcium re-uptake [29], perhaps through modulation of the function of ER localized pc-2. Several studies have shown that the isolated cytoplasmic COOH terminal of pc-1 also influences cellular calcium responses. In one study, the pc-1 truncation prolonged ligand-activated cell calcium responses [30] and in another, caused proliferation in association with subtle elevation of basal cell calcium concentration and activation of PKC [31]. Along these lines, others have reported that the pc-1 truncation activates NFAT (nuclear factor of activated T cells) signaling through increased cell calcium [32]. A similar truncated pc-1 construct transformed the cAMP

growth phenotype of renal tubular cells from suppression (normal) to proliferation (as seen in cystic epithelia); an effect replicated by maneuvers that lowered cytoplasmic calcium concentration [33, 34]. It should be noted that the cytoplasmic COOH terminal of pc-1 has the potential to act as a dominant negative with respect to native full-length protein [M. Sutters, unpubl. observations], so studies utilizing the truncation might in fact be evoking a 'polycystic' phenotype.

Other Signal Transduction Pathways Modulated by the Polycystin Complex

Interactions between the two polycystins are required for their participation in a number of other pathways with the potential to influence cellular differentiation. The effect of pc-2 to activate the transcription factor AP-1 is augmented by pc-1 [35]. Polycystin-1 activation of the JAK/STAT pathway (which inhibits proliferation through increased expression of p21) depends upon interaction with pc-2 [36]. Polycystin-1 has the potential to activate G α_i type heterotrimeric G proteins, an effect that is antagonized by co-expressed pc-2 [37].

Conclusion

Evidence is accumulating to suggest that ADPKD is the consequence of cellular de-differentiation stemming from the loss of a cell's capacity to recognize and correctly integrate signals arising in the extracellular environment. The external signals may be matrix proteins, soluble ligands or mechanical stimuli generated between cells, or arise as a consequence of deformation of the central apical cilia. Although the polycystins regulate a number of signal transduction pathways with the potential to influence cellular differentiation, much of the direct and circumstantial evidence indicates that the disease phenotype is triggered by dysregulation of intracellular calcium responses.

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