

## Review

# Drug discovery for polycystic kidney disease

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In polycystic kidney disease (PKD), a most common human genetic diseases, fluid-filled cysts displace normal renal tubules and cause end-stage renal failure. PKD is a serious and costly disorder. There is no available therapy that prevents or slows down the cystogenesis and cyst expansion in PKD. Numerous efforts have been made to find drug targets and the candidate drugs to treat PKD. Recent studies have defined the mechanisms underlying PKD and new therapies directed toward them. In this review article, we summarize the pathogenesis of PKD, possible drug targets, available PKD models for screening and evaluating new drugs as well as candidate drugs that are being developed.

**Keywords:** polycystic kidney disease; drug discovery; kidney; candidate drugs; animal model

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## Introduction

Polycystic kidney disease (PKD), an inherited human renal disease, is characterized by massive enlargement of fluid-filled renal tubular and/or collecting duct cysts<sup>[1]</sup>. Progressively enlarging cysts compromise normal renal parenchyma, often leading to renal failure. The occurrence of autosomal dominant polycystic kidney disease (ADPKD) is estimated to be between 1 in 1000 and 1 in 400 individuals by a study in Olmsted Country, MN<sup>[2]</sup>. ADPKD is caused by mutations in one of two genes (*Pkd1* and *Pkd2*) expressing the interacting polycystic proteins polycystin-1 (PC1) and polycystin-2 (PC2) in renal tubular epithelia<sup>[3, 4]</sup>. Mutation of *Pkd1* accounts for approximately 85% cases in clinically identified patients<sup>[5]</sup>. PC1 is a membrane receptor capable of binding and interacting with many proteins, including carbohydrates and lipids, and eliciting intracellular responses through phosphorylation pathways<sup>[6, 7]</sup>. PC2 is thought to act as a calcium permeable channel<sup>[8, 9]</sup>. PC1 and PC2 form a complex that localizes to primary cilia<sup>[10, 11]</sup>. The polycystin complex has a role in the regulation of the proliferation, differentiation and morphogenesis of renal tubular cells through interactions with protein complexes linked to the actin cytoskeleton, intracellular signaling cascades, and the regulation of gene transcription<sup>[12, 13]</sup> (Figure 1). In ADPKD, the thousands of large, spherical cysts of various sizes throughout the cortex and medulla are derived from

the segments of the nephron. Autosomal recessive polycystic kidney disease (ARPKD) results primarily from the mutations in a single gene, *Pkhd1*<sup>[14]</sup>. Its frequency is estimated to be one per 20000 individuals. The PKHD1 protein, fibrocystin, has been found to be localized to primary cilia and the basal bodies. The exact function of fibrocystin has not been demonstrated. In ARPKD, smaller, elongated cysts arise as ecstatic expansions of collecting ducts. Patients with PKD often require dialysis and kidney transplantation, which are exceedingly costly. There are currently no approved drug or preventive strategies for PKD.

## Mechanisms of renal cyst formation and enlargement in PKD

The development and growth of PKD cysts involve the abnormal proliferation and apoptosis of immature epithelial cells, accumulation of fluid within the cyst cavity, abnormal cell-cell/cell-matrix interactions and abnormal cilia function.

## Role of epithelial cell proliferation and apoptosis in cyst development in PKD

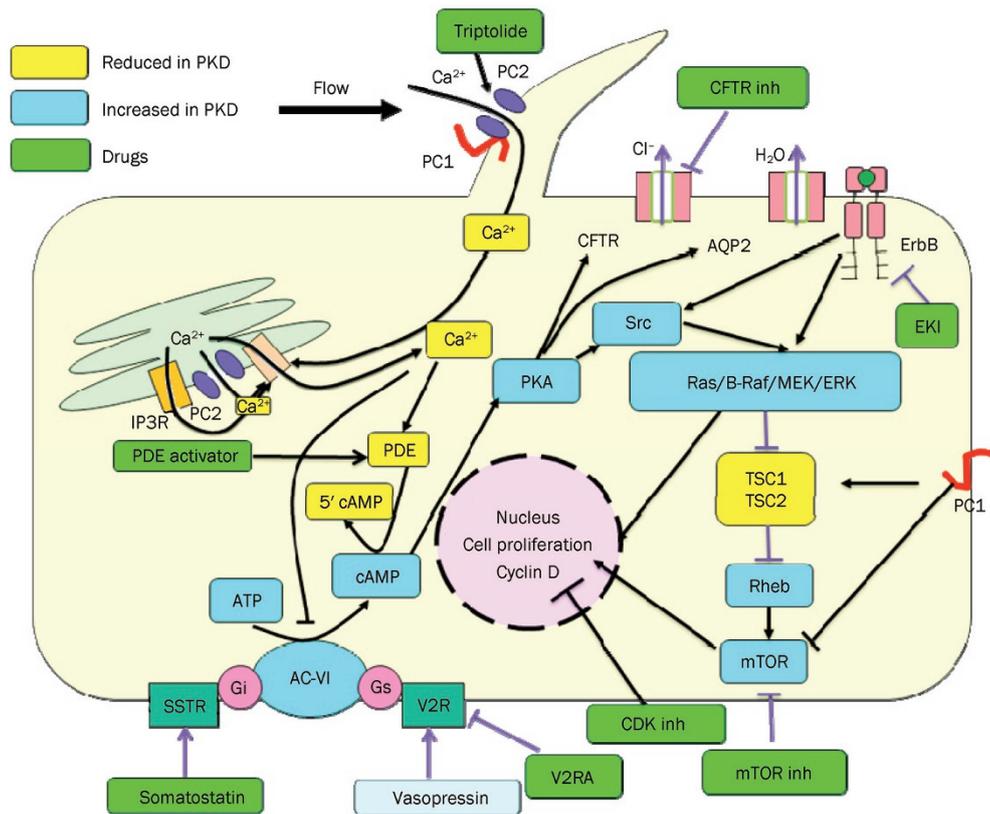
Increased apoptosis and proliferative capacity in renal epithelial cells are essential processes in PKD. While the proliferation of renal tubular epithelial cells halts before birth in normal individuals, cystic epithelia proliferate throughout life in patients with ADPKD<sup>[15]</sup>. Several genetic manipulations that increase the proliferation of tubular epithelial cells in mice result in PKD<sup>[16–19]</sup>.

Epidermal growth factor (EGF), transforming growth fac-

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**Figure 1.** A diagram depicting the effects that PC1 and PC2 exert on signaling pathways. Multiple direct and indirect interactions allow the polycystin proteins to be inhibited or stimulated. Pathways involved in cell proliferation and liquid secretion.

tor alpha (TGF- $\alpha$ ) and EGF receptor (EGFR) promote cystic epithelial proliferation and expand renal cysts. EGFR is over-expressed and mislocalized to the apical membranes of cystic epithelial cells, which leads to a sustained stimulation of cell proliferation in the cysts<sup>[20]</sup>. Increased intracellular cAMP level also plays a crucial role in cystogenesis. The reduced calcium caused by mutation of Pkd1 or Pkd2 can inhibit adenylyl cyclase 6 leading to increased cAMP. Studies have demonstrated that cAMP inhibits the proliferation of normal renal epithelial cells. In contrast, cAMP promotes the proliferation of cells derived from PKD patients<sup>[21]</sup>. The switch is caused by decreased intracellular calcium levels in a polycystic kidney leading to cAMP-mediated stimulation of the B-Raf/MEK/ERK pathway instead of inhibiting the Ras/Raf/MEK/ERK pathway like in the normal kidney<sup>[22]</sup>. B-Raf is inhibited by Akt in normal cells, while it is activated because of decreased activation of Akt in calcium-restricted cells. Inhibitors of Akt and PI3K can reproduce the effects of calcium reduction. However, activation of Akt has been found in animal models of PKD, such as Pkd<sup>-/-</sup> mice, Han:SPRD rats and jck mice. Additional growth factors, cytokines, lipid factors, and adenosine triphosphate (ATP) also participate in regulating the proliferation of renal epithelial cells<sup>[23–25]</sup>. Cell apoptosis is also a key factor in the development of PKD. Knocking out the anti-apoptotic Bcl-2 and AP-2 genes or overexpression of the pro-apoptotic gene c-myc in mice results in renal cystogenesis<sup>[26]</sup>.

#### Role of fluid secretion in cyst development in PKD

Fluid secretion is a critical pathogenic mechanism associated with cyst formation and growth in PKD. Fluid secretion, coupled with epithelial hyperplasia, is necessary and sufficient to account for the dynamics of cyst growth. In PKD, a large number of cystic lesions lack afferent and efferent tubule connections, suggesting that cysts, which arise from tubular segments, become disconnected from the glomerular filtrate. The development and expansion of cystic lesions therefore requires net transepithelial fluid secretion. An extensive body of *in vitro* data implicates epithelial chloride secretion in the generation and maintenance of fluid-filled cysts<sup>[27]</sup>. The fluid secretion is driven by mechanisms that are similar to those found in other secretory epithelia. Chloride movement drives fluid into the cyst lumen. Fluid accumulation causes cyst enlargement directly by swelling cysts and indirectly by stretching cells to promote cell division<sup>[28]</sup>.

Cystic fibrosis transmembrane conductance regulator (CFTR), a cAMP-regulated chloride channel, is present on the apical membranes of many secretory epithelia. Chloride secretion through the CFTR has been implicated in the pathway of fluid secretion in PKD. *In vitro* experiments have suggested that increased cAMP-mediated chloride secretion provides the electrochemical driving force for fluid secretion in cystic epithelia<sup>[29]</sup>. CFTR is expressed in the apical membrane of intact cysts dissected from PKD kidneys<sup>[30]</sup>. An important role of CFTR in PKD fluid secretion is also supported by the observation that interference with CFTR protein production

(by treatment of ADPKD monolayers with antisense oligonucleotide against human CFTR) dramatically reduced fluid secretion by these cells<sup>[27]</sup>. Additional evidence supporting a role of CFTR in chloride secretion was obtained from immortalized cystic murine collecting duct cell lines isolated from CFTR mutant and CFTR wild-type mice. The wild-type cell lines formed numerous fluid-filled cysts in response to EGF and forskolin when cultured in three-dimensional collagen gels, whereas the CFTR mutant cell lines failed to form cysts under identical conditions<sup>[31]</sup>. These results demonstrate that CFTR is required for *in vitro* cyst formation. In a single family affected with both ADPKD and cystic fibrosis (CF), individual members with both ADPKD and CF had less severe renal disease than those family members with only ADPKD<sup>[32, 33]</sup>. These studies suggest that *in vivo*, defective CFTR function provides partial protection against renal cyst development and enlargement and suggests that modulation of epithelial chloride secretion may have therapeutic benefit in PKD. Using type I MDCK cells as a cell culture model of cyst development and growth, Sheppard's group found that the CFTR inhibitor CFTR<sub>inh</sub>-172 (see below) retarded cyst growth. In contrast, blockers of other types of apical membrane Cl<sup>-</sup> channels, which do not inhibit CFTR, failed to inhibit cyst growth<sup>[28]</sup>. Inhibition of cyst growth by CFTR inhibitors correlated with inhibition of cAMP-stimulated Cl<sup>-</sup> current, but not cell proliferation<sup>[28]</sup>. Their studies strongly suggest that CFTR inhibitors might retard cyst growth predominantly by inhibiting fluid accumulation within the cyst lumen. In two ADPKD animal models, PCK rats and the pcy mice, renal cAMP levels were significantly higher compared to that in wild-type animals. Expression of the water channel AQP2 and vasopressin V2 receptor (VPV2R) was also increased. Administration of the VPV2R antagonist OPC-31260 lowered renal cAMP levels and halted progression or caused regression of established cysts<sup>[33, 34]</sup>.

Aquaporin (AQP)-mediated water permeability in cyst epithelia may also be involved in fluid secretion in cyst formation and progression, as fluid consists of salts and water. Normally, several AQPs are expressed in kidney: AQP1 in the proximal tubule, thin descending limb of Henle, and vasa recta; AQP2 in the apical membranes of collecting duct; AQP3 and AQP4 in the basolateral membranes of collecting duct. It has been reported that AQP1 and AQP2 are expressed in cyst epithelia from patients with PKD<sup>[35]</sup>. Gattone *et al*<sup>[36]</sup> found AQP2 and AQP3 expression in cysts in C57BL/6J-cpk/cpk mice with autosomal recessive-infantile polycystic kidney disease. High aquaporin-dependent water permeability in cyst epithelium may be important to facilitate near-isosmolar fluid secretion, particularly in growing cysts that have low surface-to-volume ratios.

#### Role of cell-cell/cell-matrix interactions in cyst development in PKD

PC1 has been detected in tight junctions, adhesions junctions, desmosomes, focal adhesions, apical vesicles, and primary cilia<sup>[37, 38]</sup>. A study has shown that PC1 mediates cell-cell adhesion through the formation of strong homophilic interaction of

its Ig-like domains<sup>[39]</sup>. A significant downregulation of Pkd1 mRNA is detected in MDCK cysts compared to tubules, which leads to a striking reduction of membrane PC1 and mislocalization to the cytoplasmic pools<sup>[40]</sup>. It has been demonstrated that a controlled level of PC1 expressed at cell-cell junction is critical for normal tubular differentiation. In normal renal cells, PC1 forms a complex with the protein E-cadherin and its catenins. However, in primary cells from ADPKD patients, the PC1/E-cadherin/ $\beta$ -catenin complex was disrupted and was accompanied by increased PC1 phosphorylation, reduced E-cadherin and upregulated normal mesenchymal N-cadherin<sup>[41]</sup>.

Renal epithelial cells in ADPKD show increased PC1 adhesion to type I collagen compared with normal human epithelia<sup>[42]</sup>. The defects reduce the cell migratory capacity required for kidney morphogenesis<sup>[43]</sup>. The PC1 protein has been proven to regulate the relationships between the cell and matrix through interacting with  $\alpha$ 1 $\beta$ 2 integrin, vinculin, paxillin, p130-cas, talin and focal adhesion kinase (FAK)<sup>[42]</sup>. The basement-membrane composition and expression of matrix metalloproteases and their inhibitors are abnormal in PKD kidneys. It has been demonstrated that inactivation of several matrix adhesion receptors and focal adhesion complex-associated proteins result in cystogenesis<sup>[44-46]</sup>.

#### Role of cilia in cyst development in PKD

Renal cilia are microtubule-based, membrane-bound projections on the epithelia of the renal tubule and duct. Renal cilia have been reported to be mechanosensors and respond to flow by increasing intracellular calcium<sup>[47]</sup>. Several studies support that PC1 and PC2 localize to primary cilia<sup>[10, 38]</sup> and form a subfamily of transient receptor potential channels that are responsible for sensing flow and regulating levels of intracellular calcium<sup>[48]</sup>. The bending of cilia causes calcium influx into the cell through the PC2 channel<sup>[42]</sup>. The mechanosensory response is lost in cells with mutated PC1<sup>[48]</sup>. Many cellular functions that are related to PKD, such as gene expression, cell cycle, differentiation and apoptosis, are regulated by intracellular calcium concentration.

The dysfunction of cilia has a close relationship with cell cycle progression<sup>[49, 50]</sup>. PC1 upregulates p21 (waf1) through activating the JAK-STAT pathway and results in cell cycle arrest in G<sub>0</sub>/G<sub>1</sub><sup>[23]</sup>. The IFT88/Polaris protein, which is localized to cilia, has been demonstrated to be tightly associated with the centrosome during cell cycle transition<sup>[51]</sup>. Overexpression of IFT88/Polaris prevents G<sub>1</sub>/S transition and induces cell death. In contrast, deletion of IFT88/Polaris promotes cell cycle progression<sup>[51]</sup>. PC2 also can regulate the cell cycle through direct interaction with Id2, a member of the helix-loop-helix (HLH) protein family, which has been proven to regulate cell proliferation and differentiation<sup>[52]</sup>.

#### Experimental models for screening and evaluating new drugs for PKD

Several common experimental models that have been used to screen and evaluate the new PKD drugs at the cell, organ and

whole animal levels are described in subsequent sections.

#### Madin-Darby canine kidney (MDCK) cyst model

MDCK type I cells provide a useful *in vitro* model of cystogenesis for screening candidate inhibitors of cyst formation and growth (Figure 2). MDCK cells cultured in three-dimensional collagen gels with forskolin produce a polarized, single-layer, thinned epithelium surrounding a fluid-filled space similar to the cysts in PKD<sup>[53]</sup>. MDCK cells in cysts undergo proliferation, fluid transport and matrix remodeling, as seen in tubular epithelial cells cultured from PKD kidneys. Cyst formation and growth are cAMP-dependent, which is thought to independently increase cell proliferation and activate CFTR-facilitated transepithelial fluid secretion<sup>[28]</sup>. Recognizing its limitations, such as differences between MDCK cells versus renal epithelial cells and cell cultures versus intact kidneys, the MDCK cyst model may be used to identify cyst inhibitors that reduce cyst formation and enlargement without demonstrable cell toxicity or inhibition of cell proliferation.

#### Embryonic kidney cyst model

The embryonic kidney culture model permits organotypic growth and differentiation of renal tissue in defined medium without the confounding effects of circulating hormones and glomerular filtration<sup>[54]</sup>. In the absence of 8-Br-cAMP, kidneys cultured on porous cell culture inserts increase in size over 4 d, whereas numerous cystic structures were seen in the presence of 8-Br-cAMP (Figure 3). Although embryonic kidney cultures probably represent a better PKD model than MDCK cells, they are avascular and non-perfused and therefore are not exposed to the same environment as the *in vivo* kidney.

#### PKD mouse models

Pkd1<sup>flox/-</sup>;Ksp-Cre mice, are kidney-selective Pkd1 knockout mice that manifest a fulminant course with the development of large cysts (Figure 4), renal failure in the first 2 weeks of life and death by 20 d. This model is suitable to evaluate the efficacy of cyst inhibitors on retarding the growth of cysts in the distal segments of the nephron, including the medullary thick ascending limbs of the loops of Henle, distal convoluted tubule and collecting ducts. In humans, ADPKD develops slowly and causes renal failure at an average age of over 50 years. For experimental studies, this relatively severe model of ADPKD has been used, rather than mouse models in which disease develops more slowly because of the shorter time required for compound administration and the greater likelihood of observing an immediate benefit. Testing cyst inhibitors in the ADPKD mouse model should be of further utility in predicting efficacy in human ADPKD. The CFTR inhibitors significantly reduced cyst formation and clinical signs of PKD, as assessed by lower kidney weights and serum creatinine and urea concentrations in this mouse model<sup>[55]</sup>.

Pkd1<sup>flox</sup> mice and Ksp-Cre transgenic mice have been generated as described<sup>[56, 57]</sup>. Ksp-Cre mice express Cre recombinase in the kidney under the control of the Ksp-cadherin promoter<sup>[58]</sup>. Pkd1<sup>flox/-</sup>; Ksp-Cre mice were generated by cross-

breeding Pkd1<sup>flox/flox</sup> mice with Pkd1<sup>+/-</sup>;Ksp-Cre mice<sup>[56]</sup>. Neonatal mice (age 1 d) were genotyped by genomic PCR. Test compound or saline DMSO vehicle control were administered by subcutaneous injection on the backs of neonatal mice four times a day for 3 or 7 d using a 1 mL insulin syringe beginning at age 2 d. Pkd1<sup>flox/+</sup>; Ksp-Cre or Pkd1<sup>flox/+</sup> mice from the same litter were used as controls. Body weight was measured at d 5. Blood and urine samples were collected to measure the test compound concentration and renal function. The kidneys were removed, weighed, and fixed for histological examination or homogenized to determine the test compound content.

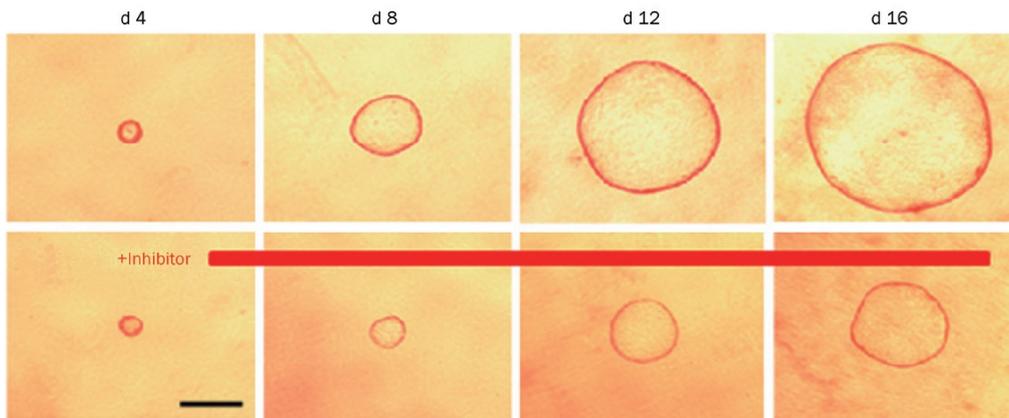
Many other mouse models of PKD have been described in which the mutant phenotypes result from spontaneous mutations or gene-specific targeting in mouse orthologs of human PKD genes. These murine phenotypes closely resemble human PKD with common abnormalities observed in the tubular epithelia, interstitial compartment, and extracellular matrix of cystic kidneys<sup>[59]</sup>.

Pkd1 and Pkd2 knockout mouse models, which are homologs of human genes, have been generated by targeted mutagenesis<sup>[59, 60]</sup>. In most of these models, heterozygous mice develop renal, biliary, and pancreatic cysts at age 4–19 months. Disease progression is rapid, with embryonic lethality occurring in most homozygous mutants.

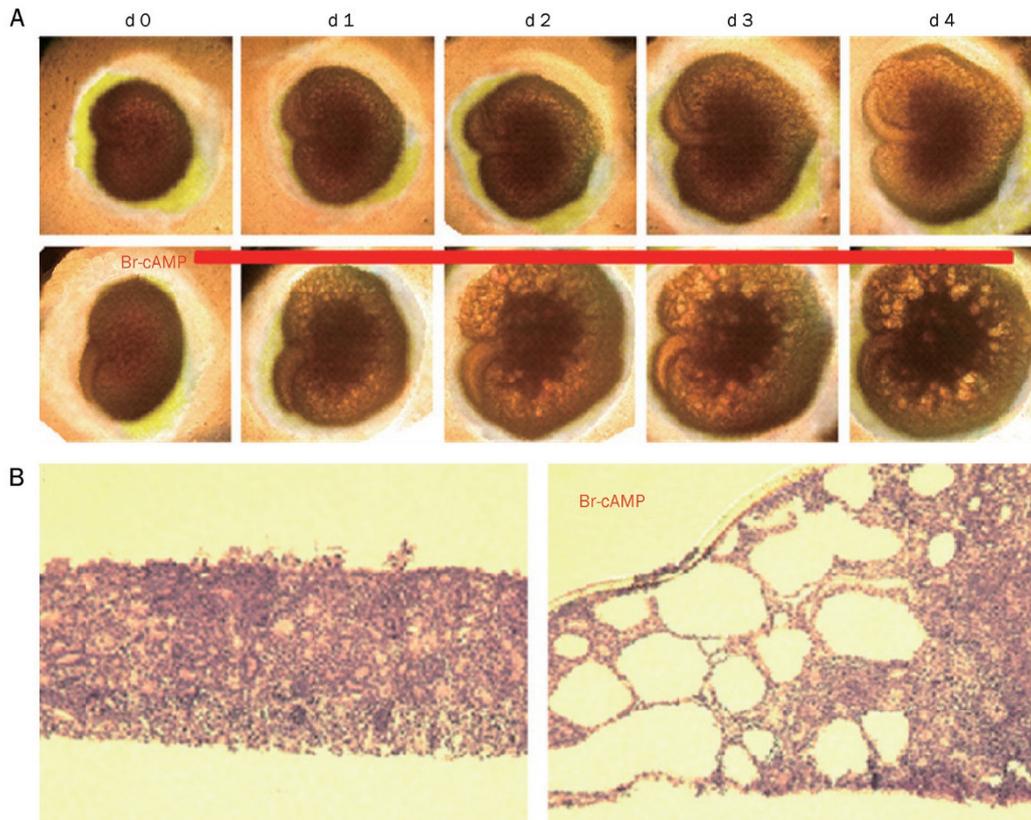
In the mouse models arising from spontaneous mutations, PKD is generally transmitted as an autosomal recessive trait. Several of these models with cysts distributed along the entire nephron and slower disease progression closely recapitulate human ADPKD<sup>[59]</sup>. One of them is the murine autosomal recessive juvenile cystic kidney (jck)<sup>[61]</sup>. The jck locus maps to chromosome 11. The mutant allele has a missense change in Nek8, which encodes NIMA (for 'never in mitosis' A)-related kinase 8<sup>[62]</sup>. In homozygous mutant mice, focal renal cysts are evident as early as 3 d of life, and the renal cystic disease is slowly progressive but not evident by kidney palpation until age 4 to 5 weeks. Histological analysis of jck mutant kidney tubules showed the defects were specific to the connecting segment and collecting duct cells. The proximal tubule cells appeared morphologically normal. Cell membrane and cytoplasmic disruption could be observed in collecting ducts from mutant mice at 2–3 weeks of age. No histological abnormalities in other organs have been described. The mutant mice are fertile and generally survive for 4 months or more.

Another PKD mouse model arose spontaneously by mutation of the "congenital polycystic kidney" (cpk) gene with locus mapping to mouse chromosome 12<sup>[63]</sup>. Cys1, the cpk gene, encodes cystin, which localizes to the primary apical cilia on collecting duct cells. Mutant mice develop massive renal cystic disease and progressive renal insufficiency in a pattern that resembles human ARPKD. Initial cystic changes are evident at approximately embryonic d 16 and localize primarily to the proximal tubule. With progressive postnatal age, the cystic changes predominantly involve the collecting duct. Death occurs by 3–4 weeks of age due to uremia<sup>[64]</sup>.

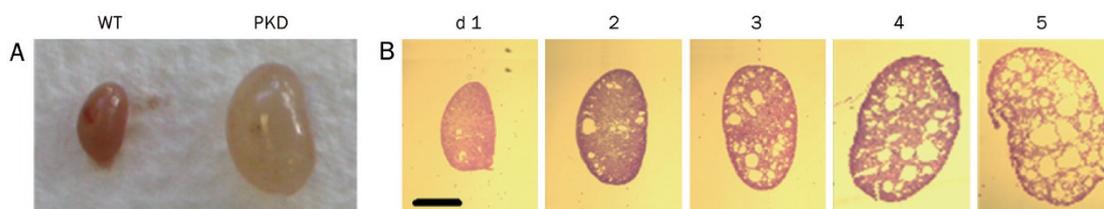
PKD in the kat mouse model is caused by a spontaneous mutation occurring in the Nek1 gene, which encodes NIMA-



**Figure 2.** MDCK cyst growth in collagen gels. Light micrographs were taken at indicated days after cell seeding of MDCK cells exposed continuously to 10  $\mu\text{mol/L}$  forskolin without (top) or with cyst inhibitor (bottom). Each series of photographs shows the same cyst on successive days in culture.



**Figure 3.** Embryonic kidney cyst model. Embryonic kidneys at d E13.5 were cultured for 4 d. (A) Kidney appearance by transmitted light microscopy for cultures in the absence (top) or continued presence (bottom) of 100  $\mu\text{mol/L}$  8-Br-cAMP. Each series of photographs shows the same kidney on successive days in culture. (B) Histology (hematoxylin and eosin staining) of embryonic kidneys.



**Figure 4.**  $\text{Pkd1}^{\text{flox}/-}; \text{Ksp-Cre}$  PKD mouse model. (A) Kidney from wildtype (left) and  $\text{Pkd1}^{\text{flox}/-}; \text{Ksp-Cre}$  PKD mouse (right) at age 5 d. (B) Histology (hematoxylin and eosin staining) of kidneys from  $\text{Pkd1}^{\text{flox}/-}; \text{Ksp-Cre}$  PKD mice at ages 1 to 5 d.

related kinase 1. In Nek1kat-2J homozygotes, fluid-filled cysts and dilated proximal tubules and Bowman spaces are found as early as 1 month of age. The bilateral renal cystic disease involves all levels of the nephron by 3 months of age. Disease progression, including growth of cysts and an increase in the number of cysts, is similar to that in ADPKD.

As a model of ADPKD, the Han:SPRD-cy rat has been used for research extensively<sup>[65-67]</sup>. The gene locus maps to chromosome 5<sup>[68]</sup>. The spontaneous mutation occurs in the Sprague-Dawley strain. In male Cy/+ rats, the kidneys enlarged more rapidly, and interstitial fibrosis is more pronounced<sup>[69]</sup>. The Han:SPRD Cy/+ rat can be studied for the efficacy of long-term medical therapy. In this model, the renal cyst exclusively develops in the proximal tubules instead of the whole renal segment. Other mouse models, bpk, jcpk, orpk, inv and pcy, also resemble human PKD with respect to renal cyst pathology and disease progression<sup>[60]</sup>. Because the murine models share common pathogenic features with human PKD, it is assumed that there are common molecular pathways involved in PKD progression in humans and mice. The jck, cpk, and kat mouse models are commercially available from the Jackson Laboratories.

The dynamics of cyst growth differ in the various models. These differences provide a unique opportunity to study the mechanism of cyst formation. The Nek8jck mouse model can be used mainly to test the preventive role of cyst inhibitors in the formation of cysts in collecting ducts of young mice. The Cys1cpk mouse model is suitable to test the role of cyst inhibitors on the progression of cysts and to compare the effects of treatments on cysts derived from different cell types in all levels of the nephron. The Nek1kat mouse model has been proposed to study the roles of cyst inhibitors on cysts derived from proximal tubules. Heterozygous Pkd2<sup>WS25</sup> mice, an ADPKD model generated by targeted mutagenesis, can be used to test the prevention and the treatment with cyst inhibitors on the development of cysts in the kidney and other organs.

Cyst progression can be evaluated by measuring the size and number of cysts in the kidney. At first, the ratio of kidney weight to body weight can be measured. Development of cysts should increase kidney weight. For light microscopic analysis, transverse tissue sections, including cortex, medulla and papilla, can be stained with H&E to measure cyst size and number. The analysis can be performed by a reviewer who is blinded to the identity of the treatment modality. To quantitatively evaluate cyst growth, cyst size can be recorded on the following scale: 0, <0.05 mm (It is difficult to distinguish the cysts from normal renal tubules); 1, 0.05–0.3 mm; 2, 0.3–0.6 mm; 3, 0.6–0.9 mm; 4, 0.9 mm–1.2 mm; and 5, >1.2 mm. The number of cysts can be counted in the cortex, medulla and papilla. In some experiments, the origin of renal tubule cysts can be determined by segment-specific lectin binding using Dolichos biflorus agglutinin (DBA) as a marker for collecting ducts and Lotus tetragonolobus (LTA) as a marker for proximal tubules as described previously<sup>[70]</sup>. The numbers of LTA-positive and DBA-positive cysts can be counted in serial

sections of bisected whole-mount kidneys from each animal. Proximal tubule cysts can be identified by LTA binding, and collecting duct cysts can be identified by DBA binding. A minimum of 10 sets of serial sections evenly spaced through the kidney from the cortex to the inner medulla can be used to determine the ratio of proximal tubule to collecting duct cysts.

### Candidate drugs under research and development

Based on the mechanism of renal cyst development and the pathogenesis of PKD, some chemical and natural compounds have been discovered to have inhibitory activity on renal cysts and to slow PKD progression. Some classes of candidate PKD drugs have been described according to the drug targets in PKD as follows.

#### Vasopressin 2 receptor (V2R) antagonist

Studies were conducted to target cAMP pathways and take a step further by demonstrating the upregulation of vasopressin and the inhibition of cytotogenesis by V2R antagonists OPC-31260 in cpk mice, ARPKD (PCK rat), ADPKD (Pkd2WS25 mice) and adolescent nephronophthisis (pcy mouse)<sup>[34, 35, 37]</sup>. As OPC-31260 is a weak antagonist for human V2R, clinical trials with tolvaptan, which has a higher affinity for human V2R, are underway. Tolvaptan was also effective in animal models of ARPKD, ADPKD, and nephronophthisis<sup>[71-73]</sup>. The Tolvaptan Efficacy and Safety in Management of PKD and Outcomes (TEMPO) program is currently active<sup>[74, 75]</sup>. Phase 2a studies to determine the response to increasing doses of tolvaptan (15, 30, 60, and 120 mg) in patients with ADPKD and normal renal function have been completed<sup>[75, 76]</sup>. A 3-year phase 3, placebo-controlled, double-blind study in 18- to 50-year-old patients with ADPKD to determine long-term safety and efficacy has been initiated and will be completed in 2011.

#### Renin angiotensin aldosterone system (RAAS) antagonist

Angiotensin-II (AT-II) has been demonstrated to promote cellular proliferation, apoptosis, and the production of TNF- $\alpha$  and other pro-inflammatory cytokines<sup>[77]</sup>. RAAS also plays an important role in hypertension. So, RAAS antagonism can prevent cellular proliferation and inflammation and treat hypertension in PKD. Angiotensin-converting enzyme (ACE) inhibitors, which are RAAS antagonists, have been proven to reduce cyst enlargement and blood pressure and improve renal function in Han:SPRD rats<sup>[78, 79]</sup>. A randomized 7-year study showed that ACE inhibitors prevented left ventricular hypertrophy better than calcium channel blockers in 75 hypertensive ADPKD patients<sup>[80]</sup>. An earlier longitudinal study has shown slower renal progression in those treated only with ACE inhibitor compared to only diuretics. Two HALT PKD trials that are randomized, double-blind, and placebo-controlled are underway to test the impact of intensive blockade of RAAS in ADPKD patients with ACE inhibitor or angiotensin receptor blocker (ARB)<sup>[81]</sup>.

**Epidermal growth factor receptor (EGFR) tyrosine kinase inhibitor**  
EGF is an important factor in cyst epithelial cell proliferation

and cystogenesis. EKI-785, an EGFR tyrosine kinase inhibitor, has been shown to be effective in reducing cyst formation and decreasing mortality in murine ARPKD<sup>[82]</sup>. EKI-785 and another EGFR tyrosine kinase inhibitor EKB-569 attenuate the development of PKD in Han:SPRD rats<sup>[66]</sup>. Contrary to other murine models of ARPKD, overexpression and mislocalization of EGFR are not found at the apical membrane of cystic cells in PCK rats<sup>[64]</sup>. This may be the reason that EKI-758 and EKI-569 have no efficacy in PCK rats<sup>[64]</sup>.

#### **Peroxisome proliferator-activated receptor- $\gamma$ (PPAR $\gamma$ ) agonist**

Proliferation is recognized as an important factor for cysts development in PKD. PPAR $\gamma$ , a member of the superfamily of nuclear hormone receptor transcription factors, has been demonstrated to suppress cell growth and promote differentiation and apoptosis in various cancer cells<sup>[83]</sup>. Thus, it may be effective in treating PKD. A recent study showed that the expression of PPAR $\gamma$  was greater in ADPKD kidneys and cyst-lining epithelial cells compared to normal kidneys and human kidney cortex cells<sup>[84]</sup>. Rosiglitazone, a PPAR $\gamma$  agonist, significantly inhibits the proliferation of ADPKD cystic epithelial cells by causing a G<sub>0</sub>/G<sub>1</sub> arrest. Short-term treatment in Han:SPRD rats with rosiglitazone has been shown to attenuate the development of kidney cysts and improve renal function, while long-term administration with rosiglitazone can prolong survival in Han:SPRD rats<sup>[67]</sup>.

#### **Somatostatin**

Octreotide, a kind of somatostatin, has been shown to inhibit hepatic and renal cystogenesis in PCK rats by decreasing cAMP accumulation<sup>[85]</sup>. A clinical trial has shown that octreotide safely slows renal volume expansion in 6-month therapy for 13 ADPKD patients<sup>[86]</sup>. Recently, octreotide has been tested as long-term treatment for polycystic kidney and polycystic liver disease in a clinical trial.

#### **Phosphodiesterase (PDE) activator**

In PKD, cAMP has been proven to be a critical intracellular second messenger involved in cytotogenesis. The level of cAMP is largely regulated by the PDE superfamily through hydroxylation. In mixed cortical tubules and microdissected tubular segments, 50%–70% of PDE activity is inhibited by an inhibitor of the calcium-calmodulin-sensitive PDE1<sup>[87]</sup>. PDE1 is responsible for cAMP and cGMP activity. The reduction of intracellular calcium in PKD may increase cAMP by dysregulating PDE1. PDE3 inhibited by increased cGMP are cAMP-specific PDEs. PDE3 may also be involved in cAMP accumulation in renal cells of PKD kidneys. In mesangial cells, PDE3 inhibitors increase cAMP levels and activate PKA, block phosphorylation of Raf-1 on serine 338 and suppress Raf-1 kinase activity<sup>[88]</sup>. PDE inhibitors stimulate MDCK cell proliferation. A recent study showed that the protein levels of PDE1, PDE3, and PDE4 are significantly reduced in the cysts of PCK and Pkd2<sup>-1/WS25</sup> kidneys compared with wild-type kidneys<sup>[89]</sup>, which indicates that a PDE activator may inhibit cystogenesis.

#### **Src inhibitor**

Src has been confirmed to be an important intermediary in cAMP pathways that promote epithelial proliferation in PKD and also a critical mediator in the activation of the EGFR axis. Src activity has a relationship with PKD progression in BPK mice and PCK rats<sup>[90]</sup>. SKI-606 can inhibit Src activity in a highly specific manner. SKI-606, which is also in clinical trials for breast cancer and malignant tumors, significantly improves renal and biliary lesions in BPK and PCK rodent models of ARPKD<sup>[90]</sup>. Thus, Src can be a prospective therapeutic target in PKD.

#### **Raf inhibitor**

Sorafenib, a small molecule Raf inhibitor, has been demonstrated to inhibit the proliferation of cells derived from the cysts of human ADPKD kidneys<sup>[91]</sup>. Sorafenib has also been proven to treat renal cell carcinomas and prolong survival time<sup>[92]</sup>. Cyst growth in human ADPKD cystic cells cultured within three dimensional collagen is completely inhibited by sorafenib<sup>[91]</sup>. This study suggests that the inhibition of the Raf kinases may be an effective therapy for PKD.

#### **Mitogen extracellular kinase (MEK) inhibitor**

MEK is an important mediator in EGFR and cAMP signaling. PD98059, an inhibitor of MEK, has been shown to completely prevent ADPKD cellular proliferation in response to cAMP agonists<sup>[21]</sup>. Another MEK inhibitor, PD184352, improved renal function and reduced the expression of P-ERK and ERK in pcy mice<sup>[93]</sup>. However, U0126, an inhibitor of MEK1/2 that blocks phosphorylation of ERK, did not change the rate of cyst growth in Pkd1<sup>fllox/-</sup>:ksp-Cre mice<sup>[94]</sup>. More studies on MEK inhibitor efficiency in PKD are needed.

#### **Mammalian target of rapamycin (mTOR) inhibitor**

In human ADPKD patients and mouse models, the mTOR pathway is abnormally activated in cyst-lining epithelial cells. It has been shown that the cytoplasmic tail of PC1 interacts with tuberlin<sup>[95]</sup>. Recently, another experiment<sup>[96]</sup> directly showed that PC1 was able to inhibit the mTORC1(mTOR complex-1) cascade that regulates cell growth and proliferation, ribosome biogenesis and translation of a subset of mRNAs, cellular energy responses and autophagy<sup>[97, 98]</sup>. Mutations in PC1 therefore lead to persistent activation of mTOR, which promotes cell growth and proliferation and cyst expansion in PKD. Also, mTOR is activated by increased ERKs through inhibiting tuberlin in the renal cells of ADPKD. Rapamycin, an inhibitor of mTOR, was shown to be highly effective in reducing renal cystogenesis in the bpk and orpk-rescue mouse models<sup>[95]</sup>. In another study, long-term rapamycin treatment in Han:SPRD rats resulted in a normalization of kidney volume, renal function, blood pressure and heart weight<sup>[65]</sup>. Treatment of human ADPKD transplant recipient patients with rapamycin showed a significant reduction in polycystic kidney volumes<sup>[95]</sup>. A two-year, placebo-controlled trial of another mTOR inhibitor, everolimus, involving 433 patients with ADPKD has been finished. Everolimus slowed the increase in total kidney volume, but the estimated GFR was

not improved<sup>[99]</sup>.

#### **Cystic fibrosis transmembrane conductance regulator (CFTR) inhibitor**

CFTR<sub>inh</sub>-172<sup>[59]</sup>, a thiazolidinone, has been shown to slow cyst growth in a MDCK cell culture model of PKD<sup>[28]</sup> and in metanephric kidney organ cultures<sup>[55]</sup>. CFTR<sub>inh</sub>-172 maintains the channel closed state, probably by binding to a cytoplasmic domain of CFTR according to patch-clamp analysis<sup>[100]</sup>. The other kind of CFTR inhibitors is the glycine hydrazides, which directly bind to the CFTR pore at a site near its external entrance<sup>[101]</sup>. In an experiment screening CFTR inhibitors for PKD<sup>[56]</sup>, tetrazolo-CFTR<sub>inh</sub>-172, a tetrazolo-derived thiazolidinone, and Ph-GlyH-101, a phenyl-derived glycine hydrazide, were found to almost completely suppress cyst growth without affecting cell proliferation. These compounds also showed a remarkable inhibition of cyst number and growth in an embryonic kidney cyst model. Subcutaneous delivery of tetrazolo-CFTR<sub>inh</sub>-172 and Ph-GlyH-101 to neonatal kidney-specific Pkd1 knockout mice for 7 d prominently slowed kidney enlargement, cyst expansion and renal function impairment.

#### **Cyclin-dependent kinase (CDK) inhibitor**

As we discussed previously, cilia has a close link with the cell cycle progression. The CDK inhibitor roscovitine effectively inhibited cyst formation through cell cycle arrest in jck and cpk mouse models of PKD<sup>[102]</sup>. Roscovitine has also been detected to be active against cysts originating from different parts of the nephron<sup>[102]</sup>. Roscovitine significantly downregulates cAMP and aquaporin 2<sup>[103]</sup> and increases p21<sup>[104]</sup>, leading to decreased renal tubular epithelial cell proliferation.

#### **TNF- $\alpha$ interventions**

A recent study has demonstrated that TNF- $\alpha$  promotes the progression of PKD<sup>[105]</sup>. Treating inner medullary collecting duct (IMCD) cells with TNF- $\alpha$  increases the expression of FIP-2 and shows a striking loss of PC2 from its normal location. FIP-2 plays a role in transporting protein from the apical membrane and regulating epithelial cell polarity. TNF- $\alpha$  causes cystogenesis in the wild-type murine embryonic kidney, which is exacerbated in the Pkd2<sup>+/-</sup> embryonic kidney. Pkd2<sup>+/-</sup> mice injected with TNF- $\alpha$  have increased cyst development (the frequency was 6/14), while 50 Pkd2<sup>+/-</sup> mice treated with the TNF- $\alpha$  inhibitor ethanercept did not develop cysts. Another study has found that TNF- $\alpha$  can activate the mTOR pathway<sup>[106]</sup>, which plays an important role in PKD development. These studies suggest that inhibition of TNF- $\alpha$  can slow cyst formation in PKD.

#### **Glucosylceramide synthase inhibitor**

Glucosphingolipids have been proven to play an important role in regulating cell proliferation and apoptosis<sup>[107]</sup>. Recently, a study demonstrated that the glucosylceramide (GlcCer) synthase inhibitor Genz-123346 effectively inhibited cystogenesis in Pkd1<sup>-/-</sup>, jck and pcy mice<sup>[108]</sup>. GlcCer and ganglioside GM3 levels are higher in human and mouse PKD kidneys compared

to normal kidneys. Molecular analysis of jck mice and jck cells shows that Genz-123346 prevents cyst growth by dysregulating Akt-mTOR signaling<sup>[108]</sup>.

#### **Matrix metalloproteinases (MMPs) inhibitor**

MMPs are a large family of proteinases that play an important role in remodeling extracellular matrix components and cleaving a number of cell surface proteins. Kidney tubules derived from the C57BL/6J-cpk mouse contain higher levels of MMP-2 and -9 than normal mice<sup>[109]</sup>. Serum MMP-1, -9, and tissue inhibitor of metalloproteinases-1 concentrations in patients with PKD were significantly higher compared to healthy controls<sup>[110]</sup>. MMP-14 mRNA has a higher expression in cyst-lining epithelia and distal tubules in Han:SPRD rats<sup>[111]</sup>. Treating Han:SPRD-cy/+ rats with the MMP inhibitor, batimastat, for 8 weeks caused a prominent reduction in cyst number and kidney weight<sup>[111]</sup>, which suggests that MMP inhibitor could be potential therapy for PKD.

#### **HMG-CoA reductase inhibitor**

Statins, which are HMF-CoA reductase inhibitors, are widely applied to decrease cholesterol in clinical settings. They can be used for improving renal function in PKD. Lovastatin significantly decreased cystic kidney size and improved function in heterozygous male Han:SPRD rats<sup>[112]</sup>. It may be related with lovastatin reducing farnesyl pyrophosphate, which is important in cell proliferation<sup>[112]</sup>, and lovastatin can also cause actin filament disruption, which can induce apoptosis<sup>[113]</sup>. In a double-blind cross-over study, 10 normocholesterolemic ADPKD patients treated with 40 mg/d simvastatin or placebo for 4 weeks showed that simvastatin significantly improved both glomerular filtration rate (GFR) and effective renal plasma flow<sup>[47]</sup>. Another study of 16 ADPKD patients with well-preserved renal function treated with 40 mg/d simvastatin for six months proved that simvastatin ameliorated endothelial dysfunction in ADPKD patients using high resolution vascular ultrasound<sup>[114]</sup>. A randomized open-label clinical trial was performed with 49 ADPKD patients who were treated with 20 mg/d pravastatin or no treatment for 2 years<sup>[115]</sup>. There were no significant changes in the markers of kidney function or urinary protein excretion between the two groups.

#### **Triptolide**

Triptolide is a natural product isolated from the "Thunder God Vine". It has been demonstrated to promote an increase in PC2-mediated calcium release and cytosolic calcium in the murine kidney epithelial Pkd2<sup>-/-</sup> cells and to inhibit cyst formation in Pkd1<sup>-/-</sup> embryonic mice<sup>[116]</sup>. Triptolide is an inhibitor of NF- $\kappa$ B- and NF-AT-mediated transcription, which results in reduced gene products and cell growth arrest<sup>[117, 118]</sup>. It has been proven to inhibit cell growth and increase p21 expression in Pkd1<sup>-/-</sup> kidney cells. In another study, triptolide significantly inhibited the early phases of cyst expansion and improved renal function at postnatal d 8 in a kidney-specific Pkd1<sup>flox/-</sup>; Ksp-cre mouse model of ADPKD<sup>[119]</sup>. Recently, a study showed triptolide has a pronounced effect in reduc-

ing cyst formation in a Pkd1<sup>flox/flox</sup>; Mx1Cre mouse model of ADPKD<sup>[120]</sup>.

### Curcumin

Curcumin is a natural polyphenol derived from the plant *Curcuma longa*. Numerous studies have indicated that curcumin is a highly pleiotropic molecule capable of treating various cancers. Our studies have proven that curcumin also has a significant inhibitory effect on renal cyst development<sup>[121]</sup>. Curcumin slowed cyst formation in both a MDCK cyst model and an embryonic kidney cyst model with a dose-dependent response. Curcumin inhibited forskolin-induced cell proliferation and promoted tubule formation in MDCK cells, which indicates that curcumin promotes MDCK cell differentiation. Curcumin affected intracellular signaling in the MDCK cells exposed to forskolin. Curcumin reduced signaling proteins Ras, B-raf, p-MEK, p-ERK, and c-fos and increased Raf-1 and NAB2 in MDCK cells.

### Summary

PKD is a progressive disease with a decline in renal function. The cost of treatment, dialysis, and kidney transplantation related to PKD exceeds \$1 billion in USA each year according to the Polycystic Kidney Research Foundation. Up to now, the treatment options for PKD have been limited to renal replacement therapy by dialysis or transplantation. Based on the understanding of the pathogenesis of PKD, the inhibition of cyst epithelia and cyst fluid secretion may provide a new therapeutic option in PKD. Dual or triple therapies may be highly effective in slowing PKD progression. In addition to advancing the understanding of the mechanism in which PKD develops, the functional and morphological improvement in PKD, as seen with chemical compounds, could provide a proof-of-concept for the application of new drugs in treating PKD.

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