Chapter 7

Polycystins and Molecular Basis of Autosomal Dominant Polycystic Kidney Disease

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Abstract

Autosomal dominant polycystic kidney disease (ADPKD) is the most common renal monogenic disorder. It is characterized by progressive, bilateral renal cystic expansion followed by gradual loss of renal function after decades of life, while its systemic nature is reflected by extra-renal manifestations typically involving liver and the cardiovascular system. Cyst formation is triggered by mutations in the *PKD1* or *PKD2* genes. In most cysts, if

not all, cystogenesis follows a two-hit model in developmental kidneys, while in the mature organ broad and fast cyst formation requires a third hit such as kidney injury. The first hit is represented by the germline mutation whereas a somatic total or partial inactivation of the previously normal allele constitutes the second event, a process that is consistent with the focal nature of ADPKD cystogenesis. *PKD1* encodes polycystin-1 (PC1), a likely transmembrane mechano-sensor receptor comprised of a singular combination of structural domains present in other proteins. *PKD2*, in turn, encodes polycystin-2 (PC2), a non-selective cationic channel permeable to calcium composed by six transmembrane helices and intracytosolic C- and N-termini. In the primary cilium, PC1 regulates cell calcium influx by physically interacting with PC2 through their intracytosolic domains. Disruption of calcium cellular homeostasis increases cAMP cytosolic levels and affects cell cycle, leading to increased cell proliferation and transepithelial fluid secretion. In addition, disruption of PC1 C-terminus interactions with components of Wnt, mTOR, STAT3 and JAK2/STAT1 pathways is translated into a number of intracellular pathway abnormalities. In the same line, interactions between the PC2 C- and N-termini with ERK/B-Raf, GSK3β and other partners lead to disturbed cell proliferation, apoptosis and cell polarity. Mutations in *PKD1* can result, moreover, in cell adhesion and extracellular matrix alterations, due to the role of PC1 extracellular domains in cell-cell and cell-matrix contact. Defects in the mentioned pathways can also impact oriented cell division, contributing to cyst growth.

Key words: ADPKD; Cystogenesis; *PKD1*; *PKD2*; Polycystin-1; Polycystin-2

Introduction

Autosomal dominant polycystic kidney disease (ADPKD) is the most common monogenic renal disease, presenting a worldwide prevalence of 1:400-1000 (1). ADPKD is characterized by an intense and bilateral development of renal cysts that leads to progressive organ enlargement and, eventually, loss of renal function. This entity constitutes a systemic disorder, comprising cystic and noncystic involvement of multiple organs, including liver, pancreas, blood vessels, heart and brain (2). ADPKD is a genetically heterogeneous disease originated by a germline mutation in one of two genes, *PKD1* or *PKD2* (Polycystic Kidney Disease 1 and 2) (3,4). While *PKD1* is responsible for 85% of the cases in clinically-affected families (ADPKD1) and is associated with a more severe clinical course, mutations in *PKD2* are present in the remaining 15% of the patients (ADPKD2), who generally present a milder renal functional decline and a lower renal complication rate. Indeed, the progression to end-stage kidney disease (ESKD) occurs at the mean age of 54 years for ADPKD1 affected individuals and 74 years for ADPKD2 patients (5). This difference, however, is not sufficient to distinguish the two forms clinically, since the disease is associated with a large intra- and

inter-family phenotypic variability (6). Given its slower progression to ESKD, the lack of diagnosis in asymptomatic individuals suggests a likely underestimation of the ADPKD2 true prevalence. In fact, a population study revealed an ADPKD2 prevalence of 26% (7). Additionally, *PKD1* and *PKD2* mutations may coexist in the same patient, leading to severe and early ESKD, evincing interaction between these genes (8). A third ADPKD locus was suggested based on previous linkage analyses, however further studies performed in some of such families disregarded this possibility (9).

The *PKD1* and *PKD2* genes and products

PKD1 is a 46-exon gene localized at 16p13.3, spanning a genomic region of approximately 52-kb and giving rise to a 14-kb mRNA (3). In addition to its large size, six pseudogenes highly homologous to its 5' portion are also positioned on chromosome 16, sharing up to 99% of sequence identity. This peculiarity makes *PKD1* sequencing a challenging task and turns the employment of direct mutation analysis in the clinical setting more complex. *PKD1* encodes polycystin-1 (PC1), a transmembrane likely mechano-sensor receptor that encompasses a unique combination of structural motifs present in other proteins of known functions (Figure 1). Such features give a versatile structure to PC1, with the ability to perform a large number of functions and to interact with several molecular binding partners.

The N-terminus of the PC1 polypeptide chain starts with a signal peptide and leucine-rich repeats (LRR), a motif responsible for its interaction with the extracellular matrix and cell adhesion (Figure 1). The following domains are the cell wall integrity and stress response component (WCS) domain, thought to interact with carbohydrates, and a type-C lectin motif, involved in biological processes such as cellular signaling and exocytosis. An LDL-A domain follows along the primary sequence, suggesting a possible interaction between PC1 and LDL-related molecules. At the central portion of the chain, PC1 has 16 PKD repeats that share sequence similarity with immunoglobulin-like and fibronectin type-3 domains. Such repeats are supposedly stabilized by force-induced formation of a stable intermediate state, which is consistent with a role for PC1 in mechanical coupling between cells (10). The next motif is the polycystin-1 lipoxygenase α-toxin (PLAT) domain, the most conserved motif and the family signature of the PC1-like proteins. This domain has been identified in more than 1000 proteins with functions related to lipid binding (often calcium-dependent) and, sometimes, to protein binding. In MDCK (Madin-Darby Canine Kidney) cells, however, PLAT targets PC1 to the plasma membrane in a selective binding process to phosphatidylserine and L-α-phosphatidylinositol-4-phosphate (PI4P). This event is regulated by protein kinase A (PKA)-mediated phosphorylation of the PLAT domain,

which reduces PI4P binding and recruits β-arrestins and clathrin adaptor AP2 to trigger PC1 internalization (10). Just before the first transmembrane helix comes the GAIN (G protein-coupled receptor-autoproteolysis inducing) regulatory domain. The functional role of the GAIN-mediated PC1 cleavage is yet not well understood; nevertheless, a *Pkd1* GPS cleavage mouse mutant escapes lethality but develops rapidly progressive postnatal PKD (11). At this point, the PC1 polypeptide chain comprises 11 transmembrane domains, passes to the cytoplasm, and ends with the G-protein binding and the coil-coiled domains.
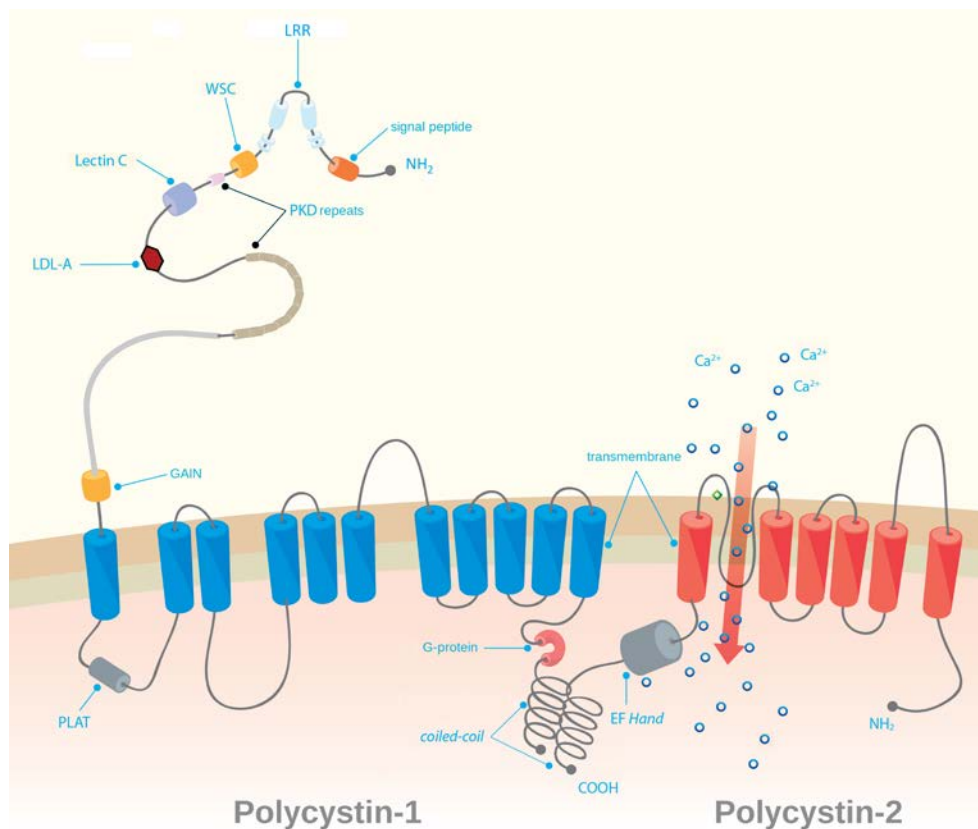

Figure 1. Domain structure of polycystins. Polycystin-1, from N- to C- terminus: signal peptide; leucine-rich repeats (LRR); WSC domain; lectin C type-3 domain; low-density lipoprotein-A domain (LDL-A); polycystic kidney disease (PKD) repeats; protein-coupled receptor auto-proteolysis inducing (GAIN) domain; Polycystin-1, Lipoxygenase, α-Toxin (PLAT) domain; transmembrane domains; G-protein domain; and coil-coiled domain. Polycystin-2, from N to C terminus: transmembrane domains; helix-loop-helix motif, calcium-binding EF-hand domain; and coil-coiled domain.

PKD2 localizes on 4q21, is smaller and encompasses 15 exons (4). However, as for *PKD1*, it presents high allelic heterogeneity and pathogenic mutations distributed along the gene (12). Its product, polycystin-2 (PC2), is a member of the transient receptor potential (TRP) superfamily, functioning as a non-selective cation channel of six transmembrane helices with high permeability to ionic calcium (Figure 1). Similarly to PC1, PC2 presents an intra-cytoplasmic C-terminal portion including a calcium-binding EF-hand and a coil-coiled subdomain which interacts with PC1 C-terminus and a number of additional binding partners (13) (Figure 1). In contrast, the PC2 N-terminus is small (223 amino-acids) and is turned to the cytosol.

The PC1 and PC2 molecular features, associated with their expression profiles, support the systemic nature of ADPKD. It must be noted that truncation mutations predominate in ADPKD, accounting for around 65% of the ADPKD1 and 83% of the ADPKD2 cases (12,14). Point mutations, in turn, are frequently found to be of uncertain clinical significance.

An interesting genomic aspect of PKD1 is its tail-to-tail chromosomal positioning to the *TSC2* gene (Tuberous Sclerosis Complex 2). Contiguous deletions involving both loci lead typically to an ADPKD-TSC associated phenotype expressing severely cystic kidneys (15). Such patients usually progress to early ESKD by the third decade of life.

PKD1 and *PKD2* also interact with genes mutated in various renal cystic diseases. All typical forms of autosomal recessive polycystic kidney disease (ARPKD) are caused by mutations in the *PKHD1* (Polycystic Kidney and Hepatic Disease 1) gene. Mice homozygous for a *Pkhd1* (the mouse orthologue to *PKHD1*) hypomorphic allele (*Pkhd1*[del3-4]) and heterozygous for a *Pkd1*-null mutation showed a more severe renal cystic phenotype than *Pkhd1*[del3-4/del3-4] animals (16). This genetic interaction was later extended to a genetic network, by breeding strategic genetically-modified mice with *Pkd1, Pkd2, Pkhd1, Sec63* and *Prkcsh* mutant alleles. The latter two genes are the mouse orthologues to human genes mutated in autosomal dominant polycystic liver disease (ADPLD) (17). The combination of *Sec63* or *Prkcsh* loss of activity with *Pkd1* or *Pkd2* null heterozygosity led to remarkable exacerbation of renal cystic burden (18). Loss of *Sec63* function associated with *Pkhd1* hypomorphic homozygosity also led to worsening of the renal cystic phenotype. This same study, in turn, revealed that PC1 overexpression was able to rescue the phenotype of cystic mice generated by inactivation of other than *Pkd1* genes. In this setting, the comprehensive analysis of all breedings allowed the proposal of a genetic network, based on the hypothesis that PC1 is the central determinant of cystic phenotype severity. Interestingly, *Sec63* and *Prkcsh* are genes involved in translocation and quality control of proteins in the

endoplasmic reticulum (ER), supporting PC1 processing as a limiting step in cyst development.

PC1-PC2 interactions and assembly

The interactions of polycystins between themselves or with other binding partners are of major interest and have been subject of a large number of studies. Such investigations are essential to determine and elucidate relevant biological pathways involved in the disease, as well as to identify potential treatment targets.

The most important and studied PC1 binding partner is PC2. This interaction has been described at several levels, from single amino acid contacts, passing through domain-domain interaction (19), to homo- and hetero-oligomeric associations (20,21). This binding process has been largely scrutinized by different experimental and computational approaches, using both *in vitro* and *in vivo* systems. Most of them point to the PC1 and PC2 intra-cytosolic domains as the critical interacting regions responsible for driving homo- and hetero-assembly. The EF-hand and the coil-coiled subdomains, in particular, represent the most important regions and constitute the key elements to understand the protein oligomerization state, as well as how they interact to each other. In the described scenario, the PC1 and PC2 homo- and hetero-interactions became a central and controversial issue in the PKD research field. PC2 seems to be required for the proper processing and trafficking of PC1 (22). Disruption of PC1-PC2 interaction, in fact, precludes PC1 to reach its mature glycosylated isoform needed to target the primary apical cilium (PAC, Figure 2). PC2 trafficking to the PAC, on the other hand, is not dependent on PC1, since the truncation of PC1 C-terminus does not impair this process (23). Transfection experiments with human embryonic kidney cells (HEK293T) showed that PC2 selectively associates with the transient receptor potential channels type 1 (TRPC1), but not type 3, to modulate calcium transient currents (19). In this study, a segment of 73 amino-acids within the PC2 C-terminus was shown to be one of the interacting regions. The fact that PC1-PC2 interactions are disrupted by native disease mutations supports biological relevance for a complex formed by these counterparts. The PC1 R4227X mutation, in fact, reduced the capacity of PC1 to interact with PC2 and abrogated calcium currents (24). Similarly, the deletion of the last 227 C-terminal amino-acids caused by the PC2 R742X mutation also led to calcium current disruption. Interestingly, this study suggested the need of PC1 to mobilize PC2 to the plasma membrane and regulate the intracellular calcium homeostasis (24).
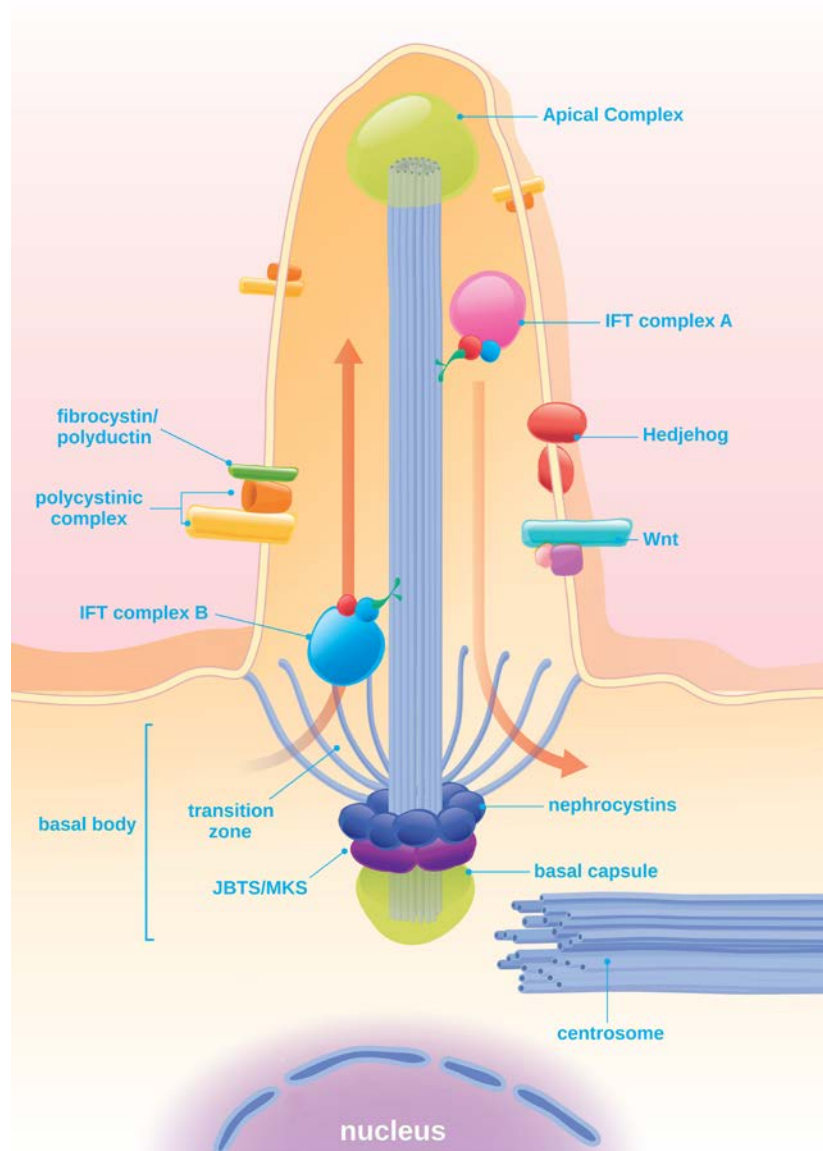
Figure 2. Basic structure of the primary apical cilium. The basal body separates PAC from the cytoplasm. This structure controls the ciliary trafficking in the transition zone and anchors the axoneme at the centrosome. JBTS (Joubert syndrome), MKS (Meckel syndrome) and nephrocystins are components of the basal body. IFT-B and IFT-A, respectively, transport protein cargo to and from the axoneme tip. PC1/PC2/FPC form a complex involved in calcium transients. FPC: fibrocystin/polyductin. Wnt (wingless integration 1) and Sonic Hedgehog are additional pathways present in the PAC.

Biophysical and biochemical studies addressed the multimerization properties of the polycystins. Atomic force microscopy (AFM) is a high resolution technique capable of producing images of big complexes and oligomers at a molecular level, such as receptors and channels. AFM imaging and single-channel patch clamp experiments supported a homo-tetrameric structure for PC2, a finding consistent with the PC2-expected functional roles (25). Other atomic force microscopy studies with PC1 and PC2, isolated from singly-transfected tsA 201 cells, indicated not only assemblies of PC1 and PC2 homotetramers, but also PC1-PC2 heterodimers with a 2:2 subunit stoichiometry following an alternative arrangement (26,27). The tetrameric arrangement is also found in other members of the TRP cation channel superfamily, such as TRPV6 (28) and TRPC1 (29). In support to this assembly pattern, a recent study reported a heteromeric TRP channel formed by TRPV4, TRPC1 and PC2 subunits (30).

Small Angle X ray Scattering (SAXS) analyses were also applied to investigate this theme. This technique takes advantage of X ray scattered by molecular surfaces. It has a wide applicability despite its low-resolution, ranging from nanoscience to soft condensed matter studies. The major advantage of SAXS over macromolecular crystallography is not to require crystals to derive molecular information, since the data are collected directly from protein solution (31). This technique, however, is sensitive to protein aggregation. In this context, SAXS has been successfully used to measure size, shape and oligomerization state of macromolecular complexes in solution. Based on the aforementioned principles, a study comprising SAXS and biochemical assays analyzed PC2, supporting a homo-tetrameric arrangement for the PC2 C-terminal intra-cytosolic domain. Such experiments also showed that the tetrameric organization does not depend on any other portion of the protein (32,33). A crystallographic study, on the other hand, supported a trimeric oligomerization for the coiled-coil subdomain of PC2 C-terminus (34), while a combined technical strategy supported this assembly for the whole PC2 C-terminus (35). Macromolecular crystallography is a very high resolution technique used to elucidate molecular structures. Its application requires molecular model building based on experimental X ray diffraction data from monocrystals (36). An additional resonance nuclear magnetic study conducted with the C-termini of PC2 and PC2-like channels also reached a trimeric arrangement (37). A similar structural organization was suggested by Size Exclusion Chromatography followed by Multi Angle Light Scattering (38), a very low resolution technique based on light scattering over the molecular surface. In this technique, the angle between the incident and scattered beams can be varied to increase the accuracy of the molecular hydrodynamic radius estimation. This information is usually introduced in the Stokes-Einstein equation and fitted against the experimental scattering intensity curve decay. This process involves assumption of a mono-modal gyration radius distribution and does not tolerate large deviations from globularity (39). Based on the available technical and

experimental information, both the trimeric and tetrameric PC2 C-terminus conformations are possible to occur *in vitro* and perhaps may coexist under specific conditions. Nevertheless, the trimeric arrangement seems less likely to be relevant *in vivo*, given that PC2 channel activity has not yet been shown for this conformation.

Polycystins in the cellular environment and the primary apical cilium

Discoveries in many renal cystic diseases and PKD animal models converged to the involvement of the primary apical cilium in cyst development (Figure 2). The observation of a common path for a number of such diseases gave rise to the concept of "ciliopathies" (40,41), a subset of disorders that include ADPKD, ARPKD, nephronophthisis, Bardet-Biedl syndrome and Meckel syndrome, among others.

PAC consists in a sensing organelle present in almost all nucleated cells in vertebrates (42) (Figure 2). It is a long projection of the plasma membrane, containing a microtubule-organized axonema running in its length and anchored in the centrosome. The PAC is separated from the cellular cytoplasm and membrane by a structure defined as basal body, which allows the cilium to maintain a unique composition of membrane proteins and to modulate signaling pathways differently from the rest of the cell.

Functional features

The PC1-PC2 complex, located at the PAC surface, appears to function as a fluid shear sensor to regulate the calcium signal. The PC1 extracellular portion is thought to be responsible for the mechano-sensor property, promoting the transduction of extracellular signal to the cytoplasm by activating PC2 through their intracytoplasmic domains. PC2, in turn, triggers the intracellular calcium release from the endoplasmic reticulum stores by modulating the inositol trisphosphate and ryanodine receptors (43,44). This regulation has been documented in mouse kidney cells. Cells bearing a *Pkd1* null mutation were unable to translate the shear stress signal into intracellular calcium transients. It must be noted, however, that a PC1L1–PC2L1 hetero-oligomeric complex has been recently shown to behave as the main calcium channel within the primary cilia in several cell types (45).

Another piece of work in cystic cells revealed that the loss of both ciliary polycystins led to defective calcium transients in response to angiotensin II and vasopressin (44). Ciliary disturbance has also been associated with cell division abnormality and incorrect planar cell polarity, suggesting that disoriented cell division may result in irregular tubule diameter and contribute to cyst development (46).

Binding partners and cell expression

Some studies showed that PC2, kinesin-2 and fibrocystin FPC (the *PKHD1* gene product), form a complex in the primary cilium, and in the perinuclear cytoplasm of renal epithelial cells (47,48). FPC stimulates PC2 channel activity in the presence of the kinesin-2 motor subunit, KIF3B, though the function of this complex is not completely understood (49). Notably, *Kif3A* knockout renal tubular epithelial cells do not have PAC, presenting cleavage of PC1 C-terminus and subsequent translocation to the nucleus to initiate AP-1 signaling (50). This pathway regulates gene expression in response to a variety of stimuli, including cytokines, growth factors and stress signals.

In a lipid bilayer electrophysiology system, α-actinin was shown to modulate the PC2 channel activity (51). In addition, PC2 and Hax-1 were shown to co-localize in the cell body of different cells. Hax-1 is a protein associated with the actin cytoskeleton, which binds the F-actin-binding protein cortactin to mediate association with other actin-binding proteins (52). PC2 was also found to direct associate with actin microfilaments of tropomyosin-1, a protein present in muscle cells (53), as well as to interact with an angiogenesis inhibitor, troponin-1 (54). Interestingly, PC2 was demonstrated to inhibit stretch-activated ion channels (SAC) and interact with filamin A, an actin crosslinking protein critical for SAC regulation (52). Yeast two-hybrid screening and immunoprecipitation assays revealed, in addition, that both PC1 and Pacsin2 C-termini domains and the neural Wiskott-Aldrich syndrome protein (N-Wasp) interact to form a protein complex (55). This complex, in turn, modulates the Arp2/3 protein complex function, involved in cell migration and actin filament nucleation, therefore contributing to the establishment and maintenance of the tubular architecture.

Mechanisms of cyst formation and growth in ADPKD

Renal cystic formation is a complex and not fully understood process in ADPKD, however it is known to involve cell clonal proliferation, increased apoptosis, abnormal epithelial cell phenotype, extracellular matrix alterations and inflammation (56). Though most cysts appear to derive from the collecting ducts, they may arise from any nephron segment. The development of such lesions follows a focal pattern, affecting less than 1% of the nephrons (57). The combination of the cited conjunctions appears to lead to focal tubular bulging, followed by the detachment of such a structure from the original nephron when it reaches a certain size. The continuous cyst expansion leads to progressive enlargement of the kidneys, alterations of their architecture and increase in kidney fibrosis; after reaching large kidney volumes, renal function loss occurs at a fast rate (56).

The two-hit, three-hit and threshold models

Although ADPKD presents dominant inheritance, its mechanism of cystogenesis is recessive at the cellular/molecular level, for most if not all cysts. Following a Knudsonian pattern, cyst formation was shown to obey a two-hit model in ADPKD human kidneys (58,59). The germline mutation constitutes the first event while the second hit is represented by a somatic mutation in the previously normal allele. According to this model, cystogenesis requires the inactivation or severe reduction of functional activities of both alleles of *PKD1* or *PKD2*. These observations are in accordance with the proposed clonal nature and focal profile of the cysts. They are also in agreement with the broad clinical variability within some ADPKD families and within the kidney itself, as well as with the increase in cyst number with age.

More recently, studies employing orthologous mouse models showed that a third hit is required for rapid and severe cyst development in mature kidneys. The inactivation of both *Pkd1* mouse alleles before 13 days of life led to massive and rapid cystogenesis, while after this age the same maneuver was followed by minor early cyst development and only late significant cystic disease (60,61). Interestingly, in another study both copies of *Pkd1* were inactivated at five weeks of age and, after three weeks, the mice were submitted to a unilateral renal ischemia-reperfusion insult (62). Remarkably, the injured kidney developed rapid and broad cystogenesis while the contralateral one did not, supporting the need for a third event for rapid cystogenesis in mature kidneys. The current thought is that this process depends on the reactivation of renal developmental programs and/or increase in cell proliferation rates triggered by renal injury. Altogether, the available studies suggest that cystogenesis arises when the *PKD1* or *PKD2* functional activity falls below a critical level in a given renal cell. This threshold model by Gallagher et al. (63) proposes, however, that this critical level can vary according to a series of factors, including the kidney developmental stage, environmental effects, increased functional demands induced by renal lesion, and genetic variants at modifier loci. Other cystogenic mechanisms have been described, however their relevance in human ADPKD has not been fully determined yet.

Role of defective intracellular calcium homoeostasis and cell response to cyclic AMP

Intracellular calcium participates in a number of cell signaling pathways, including the regulation of cyclic adenyl-monophosphate (cAMP) levels (64,65) (Figure 3). Low intra-cytosolic calcium leads to activation of adenylate cyclase 6 and inhibition of phosphodiesterases 1 and 3, favoring intracellular accumulation of this second messenger (66,67). As predicted by the molecular features of the polycystins, disruption of this pathway was shown to lead to a defective calcium homeostasis in renal tubular cells. Lack

of activity of either PC1 or PC2, in fact, impairs the calcium transient response determined by the PAC bending (68). Heterologous expression of PC1, in turn, inhibits calcium leakage through the ER membrane in MDCK cells. The high level of cellular cAMP detected in a number of ADPKD cells and animal models agrees with such findings (66,69,70). Interestingly, this calcium defective regulation also disrupts cell response to cAMP. While in normal cells cAMP induces cell cycle arrest, in ADPKD cells it leads to an abnormal proliferative response (71,72). cAMP has also been shown to participate in other components of ADPKD pathogenesis, such as inflammation, alterations in the cell polarity and, probably, extracellular matrix defects (68).

Increased cell proliferation and apoptosis

Clonal cell expansion is a major player in cyst development. Various pro-proliferative pathways are in fact upregulated in ADPKD. The significantly higher cyst epithelial cell number compared to renal tubules, observed in scanning electron micrographs, underscores this point (72). In support to this finding, several studies identified activation of the MAPK (mytogen-activated protein kinase)/ERK (extracellular-regulated protein kinase) pathway in cellular and animal PKD models (73–75) (Figure 3). This cascade transduces extracellular signal by activating small G proteins (SGP) which, in turn, lead to RAF1, LAMTOR3 and MAP3K phosphorylation. This sequential activation modulates the activity of transcriptional factors, favoring the advance of cell cycle. The inappropriate activation of this pathway in ADPKD is attributed to the accumulation of cAMP and consequent activation of PKA, apparently resulting from the defective calcium cellular handling (76). This abnormal signaling scenario activates phosphatidylinositol 3-kinase, resulting in the SGP activation (71) (Figure 3).

PKA activation also leads to tuberin (the *TSC2* gene product) phosphorylation, promoting upregulation of the mTOR (mammalian target of rapamycin) pro- proliferative pathway (77) (Figure 3). This process also appears to be responsible for the abnormal shift of glycolysis activation and intracellular ATP accumulation, favoring liver kinase B1 and AMP kinase inhibition and consequently further activation of mTOR (78). Notably, the PC1 intracytoplasmic tail directly interacts with tuberin, leading to mTOR inhibition. This may be another source of mTOR hyperactivation in ADPKD (79). PKA also contributes to cell proliferation by stabilizing β-catenin and inhibiting its degradation, which leads to canonic activation of the Wnt pathway. In addition, PKA activates CREB (cAMP responsive element-binding protein), promoting activation of the pro-proliferative STAT3 (signaling transducer and activator of transcription 3) (Figure 3) and PAX2 (paired box 2) pathways (80–82). Disruption of the JAK (Janus kinase)/STAT pathway has also been suggested to contribute to increased cell proliferation, since activation of PC1 activates JAK2 in a
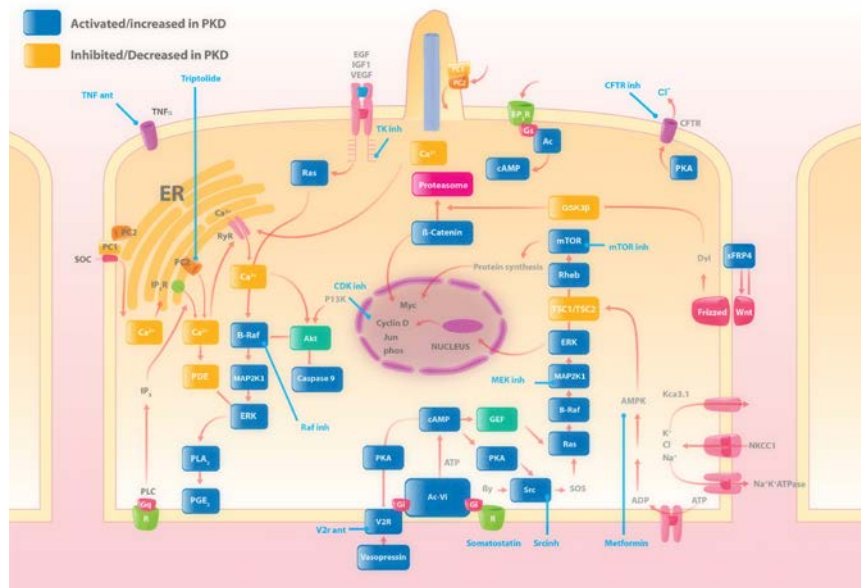
Figure 3. Cellular pathways involved in ADPKD pathogenesis. PC1 or PC2 loss of function impairs the calcium transient response mediated by PAC and ER, resulting in intracellular cAMP accumulation. An abnormal cell response to cAMP determined by the defective calcium homeostasis, in turn, leads to a pro-proliferative cellular status with activation of B-RafMAP2K1/ERK. Other alterations include loss of planar cell polarity with Wnt dysregulation and altered cellular energy metabolism, encompassing AMPK and mTOR disturbed modulation. The NKCC1 and CFTR localization pattern is essential to the observed secretory cell profile. V2R activation is a major source of cAMP production in the cell, consisting in a therapeutic target in ADPKD. TNF-α is a potential inflammatory mediator of the PKD phenotype. Adenylate cyclase (AC); adenosine monophosphate kinase (AMPK); v-raf murine sarcoma viral oncogene homolog B (B-Raf); cyclic adenosine monophosphate (cAMP); Cyclin-dependent kinase (CDK); cystic fibrosis transmembrane conductance regulator (CFTR); disheveled (Dlv); epithelial growth factor (EGF); E-prostanoid receptor 2 (EP2R); insulin-like growth factor (IGF1); endoplasmic reticulum (ER); extracellular signal-regulated kinase (ERK); guanine nucleotide exchange factor (GEF); G-protein inhibitory α subunit (Gi); G-protein q α subunit (Gq); G-protein stimulatory α subunit (Gs); inositol-triphosphate (IP3); glycogen synthase kinase 3 β (GSK3β); mammalian target of rapamycin (mTOR); Na-K-Cl cotransporter (NKCC1); Polycystin-1 (PC1); Polycystin-2 (PC2); phosphodiesterase (PDE); prostaglandin (PGE2); phospholipase A2 (PLA2); Phospholipase C (PLC); G-protein coupled receptor (R); rat sarcoma (Ras); Ras homolog enriched in brain (RHEB); ryanodine receptor (RyR); store-operated channel (SOC); secreted frizzled-related protein 4 (sFRP4); son of sevenless (SOS); sarcoma (Src); tumor necrosis factor α (TNFα); tuberous sclerosis complex 1 (TSC1); tuberous sclerosis complex 2 (TSC2); vasopressin 2 receptor (V2R); vessel endothelial growth factor (VEGF); wingless integration 1 (Wnt).

PC2-dependent manner. This process results in phosphorylation and formation of STAT1 homodimers, their translocation to the nucleus, p21 upregulation and inhibition of Cdk2 (cyclin-depend kinase-2) activity (83). In support to this mechanism, experiments conducted in the PKD mouse models jck and cpk showed that the administration of roscovitine, a Cdk inhibitor, resulted in inhibition of cystic disease (84). In parallel to high proliferation rates, ADPKD is associated to increased apoptosis. As predicted, PC1 has an anti-apoptotic effect. Pull-down experiments and NMR (nuclear magnetic resonance) structural studies showed association between the PC1 polyproline motif and the nephrocystin-1 (NPHP1) SH3 domain (85). In this system, PC1 requires NPHP1 to regulate resistance to apoptosis, but not to regulate cell cycle progression. Additionally, PC1 is capable to direct association with G proteins and, therefore, to participate in apoptosis regulation. Interestingly, a recent study reported that the induction of tumor necrosis factor-α (TNF-α)-dependent cyst epithelial cell apoptosis using a second mitochondria-derived activator of caspase (Smac)-mimetic in a *Pkd1*-targeted model led to attenuation of cyst development, bringing a different potential perspective for the role of apoptosis in ADPKD (86).

Alterations in cell polarity and fluid secretion

Cell polarity is essential for proper tubular cell function, by allowing the insertion of different sets of membrane transporters in the basal and apical membranes. The loss of this arrangement disrupts the appropriate flux of ions and water, turning the tubular epithelium pattern from absorptive to secretory. The localization of a Na-K-2Cl cotransporter in the basolateral membrane, along with the expression of cystic fibrosis transmembrane conductance regulator (CFTR) in the apical membrane (87), represents key abnormalities responsible for chloride and fluid secretion in ADPKD cysts.

Planar cell polarity is also disturbed in ADPKD. This alteration is attributed to *Wnt* canonical activation accompanied by reduction in non-canonic Wnt activity. Inappropriate spatially-oriented cell division (OCD), in turn, hinders the maintenance of tubule structure, favoring tubular dilation (88). Interestingly, in mouse models orthologous to ADPKD loss of OCD was not present before cystic formation, while *Pkhd1* deficient mice did not develop renal cysts despite loss of OCD. These data indicate that alterations of planar cell polarity are not the primary cause of cyst formation (89).

Role of inflammation in cyst growth and renal fibrosis

A number of studies support a role for inflammation in ADPKD cyst development. The observations that a PKD mouse non-orthologous to human ADPKD develops a smaller

number of renal cysts when kept in a germ-free environment, and that endotoxins are capable of rescuing the cystic phenotype in a chemically-induced PKD rat model, underscore this point (90,91). In addition, other studies in human ADPKD and rodent cystic models reported high levels of cytokines and chemokines in the cystic fluid as well as interstitial inflammatory infiltrates mainly represented by macrophages.

Data from non-orthologous PKD animal models revealed that cyst formation precedes the detection of interstitial macrophage accumulation (92). A recent study is in line with such findings, indicating a role for MIF (macrophage migration inhibitory factor) in cyst growth and suggesting a non-initial, secondary role for macrophages in this process (93). The depletion of macrophages in a *Pkd1*-targeted PKD mouse, on the other hand, led to a milder cystic phenotype and a better renal function, suggesting a macrophage-dependent effect on cyst expansion (94). In this case, and in a *Pkd2*-targeted mouse model, the alternative M2-macrophage activation was found to be predominant. It is interesting to note that this pathway is generally related to a regenerative profile, differing from the oxidative and pro-apoptotic features of the classical M1-macrophage activation. In ADPKD, however, the proliferative, remodeling and pro-fibrotic effects of M2 activation are likely to play a significant role in cyst growth, renal fibrosis and renal function decline.

STAT3 is a likely contributor to establish and keep the PKD inflammatory environment. This pathway is activated in cyst-lining cells and is known to activate the transcription of cytokines and growth factors in tumoral cells. These mediators, in turn, are capable of activating STAT3 in associated M2-macrophages, which may induce a feed-forward loop between such cells.

TNF-α was shown to misposition PC2, preventing its expression in the PAC (95). Interestingly, this cytokine is capable of inducing the formation of cystic structures in cell cultures, an effect intensified in *Pkd2*$^{+/-}$ cells. These findings suggest, therefore, a role for inflammation in cystogenesis.

Cell matrix alterations

Confocal microscopy and immunoprecipitation studies support a role for PC1 in cellular structures that mediate cell–matrix adhesion and components of various cell junctional complexes. ADPKD renal epithelial cells showed increased adhesion to type I collagen and express high levels of α2β1-integrin with overlapping colocalization with PC1 and focal adhesion proteins, as vinculin and paxillin (96). PC1 was also found in MDCK cell desmosomes, a cell structure specialized for cell-cell adhesion (97). In addition, yeast

two-hybrid experiments revealed intermediate filament protein vimentin as a strong PC1 interacting partner, as well as cytokeratins K8 and K18 and desmin (98). It must be noted that the PC1 lectin domain can bind to many extracellular matrix proteins, such as collagen type I, II and IV, a process that is amplified in the presence of calcium (99). The LRR domain appears to modulate PC1 binding to collagen I, fibronectin, laminin, and cyst fluid-derived laminin fragments (100). The observation that LRR induces reduction in cell proliferation suggests that this domain acts as a mediator of interactions between PC1 and extracellular matrix. In normal human fetal collecting tubules, immunocytochemistry and immunoprecipitation analyses showed that PC1 associates with focal adhesion proteins such as talin, vinculin, p130Cas, FAK, α-actinin, paxillin and pp60c-src (101). PC2 intra-cytosolic domain, in turn, was found in association with CD2AP, a protein adapter that regulates the assembly of focal adhesion complexes (102). Altogether, these studies place PC1 and PC2 dysfunction within the cell-matrix abnormalities observed in ADPKD.

Conclusion

The scientific information accumulated in the last two decades allowed expedited progress in the comprehension of ADPKD pathogenesis. Biochemical and molecular biology advances have provided key information to the elucidation of mechanisms involved in cyst development and disease progression. In addition to the basic and structural concepts presented and discussed in this chapter, the characterization of other cellular and systemic mechanisms has also contributed to understand fundamental features of this disorder, such as the process of renal function decline and its extra-renal manifestations. While a number of questions remain open in this research field, the fast growing knowledge brings promising perspectives for the understanding of ADPKD and additional therapeutic options.

Conflict of interest

The authors declare that they have no conflict of interest with respect to research, authorship and/or publication of this book chapter.

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