Autosomal Dominant Polycystic Kidney Disease: Recent Advances in Pathogenesis and Treatment

Ming-Yang Chang^{a, b} Albert C.M. Ong^a

^aAcademic Nephrology Unit, Sheffield Kidney Institute, School of Medicine and Biomedical Sciences, University of Sheffield, Sheffield, UK; ^bDepartment of Nephrology, Kidney Research Centre, Chang Gung Memorial Hospital, Chang Gung University, Taoyuan, Taiwan

Key Words

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Abstract

Autosomal dominant polycystic kidney disease (ADPKD) is a common genetic disorder affecting 1 in 1,000 people in the general population and accounts for up to 10% of all patients on renal replacement therapy. Numerous fluid-filled epithelial cysts arise from different nephron segments as spherical dilatations or small out-pouchings, enlarge progressively and eventually become disconnected from the rest of the renal tubule. The development of cysts is accompanied by destruction of the renal parenchyma, interstitial fibrosis, cellular infiltration and loss of functional nephrons. ADPKD is not only a kidney disease but also a systemic disorder associated with intracranial arterial aneurysms, cardiac valvular defects, colonic diverticulosis and cyst formation in other organs such as the liver, spleen and pancreas. The identification of *PKD1* and *PKD2* together with the drive to elucidate the functions of their encoded proteins, polycystin-1 (PC1) and polycystin-2 (PC2), has led to an explosion of clinical and scientific interest in this common disorder. The aim of this review is to highlight recent advances in our understanding of ADPKD pathogenesis which are leading to exciting new treatment strategies.

Molecular Genetics of ADPKD

Autosomal dominant polycystic kidney disease (ADPKD) can arise from mutations in either *PKD1* (85%) or *PKD2* (15%) with clinically indistinguishable manifestations. However, *PKD2* is usually of later onset since the mean age at end-stage renal disease (ESRD) occurs 16 years later than *PKD1* (69.1 vs. 53.0 years) [1]. So far, no apparent clustering or 'hot spot' of mutations has been identified in either gene. Most mutations are predicted to truncate the protein or lead to its degradation. However, production of a partially functional mutant protein has not been excluded. The search for possible genotype/phenotype correlations has yielded mixed results. Mutations in the 5' half of *PKD1* (relative to the median nucleotide) have been shown to be associated with slightly earlier ESRD compared to mutations in the 3' half of the gene and are also more strongly associated with aneurysm rupture [2, 3]. A clear genotype/phenotype correlation for *PKD2* has not been found [4]. The considerable intra-familial phenotypic variability, even in patients with identical mutations, reflects the strong influence of additional genetic modifiers and/or environmental factors on disease progression in ADPKD [5, 6].

Modifier genes could regulate the function of polycystin-1 (PC1) and polycystin-2 (PC2) by affecting polycystin-mediated signal transduction pathways, the rate of somatic mutations, cyst fluid accumulation or other clin-

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Dr. Albert C.M. Ong, Kidney Genetics Group
Academic Nephrology Unit, The Henry Wellcome Laboratories for Medical Research
School of Medicine and Biomedical Sciences, University of Sheffield
Beech Hill Road, Sheffield S10 2RX (UK)
Tel. +44 114 271 3402, Fax +44 114 271 1711, E-Mail a.ong@sheffield.ac.uk

ical factors associated with cyst initiation or disease progression [7]. In human ADPKD, these modifier genes are not well defined although several candidate loci have been investigated in small populations [8]. Confirmatory studies are required to clarify the modifying effects of these genes in larger well-defined ADPKD populations.

Mutational Mechanisms Underlying Cystogenesis

The mutational mechanism underlying cyst initiation in ADPKD remains controversial. Strong evidence for a two-hit mechanism has been presented which could help explain the focal nature of cyst development and the variability of the cystic phenotype [9, 10]. Nevertheless, there is accumulating data that haploinsufficiency itself may be sufficient to elicit a switch to a cystic phenotype. The strongest experimental evidence for this model has come from the generation of *Pkd1* hypomorphs where a reduction, but not absence, of *Pkd1* was sufficient to initiate renal and, in one study, extrarenal (vascular) features of ADPKD [11, 12]. Overexpression of *PKD1* itself leads to cyst formation, implying that gene dosage is important [13, 14]. Of interest, *Pkd1* heterozygous mice develop inappropriate anti-diuresis, and increased proliferative activity has been observed in the non-cystic tubules of *Pkd2* heterozygous mice [15, 16]. Both *Pkd1* heterozygous collecting ducts and *Pkd2* heterozygous vascular smooth muscle cells have lower intracellular Ca^{2+} levels [15, 17]. These findings appear to precede loss of a flow-sensitive (cilia) Ca^{2+} response observed in PC1 null cells as the latter is retained in PKD1 or *Pkd1* heterozygous tubular cells [18]. Thus, current evidence indicates that loss of a single allele of either *PKD1* or *PKD2* is sufficient to initiate phenotypic changes in tubular cells, activating a pathway which ultimately leads to cyst formation.

Functions of the Polycystin Proteins

Polycystin-1 (PC1)

PC1 is a 4,302-amino acid type I membrane glycoprotein containing a long N-terminal extracellular domain of over 3,000 amino acids, 11 transmembrane domains and a short cytoplasmic C-terminus (198 amino acids) [19]. PC1 interacts with PC2 via its C-terminus to form a heterodimeric complex. This primary interaction is critical for the functional regulation of both proteins [20].

A number of potential structural domains in the N-terminus may directly or indirectly (through E-cadherin

or β_1 -integrin) mediate cell–cell or cell–matrix interactions [7]. A direct role of PC1 in mediating cell–cell adhesion via homophilic interactions of the PKD domains has been demonstrated [21]. Heterophilic interactions of other domains (LRR, C-lectin) with purified extracellular matrix proteins have also been described. Of interest, the entire N-terminal ectodomain has the biomechanical properties of a mechanosensor [22]. Potentially it could sensor laminar flow or the force of coupling between cells or cell–matrix attachments during tubulogenesis. These force-triggered events could in turn activate a PC2-dependent Ca^{2+} signal and/or PC1-dependent signals. In addition to Ca^{2+} , the latter could include activation of monomeric GTPase proteins (Ras, Rho), heterotrimeric G proteins (Gi and Go), JAK/STAT, phosphatidylinositol 3-kinase (PI3-K)/Akt/mammalian target of rapamycin (mTOR) and activator protein 1 (AP-1)/mitogen-activated protein kinases (MAPK) pathways [7]. Finally, PC1 may undergo a stimulus-triggered proteolytic cleavage event to release its C-terminal tail – a process termed ‘regulated intramembrane proteolysis’ or RIP [23]. This fragment could translocate to the nucleus to directly initiate gene transcription.

Polycystin-2 (PC2)

PC2 is a 968-amino acid type II membrane glycoprotein with six transmembrane domains and intracellular N- and C-termini. PC2 (or TRPP2) has significant homology to the transient receptor potential (TRP) family of store-operated calcium channels and is likely to function similarly as a non-selective calcium channel [24]. Unlike *Pkd1* mice, *Pkd2* knockout mice were unexpectedly found to have a defect in left–right patterning of the body axis: PC2 (but not PC1) is expressed by monocilia at the mouse embryonic node and may mediate the asymmetric Ca^{2+} signal proximal to the establishment of left–right asymmetry [25]. Similarly, PC2 (together with PC1) has been shown to mediate flow-induced Ca^{2+} signals originating from primary cilia of kidney epithelial cells [26].

The subcellular location of PC2 has been an area of controversy with immunolocalisation to the endoplasmic reticulum (ER), lateral plasma membrane and primary cilia. PC2 does not contain a classical ER retention signal; rather, a stretch of acidic residues containing a casein kinase 2 phosphorylation site (Ser⁸¹²) appears to mediate its retrograde trafficking between the ER, Golgi and plasma membrane compartments [27]. A second phosphorylation site for PC2 within its N-terminal domain (Ser⁷⁶) is critical for its localization in the lateral plasma

membrane but not in the primary cilia [28]. A ciliary localisation signal in its N-terminus has also been described [29]. These three distinct non-overlapping recognition motifs could determine the subcellular location of PC2 and hence its function within different compartments. Finally, there is evidence that PC2 channel activity can be modulated by its interaction with the actin (lateral membrane?) or microtubule (cilia?) cytoskeleton [30, 31].

Mechanisms of Cyst Formation

Human and experimental data suggest several potential mechanisms that could lead to cyst formation in ADPKD: (1) increased cell proliferation and apoptosis; (2) enhanced fluid secretion; (3) abnormal cell–matrix interactions; (4) alterations in cell polarity, and (5) abnormal ciliary structure or function.

Cell Proliferation and Apoptosis

A primary increase in tubular proliferative activity, e.g. through the transgenic expression of oncogenes or growth factors, can lead to cyst formation [32]. PKD1 cystic epithelial cells are more sensitive to the mitogenic effect of growth factors and cAMP in vitro – these effects are dependent on Ras and Raf activity [33]. ADPKD cystic cells also have lower intracellular Ca^{2+} concentrations and this appears to correlate with their responsiveness to cAMP via a B-Raf/ERK pathway [34]. A potential approach to inhibit cAMP-dependent cell proliferation could be by restoring intracellular Ca^{2+} regulation in these cells – this may be the basis of action of triptolide, a recently described natural compound, on cyst formation in Pkd1 null mice [35].

A primary defect in apoptotic regulation (e.g. bcl-2 null mice) can result in a cystic phenotype [36]. In human ADPKD tissue, high levels of c-myc expression have been found [37]. Moreover, kidney-specific overexpression of c-myc (SBM mice) leads to a cystic phenotype and an increase in both proliferation and apoptosis [38]. Conversely, transgenic expression of human PC1 in MDCK cells can induce growth inhibition and resistance to apoptosis suggesting that, like c-myc, PC1 could exert independent effects on both cellular processes [39].

Fluid Secretion

In patients with ADPKD, the tubular epithelium appears to switch from an absorptive to a secretory phenotype. Tubular secretion is primarily mediated by trans-epithelial Cl^- secretion with passive Na^+ and water move-

ment [40]. Chloride transport could be mediated by several channels including the cystic fibrosis transmembrane conductance regulator and Ca^{2+} -sensitive Cl^- channels [41, 42]. Intracellular cAMP and extracellular ATP may be involved in regulating this process [43, 44]. Recently, forskolin, a potent stimulator of adenylate cyclase, has been isolated from human ADPKD cyst fluid samples [45].

Cell–Matrix Interactions

A primary defect in laminin synthesis has recently been shown to cause PKD in a study describing a hypomorphic allele of laminin α_5 [46]. ADPKD cyst epithelia sit on an expanded basement membrane and abnormal increases in basement membrane components (laminin, fibronectin, type IV collagen, and heparan sulphate proteoglycan), and interstitial type I collagen have been reported. These changes in basement membrane composition could contribute to cyst initiation or expansion. PC1-deficient cystic cells have been shown to adhere more strongly to type I collagen by a β_1 -integrin-dependent mechanism and may mediate resistance to anoikis [47, 48].

Cell Polarity

Alterations in apicobasal or planar cell polarity have been described in several cystic cell models, although not always consistently. Mislocation of E-cadherin (to the apical surface) could impair the subsequent targeting of protein assemblies crucial for basolateral trafficking and could account for the observation of mislocalised basolateral proteins (e.g. EGF receptor, Na^+ - K^+ -ATPase) in ADPKD cyst epithelia [49]. Alternatively, abnormalities in planar cell polarity (Wnt signalling in NPHP2/inv mice) or oriented cell division (spindle orientation in HNF- β 1 mice) during tubular lengthening could be key mechanisms underlying cyst formation in some recessive models of PKD or nephronophthosis [50, 51].

Ciliary Structure and Function

A recent major theme in the elucidation of PKD pathogenesis is the association between structural abnormalities of the primary cilia (a previously neglected organelle) and a cystic phenotype [52]. Among several seminal observations were the findings that the worm pkd1 and pkd2 orthologues are functionally important in mechanosensory neuronal cilia and that PKD mice hypomorphic for Tg737/polaris have short kidney primary cilia [53, 54]. Many cystoproteins have since been immunolocalised to primary cilia or centrosomes (which give rise to primary cilia) and

Table 1. Comparison of potential treatments for PKD

Compound	Mechanism	Molecular target	PKD models	Potential side effects	Effect on liver cysts	Reference No.
Rapamycin	Antiproliferative	mTOR	Han:SPRD rat orpk mice bpc mice Human	Oral mucositis, tremor, hypertension	Unknown	65
Vasopressin V2R antagonist	Inhibit fluid secretion and cell proliferation	cAMP	pcy mice PCK rat Pkd2 ^{WS25/-}	Hypernatremia, thirst	No	60
Somatostatin	Inhibit fluid secretion and cell proliferation	cAMP	PCK rat Human	Diarrhoea, anorexia, gallstones	Yes	61
Triptolide	Antiproliferative	Ca ²⁺ signalling (PC2-dependent)	Pkd1 ^{-/-} mice (from E10.5)	Male infertility	Unknown	35
Roscovitine	Antiproliferative	Cyclin-dependent kinases (Cdk)	cpk mice jck mice	Nausea, transient elevation of creatinine, hypokalemia	Unknown	58
Tyrosine kinase inhibitor	Antiproliferative	EGF receptor	bpc mice Han:SPRD rat	Diarrhoea, rashes	No	66, 67
Batimastat	Inhibit matrix degradation	Metalloproteinases	Han:SPRD rat	Arthralgia	Unknown	68
Caspase inhibitor	Antiapoptosis	Caspases	Han:SPRD rat	Diarrhoea	Unknown	69
Pioglitazone	Antiproliferative	Peroxisome-proliferator-activated receptor- γ (PPAR- γ)	Pkd1 ^{-/-} mice (from E7.5)	Hypoglycemia, weight gain, fluid retention	Unknown	70

Fig. 1. Hypothetical scheme of signalling abnormalities and molecular targets in ADPKD. Major inhibitors are indicated by the pink arrowheads. The yellow arrows indicate key changes in signalling intermediates. CFTR = Cystic fibrosis transmembrane conductance regulator; mTOR = mammalian target of rapamycin; PC1 = polycystin-1; PC2 = polycystin-2; V2R = vasopressin V2 receptor; SSTR = somatostatin receptor.

some have been associated with structural or functional abnormalities of these organelles. Indeed, kidney-specific inactivation of Kif3A, a protein involved in the maintenance of renal ciliary structure, can itself lead to PKD [55]. In PKD1, the major ciliary abnormality does not appear to be structural, but rather functional: Pkd1 null collecting duct cells or human cyst-lining cells have defects in flow-induced ciliary Ca²⁺ signals [18, 26, 56]. How abnormalities in ciliary Ca²⁺ signals lead to the complex cystic phenotype of ADPKD cells (see above) and hence cystogenesis itself is the focus of intense investigation.

Treatment Targets for ADPKD

Figure 1 summarises some of the major advances in our present understanding of the cellular mechanisms underlying the cystogenesis in ADPKD. These in turn have led to some interesting new molecular targets as potential treatments (table 1). Overall, these approaches can be summarised into two major ones: targeting cell proliferation and lowering cAMP.

In view of the pronounced increase in tubular proliferation, several groups have attempted an anti-prolifera-

tive strategy in experimental models. Examples of these are the use of rapamycin (to inhibit mTOR), R-roscovitine (to inhibit cdk2) and EGF receptor tyrosine kinase inhibitors. Inhibition of mTOR by rapamycin decreased cell proliferation and prevented cyst enlargement in several models including the Han:SPRD rat, *orp*k-rescue and *bpk* mouse models [57]. Treatment with the cyclin-dependent kinase inhibitor R-roscovitine effectively arrested cystic disease in *jck* and *cpk* mice [58]. Administration of EGF receptor tyrosine kinase inhibitors, EKB-569 and EKI-785, markedly reduced cyst formation in *bpk* mice and Han:SPRD rats, though not in the PCK rat. Alternative approaches to block downstream mitogenic signalling intermediates (e.g. ERK) have also been attempted [59].

In view of its stimulatory effects on cell proliferation and fluid secretion, a second major approach has been to target renal cAMP production. In landmark studies, administration of the vasopressin V2 receptor (V2R) antagonist, OPC31260, effectively inhibited disease development and progression in three rodent models – *pcy* mice, PCK rats and *Pkd2*^{WS25/-} mice [60]. Vasopressin antagonism was associated with a decrease in kidney cAMP concentrations. Similarly, somatostatin treatment (which can inhibit cAMP-stimulated chloride secretion) led to a slowing in the rate of cyst volume expansion in a small study of ADPKD patients and in PCK rats [61]. The role of vasopressin action in modifying cyst expansion in ADPKD has been given added weight by recent studies which indirectly suppressed (high fluid intake) [62] or enhanced its action (endothelin B receptor blockade) [63] on the collecting duct.

Table 1 summarises other potential approaches that have been attempted in PKD models. Other features associated with cyst enlargement such as apoptosis, inflammation or interstitial fibrosis could represent alternative treatment targets. One note of caution is necessary when directly extrapolating results from cystic rodent models for human ADPKD. Clearly not all cystic diseases are the same. Apart from obvious differences in genotype, cysts may originate from different nephron segments and this could influence the effectiveness of different drugs [64]. We suggest that preclinical testing of candidate drugs should include the use of orthologous models of ADPKD as a minimum.

At present, blood pressure control remains the major treatment modality though currently, at least 3 major clinical trials seeking to alter the natural history of the disease (tolvaptan, rapamycin and somatostatin) are in progress. Given the complexity of the cystic disease process, it seems likely that a combination strategy will be required for maximal therapeutic benefit. The next decade holds much promise of an effective treatment for a once untreatable disease.

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