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Potential Pharmacological Interventions in Polycystic Kidney Disease

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Abstract

Polycystic kidney diseases (autosomal dominant and autosomal recessive) are progressive renal tubular cystic diseases, which are characterised by cyst expansion and loss of normal kidney structure and function. Autosomal dominant polycystic kidney disease (ADPKD) is the most common life- threatening, hereditary disease. ADPKD is more prevalent than Huntington's disease, haemophilia, sickle cell disease, cystic fibrosis, myotonic dystrophy and Down's syndrome combined. Early diagnosis and treatment of hypertension with inhibitors of the renin-angiotensin-aldosterone system (RAAS) and its potential protective effect on left ventricular hypertrophy has been one of the major therapeutic goals to decrease cardiac complications and contribute to improved prognosis of the disease. Advances in the understanding of the genetics, molecular biology and pathophysiology of the disease are likely to facilitate the improvement of treatments for these diseases. Developments in describing the role of intracellular calcium ($[Ca^{2+}]_i$) and its correlation with cellular signalling systems, Ras/Raf/mitogen extracellular kinase (MEK)/extracellular signal-regulated protein kinase (ERK), and interaction of these pathways with cyclic adenosine monophosphate (cAMP) levels, provide new insights on treatment strategies. Blocking the vasopressin V_2 receptor, a major adenylyl cyclase agonist, demonstrated significant improvements in inhibiting cytogenesis in animal models. Because of activation of the mammalian target of rapamycin (mTOR) pathway, the use of sirolimus (rapamycin) an mTOR inhibitor, markedly reduced cyst formation and decreased polycystic kidney size in several animal models. Caspase inhibitors have been shown to decrease cytogenesis and renal failure in rats with cystic disease. Cystic fluid secretion results in cyst enlargement and somatostatin analogues have been shown to decrease renal cyst progression in patients with ADPKD. The safety and efficacy of these classes of drugs provide potential interventions for experimental and clinical trials.

Autosomal dominant and recessive polycystic kidney diseases (PKDs) are characterised by cyst expansion and loss of normal kidney structure and function. Autosomal dominant polycystic kidney disease (ADPKD) is the most common life-threatening, hereditary disease. ADPKD affects 1 : 400 to 1 : 1000 live births and leads to end-stage renal disease in 50% of patients by the fifth decade of life. The prevalence of autosomal recessive kidney disease (ARPKD) is ≈1 : 20 000 live births.[1] In 1841, Rayer wrote, "The cystic degeneration of the kidneys, once it reaches the point where it can be

recognised or suspected during life, is an illness without cure".[2] For nearly one and a half centuries, this statement has rung true.

ADPKD is genetically heterogeneous with separate PKD loci on chromosome 16 (*PKD1*), which accounts for ≈85% of ADPKD patients and on chromosome 4 (*PKD2*), accounting for 15% of patients.

Polycystin (PC)-1 encoded by *PKD1* has a large N-terminal extracellular region, 7–11 transmembrane domains and a C-terminal cytoplasmic tail.[3] It has been suggested that PC-1 may play a role in cell-cell or cell-matrix interactions.[4] The C-terminal tail of PC-1 contains tyrosine and serine sites that can be specifically phosphorylated by c-Src, focal adhesion kinase, protein kinase A (PKA) and protein kinase X as well as heterotrimeric G protein activation. Proline-rich sites suggest that PC-1 might be involved in intracellular signalling pathways (Wnt and G protein signalling) resulting in modulation of gene transcription. PC-1 also may interact physically and regulate the function of PC-2. PC-1 has been detected in tight junctions, adherens junctions, desmosomes, focal adhesions, apical vesicles and primary cilia.[5,6]

PC-2 is encoded by *PKD2* and is smaller than PC-1. It contains an N-terminal cytoplasmic region, 6 transmembrane domains and a C-terminal cytoplasmic tail. The transmembrane domain has homology to PC-1 and transient receptor potential (TRP) channel subunits.[7] This domain acts as a Ca^{2+}-permeable cation channel.[8-10] The physiological location of PC-2 is known to be in endoplasmic reticulum, and is also found in the basolateral plasma membrane, lamellopodia, primary cilia and mitotic spindle (figure 1).[11,12]

The recently identified ARPKD gene, *PKHD1*, on chromosome 6, encodes fibrocystin, which (like PC-1) resembles a receptor protein with multiple protein-protein interaction domains. Fibrocystin is found in primary cilia, basal bodies and plasma membrane.[14,15]

PC complexes and fibrocystin are essential for maintenance of the differentiated, polarised, predominantly reabsorptive phenotype in tubular epithelial cells, which normally have low rates of proliferation and apoptosis.

In recent years, genetic and molecular biology findings have stimulated a great deal of exciting basic research in ADPKD. However, therapies to decrease morbidity and mortality in ADPKD patients have yet to emerge from these findings. In this review, we briefly summarise the observations that have already been made and discuss therapeutic strategies for PKD that deserve further investigation.

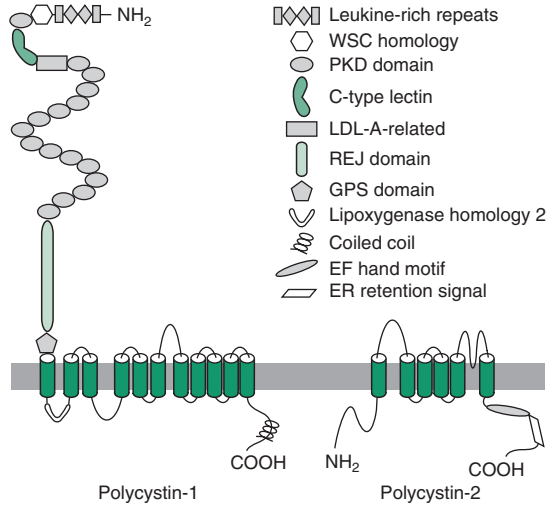

Fig. 1. Structures of polycystin-1 and polycystin-2 (reproduced from Igarashi and Somlo,[13] with permission). **ER** = endoplasmic reticulum; **GPS** = gene protein-coupled receptor proteolytic site; **LDL-A** = low-density lipoprotein class A; **PKD** = polycystic kidney disease; **REJ** = receptor for egg jelly; **WSC** = cell-wall integrity and stress-response component.

1. Renin-Angiotensin-Aldosterone System

Hypertension is an early and frequent finding of ADPKD, affecting ≈60% of the patients prior to any loss of renal function.[16] The median age at diagnosis of hypertension in ADPKD patients was found to be 32 years for males and 34 years for females.[17] Hypertension is an important risk factor for cardiovascular disease, which is the most common cause of death in patients with ADPKD.[18] Since hypertension occurs at an earlier age in ADPKD patients but is frequently undiagnosed until a later age, there is a highly significant correlation between left ventricular hypertrophy (LVH) and mean arterial pressure. Among 116 ADPKD patients with an average age of 44 years, LVH was found in 46% of men and

37% in women. LVH was even detected in 23% of normotensive ADPKD patients.[19]

Cyst enlargement in ADPKD observed by angiography is associated with compression of adjacent parenchymal and vasculature.[20] This results in ischaemic pathological findings including histological markers of glomerular ischaemia in ADPKD patients.[21] These findings supported the possibility of activation of the renin-angiotensin-aldosterone system (RAAS). Moreover, in ADPKD, an abnormal distribution of renin-containing cells have been located along the arterioles and within cyst walls.[22] Several studies have indicated an increase in renin in both kidney tissue and cyst fluid from patients with ADPKD.[23] More recently, studies have shown the presence of angiotensinogen, angiotensin converting enzyme (ACE), angiotensin II type 1 receptor and angiotensin II within cysts and tubules of ADPKD kidneys thus implicating an intrarenal role of the RAAS (figure 2).[24]

The RAAS can activate the sympathetic nervous system[25] and increased plasma catecholamine levels have been observed in hypertensive ADPKD patients.[26] In earlier studies, the blood pressure (BP) response to an angiotensin II receptor antagonist (angiotensin II receptor blocker [ARB]), saralasin, concluded that plasma renin activity (PRA) remained within the normal range in ADPKD patients. A significant decrease in both systolic BP (SBP) and diastolic BP (DBP) was observed in patients with unilateral renal artery stenosis but not in ADPKD patients.[27] However, sodium retention with plasma volume expansion in hypertensive ADPKD may secondarily suppress the RAAS.[28] Thus, both the RAAS and sodium retention are involved in ADPKD in a similar manner to the pathogenesis of hypertension that occurs in bilateral renal stenosis.

The first indication of RAAS activation in ADPKD resulted from clinical studies with the ACE inhibitor captopril. Significant stimulation of PRA was observed in hypertensive but not normotensive patients with ADPKD.[29] Because increased BP is known to suppress PRA, further studies were conducted to examine the role of RAAS by comparing ADPKD hypertensive patients with essential hypertensive patients, who were similar in age, gender, renal function, urinary sodium excretion and level of BP.[30] The ADPKD patients demonstrated significantly higher PRA and plasma aldosterone levels in the supine and upright positions, as well as after ACE inhibition with captopril. Also, after 6 weeks of ACE inhibition with enalapril, ADPKD hypertensive patients exhibited a significantly greater increase in renal plasma flow, due to a decrease in renal vascular resistance, than the patients with essential hypertension.[31,32]

A retrospective study was undertaken in ADPKD hypertensive patients who were treated either with ACE inhibitors or diuretics. During a mean follow-

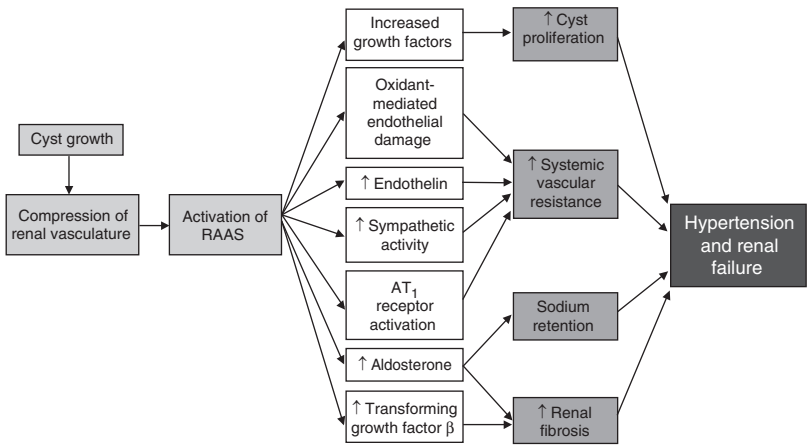

Fig. 2. Multifactorial pathogenic role of angiotensin II in the hypertension and renal disease association with autosomal dominant polycystic kidney disease. **AT_1** = angiotensin II type 1; **RAAS** = renin-angiotensin-aldosterone system; ↑ indicates increase.

up of 5 years, patients who were receiving diuretics without any ACE inhibitors had a faster decrease in glomerular filtration rate (GFR) and increase in serum creatinine levels as compared with patients who were receiving only ACE inhibitors. The diuretic group also needed more additional antihypertensive drugs for comparable BP control than the ACE inhibition group (64% vs 21%; $p < 0.05$), even though there were no differences in SBP and DBP at baseline or at the end of 5 years.[33]

Proteinuria is a predictor for renal disease progression. Although proteinuria is less frequent and severe in ADPKD than in glomerular disease, a correlation with renal progression has been demonstrated.[34] In a prospective randomised trial, the effects of an ACE inhibitor, enalapril, versus a calcium channel antagonist, amlodipine, on urinary albumin excretion was examined. Over 5 years of this study, the urinary albumin-creatinine ratio increased in the amlodipine- but not enalapril-treated patients.[35]

As mentioned previously, the early onset of hypertension in ADPKD, even in childhood, is highly associated with LVH, and increased cardiovascular risk and mortality. A randomised prospective study on 75 ADPKD patients demonstrated that patients who underwent rigorous BP control (<120/80mm Hg) versus standard (135–140/85–90mm Hg) BP control over a 7-year follow-up period had a decreased left ventricular mass index. This study also demonstrated a significantly greater decrease in LVH with enalapril compared with amlodipine.[36,37]

2. Vasopressin Receptor

Impaired renal concentrating capacity is one of the earliest findings in ADPKD patients.[38-41] Recent studies in children with ADPKD have shown a 60% decrease in urine concentration after the administration of 1-desamino-[D-Arg8]-vasopressin.[42] This concentrating defect also occurs in ARPKD. Although the mechanism of this defect is not well understood, the increased plasma vasopressin levels in ADPKD patients indicate a vasopressin-resistant nephrogenic diabetes insipidus.[43,44] Cultured epithelial cells from human ADPKD cysts exhibit a significant response with desmopressin by increasing cyclic adenosine monophosphate (cAMP) activity, suggesting a predominant collecting duct origin for cysts.[45] Increased plasma vasopressin levels in ADPKD, particularly in hypertensive patients,[46] may be a compensatory mechanism for the reduced urine concentration. The resultant increase in renal cAMP could increase both cyst proliferation and fluid secretion into the cysts.

On the basis of the observed vasopressin V_2 receptor (VPV2R) activation of adenylyl cyclase and cAMP in cysts originating from principal cells of the collecting duct in nephronophthisis, ARPKD and ADPKD, recent studies examining the effect of a non-peptide vasopressin antagonist mozavaptan (OPC-31260) have been conducted in murine cystic models. The results indicated prevention of the renal cAMP accumulation, inhibition of renal cystogenesis, decreased kidney weights and decreased blood urea nitrogen (BUN).[47,48] Administration of the antagonist, even after the cystic disease was already established, halted the progression or caused regression of the renal cysts. Additional studies were conducted to examine the effects of tolvaptan (OPC-4106), a more potent VPV2R antagonist. Similar results were observed with this agent in addition to Ras and extracellular signal-regulated kinase (ERK) inhibitory effects.[49] Since hepatocytes lack VPV2Rs, neither antagonist exerted an effect on fibropolycystic liver disease.[47,48] These drugs are attractive since they are highly selective for the renal VPV2R and are generally safe. The administration of these agents in phase I or II clinical trials has not resulted in any adverse effects compared with placebo, except mild to moderate thirst that was well tolerated by all participants.[50]

Several VPV2R antagonists are currently in phase III efficacy and safety trials for hyponatraemia in patients with the syndrome of inappropriate antidiuretic hormone secretion and for patients with disorders of water retention such as congestive heart failure and cirrhosis.

Recently, it has been demonstrated that increased water intake in PKD rats for 10 weeks slowed the growth rate of cysts, reduced kidney weights and improved renal function.[51] This effect might be due to suppression of arginine vasopressin with resultant inhibition of cAMP-dependent activity of B-Raf/mitogen extracellular kinase (MEK)/ERK pathway.[52] Because inhibitory effect of caffeine on phosphodiesterase results in a rise of cAMP, caf-

feinated beverages may be advised to be avoided in ADPKD patients.[53]

3. Endothelin

Recent studies have shown increased plasma and cyst fluid levels of endothelin in ADPKD patients, *cpk/cpk* mice models, and Han:SPRD PKD rats.[54-56] These findings suggested that cyst growth causes focal ischaemia, which is one of the most potent stimuli of the production and activity of the endothelin-1 (ET-1) protein *in vivo*.[57-60] ET-1 has two subtypes of receptors (ET_A and ET_B). Although ET-1 levels are increased in renal tissues of Han:SPRD PKD rats, the expression of ET_A and ET_B receptors has been found to be reduced in *cpk/cpk* mice.[61] One study showed that endothelin activation of the ET_B receptor can inhibit the vasopressin-mediated cAMP accumulation in collecting ducts derived from Han:SPRD PKD rats.[62] Several studies have been conducted to evaluate the effects of acute- and long-term use of endothelin receptor antagonists in the Han:SPRD PKD rat models.[63] Acute blockade of endothelin receptors with bosentan, a non-selective endothelin receptor antagonist, markedly decreased mean arterial pressure and increased GFR and renal plasma flow in Han:SPRD PKD rats.[64] In another study, long-term treatment with both endothelin receptor antagonists (darusentan [LU 135252] and LU 224332) did not show any significant effect on glomerulosclerosis in heterozygous (Cy/+) Han:SPRD PKD rats. Rather, interstitial fibrosis was enhanced in darusentan-treated as well as LU 224332-treated Han:SPRD PKD rats. This blockade, especially of the ET_A receptor, significantly increased kidney weight, number of renal cysts and cyst surface area accompanied by markedly increased cell proliferation rate in tubular cells. Therefore, these results do not support the use of endothelin receptor antagonists in ADPKD patients.[65] The recent studies have shown a correlation between ET-1 (*EDN1*) gene polymorphisms and renal progression of ADPKD.[66]

4. Intracellular Calcium

It has been shown that mutant PC-1 and PC-2 proteins play a critical role in intracellular calcium $[Ca^{2+}]_i$ homeostasis. As already mentioned, the C-terminal tail of PC-1 physically interacts with, and regulates the function of, PC-2. PC-1 also might activate a number of intracellular pathways including G-protein, Wnt and Janus kinase-signal transducer and activator of transcription (JAK/STAT) signalling.[67] The PC-1 in the plasma membrane might allow interaction with PC-2 in the adjacent endoplasmic reticulum, similar to the coupling of TRP channels in the plasma membrane and 1, 4, 5-triphosphate (IP3) receptors in the endoplasmic reticulum.[68] PC-2 also shows a physical interaction with the TRPC1α subunit of store-operated Ca^{2+} channels.[69] Overexpression of PC-2 in $LLC\text{-}PK_1$ cells (renal epithelial cell line originally derived from porcine kidney) increases IP3-mediated Ca^{2+} release from intracellular stores in response to vasopressin stimulation.[70] In vascular smooth muscle cells from *Pkd2* +/− mice (with half normal PC-2 content), capacitative Ca^{2+} entry and sarcoplasmic reticulum Ca^{2+} stores are significantly reduced.[71] B-lymphoblastoid cells derived from patients with *PKD1* or *PKD2* mutations exhibit reduced $[Ca^{2+}]_i$ responses to platelet-activating factor.[72]

Recent studies have demonstrated the role of the PC complex in primary cilia and its effect on mediating Ca^{2+} influx in response to mechanical stimulation.[73,74] Every tubular epithelial cell, except the intercalated cells of the renal collecting ducts, has a single primary cilium. The primary cilium in differentiated renal epithelial cells has mechanosensory and chemosensory functions.[75,76] Disruption of primary cilia in principal cells of mice by a kidney-specific knockout of a kinesin II subunit (*KIF3A*) results in rapid development of polycystic kidneys.[77] Inactivation of hepatocyte nuclear factor-1β, a transcription factor in the ciliary and basal body proteome, downregulates the expression of five PKD-associated proteins (uromodulin, fibrocystin, PC-2, nephrocystin-1 and Polaris) and results in polycystic kidneys.[78,79] Stimulation of primary cilia, either mechanically or in response to flow, increases the $[Ca^{2+}]_i$ mediated by the PC-1/PC-2 complex. This complex triggers Ca^{2+} release from the endoplasmic reticulum via ryanodine receptors.[80] Ryanodine and/or inositol IP3 receptor antagonists can block this release of Ca^{2+}.[81,82] Flow sensed by the primary cilium is translated into calci-

um transients, upregulation of inversin expression and inhibition of canonical Wnt signalling. Wnt, through Frizzled, regulates Dishevelled, which displaces glycogen synthase kinase (GSK)-3β from axin and blocks proteosome-mediated degradation of β-catenin. Free cytoplasmic β-catenin can translocate into the nucleus, form a complex with transcription factors of the T-cell factor-1 family and regulate expression of Wnt target genes. Inversin switches off canonical Wnt/β-catenin signalling by targeting Dishevelled for proteosomal degradation. PKA can phosphorylate and inactivate GSK-3β, thus promoting Wnt/β-catenin signalling.[83,84] There is a link between cAMP and Wnt signalling through the activation of PKA and protein kinase B. On the other hand, Ca^{2+} release from internal stores might downregulate Wnt/β-catenin signalling by enhancing movement of nuclear-β-catenin into the cytoplasm and promoting degradation by a calpain-mediated mechanism.[85] Therefore, drugs that downregulate canonical Wnt signalling decrease cAMP formation or increase Ca^{2+} release from internal stores might provide treatments for PKD.

$[Ca^{2+}]_i$ controls many aspects of cellular processes, including proliferation through hormone-mediated or growth factor processes. Many hormones act via G-protein coupled signalling. Induction of Gq proteins may stimulate IP3, increasing the levels of $[Ca^{2+}]_i$ and activating of phospholipase C.

Growth factors (e.g. epidermal growth factor [EGF]) act through tyrosine kinase receptors. This results in dimerisation and autophosphorylation of these receptors, such as the EGF receptor (EGFR), which activates signalling proteins such as Ras proteins and phosohatidylinositol 3-kinase. Ras proteins mediate proliferation through the mitogen-activated protein kinase (MAPK)/ERK signalling cascades.[86] These cascades contain three protein kinases acting in series, a MAPK kinase kinase (MAPKKK) that activates MAPK kinases (or MEKs), which phosphorylate MAPKs (or ERKs). Activated ERKs translocate to the nucleus and phosphorylate transcription factors. There are several MAPK subfamilies, including the ERK1/2 cascade and the Raf family of serine/threonine protein kinases, including Raf-1 and B-Raf, which function as MAPKKK. In addition, Raf proteins interact with Rap, another guanosine triphosphatase (GTPase) of the Ras superfamily.[87] Ras activates both Raf-1 and B-Raf, and Rap also activates B-Raf, but has an inhibitory effect on Raf-1. Raf activation is regulated by phosphorylation and binding of 14-3-3 proteins to phosphorylated motifs of Raf-1 or B-Raf. Ca^{2+} inhibits dimerisation of 14-3-3 proteins that are essential for binding to these motifs.[88,89] The importance of Ras/MEK signalling pathways in the development of PKD have been demonstrated by increased levels of phosphorylated Raf-1 and ERK in renal cells of animal models. This pathway, therefore, might be a valuable site for therapeutic agents.

On the other hand, it has been shown that Ca^{2+} plays an inhibitory role on AC6, the predominant isoform of adenyl cyclase and phosphodiesterase (PDE)-1 in the collecting duct principal cells. It has been found that only capacitative Ca^{2+} entry shows this inhibitory effect.[90,91] Moreover, PDE1, a group of isozymes, which are dependent for their activity on Ca^{2+} and calmodulin, will respond to direct selective inhibitors, such as vinpocetine or 8-MeoM-IBMX.[92] In another study, as a result of the importance of inhibition PDE activity, it has been shown that using either verapamil or the calcium chelator BAPTA-AM significantly increases cAMP concentrations in the wild-type vascular smooth muscle cells.[93]

5. Cyclic Adenosine Monophosphate

Recent studies established that, in addition to the ability of cAMP to promote the secretion of solutes and fluid into the ADPKD cysts, [94,95] it plays a major role in cystogenesis and proliferation of cells derived from polycystic kidneys.[95,96] This proliferative effect of cAMP only occurs in ADPKD epithelial cells, not in normal kidney cells. The effect of cAMP is complementary and additive to that caused by EGF. An EGF inhibitor, genistein (a tyrosine kinase inhibitor), did not have any effect on cAMP-dependent proliferation. On the other hand, a cAMP-dependent PKA inhibitor blocked only the cAMP pathway.[96,97] Both pathways were blocked by a MEK receptor inhibitor. As cAMP stimulates the ERK cascade, it thus causes proliferation of ADPKD cells while it has inhibitory effects on normal renal cells.[97,98] PKA has two isoforms, PKA1 and PKA2. They have identical catalytic (C) subunits, but their regulatory (R) subunits, termed

R1 in PKA1 and R2 in PKA2, are different. There is a functional cross activation between PKA1 expression and EGFR.[99] Recently, it has been described that PKA1 through Rap-1 and B-Raf may activate the ERK pathway.[100] The proliferative effects of cAMP and PKA1 have valuable therapeutic importance. Selective cAMP analogues, 8-Cl-cAMP, which can downregulate R1 and upregulate R2 expression at the transcriptional level, have been shown to inhibit growth and induce differentiation in human neoplastic cells. Interaction of EGFR activation and PKA1 expression suggest that the PKA1 and EGFR tyrosine kinase inhibitors may have a cooperative inhibitory effect on renal cancer cell growth in mice models.[101]

Moreover, PKA plays an important role in translocation of aquaporin-2 (AQP2) to the apical cell membrane. As mentioned, reduction in cAMP and/or expression of AQP2 messenger RNA is important to various forms of acquired and congenital nephrogenic diabetes insipidus. In addition to PKA-dependent phosphorylation of AQP2, RhoA is also important. RhoA depolymerises F-actin, which is essential for translocation of AQP2 to the apical membrane of the principal cells in the collecting duct.[102-106]

It has recently been discovered that cAMP also activates protein kinase X (PRKX). The *PRKX* gene family is expressed normally only during human metanephric kidney development. They may play an important role in the regulation of epithelial motility and cellular migration in the developing kidney. Interestingly, there is an over-expression of the *PRKX* gene family in ADPKD. Thus, molecular inhibitors of cAMP-dependent PRKX might be effective in cystic kidney diseases.[107]

As mentioned in section 2, multiple hormones acting through G-protein coupled receptors regulate cAMP levels. Vasopressin and catecholamine are the main hormonal adenylyl cyclase modulators. Vasopressin acts on adenylyl cyclase via its two G-protein, coupled receptors (V_2 and V_1). cAMP can either stimulate or inhibit MAPK/ERK signalling and cell growth depending on the cell involved. This ability is more important for PKD because cAMP significantly increases B-Raf kinase activity and ERK phosphorylation only in polycystic kidney cells.[108] In contrast, cAMP in wild-type derived cortical collecting tubule cells in the presence of Ca^{2+}, inhibits ERK phosphorylation and proliferation. The mechanisms have not been well understood. Nevertheless, the relationship between cAMP and $[Ca^{2+}]_i$ homeostasis, especially in association of Raf-1 or B-Raf, provides a strong rationale for agents targeting either the PDE or the cAMP pathway in PKD.

6. Epidermal Growth Factor Receptor

A large body of evidence has provided strong support for an important role of the EGF/transforming growth factor (TGF)-α/EGFR axis in promoting tubular epithelial cell proliferation, cyst formation and enlargement.[109-111] Previous studies have shown that renal cyst fluids from ADPKD patients, ARPKD patients and murine and rat models of PKD contain EGF-like peptides in mitogenic concentrations.[112-114] EGF-related peptide ligands relate to a large family of tyrosine kinase receptors known as ErbB receptors.[115] EGF and TGF-α belong to this family. The EGFR, also known as ErbB1, is the receptor for EGF and TGF-α. Binding of an EGF-like peptide to the extracellular domain of an ErbB receptor activates tyrosine kinase and causes autophosphorylation. This results in stimulation of intracellular signalling cascades and particular transcription factors, which results in either cell proliferation or differentiation.[116] EGF and TGF-α have cystogenic characteristics.[117,118] In human ADPKD and ARPKD, and in murine PKD models, EGFR and ErbB2 are overexpressed and mislocalised to the apical membrane of the cystic epithelium cells.[119-122] These abnormal receptors have a high affinity for EGF and result in auto activation of signalling cascades, which increase cell proliferation.[121] Inhibition of EGFR tyrosine kinase activity, genetically and/or pharmacologically, significantly inhibits cyst development in bpk and orpk mice, two rapidly progressive recessive PKD models.[123,124] Administration of EKI-785 and pelitinib (EKB-569) [EGFR tyrosine kinase inhibitors] in Han:SPRD PKD rats, which is an autosomal-dominant model, slows progressive renal cystic disease in this model and results in lower kidney weights, serum BUN, cyst volumes and fibrosis score.[125] On the background of the substantial role of EGFR tyrosine kinase, several selective therapeutic inhibitors with

limited adverse effects are in phase I or II clinical trials.

7. Mammalian Target of Rapamycin

PC-1 is widely expressed in epithelial cells, vascular smooth muscle, cardiac myocytes and other locations. The C-terminal cytoplasmic tail of PC-1 interacts with a component of the tuberous sclerosis complex, tuberin, encoded by the *TSC2* gene. Expression of tuberin might be essential for PC-1 targeting to the plasma membrane.[126] A synergistic activity of PC-1 and tuberin, as a constitutive complex, has been associated with the more severe and earlier onset of PKD in patients with *PKD1* and *TSC2* mutant genes compared with only *PKD1* mutations.[127] PC_1 also regulates the activity of mammalian target of rapamycin (mTOR) kinase.[128,129] mTOR, a serine threonine kinase, plays an important role in protein translation, [130-132] cell growth and proliferation, and is up-regulated in several types of tumours.[133]

Sirolimus (rapamycin) and its analogues, temsirolimus (CCI-779) and everolimus (RAD001), have exhibited anticancer activity with only mild adverse effects in phase I and II clinical studies.[132] Recent studies indicate that ADPKD patients have increased mTOR activity.[127] On the basis of the hypothesis that mTOR in renal cystic cells leads to abnormal cyst formation, several studies have been conducted to evaluate the effects of mTOR inhibitors. Sirolimus is a US FDA-approved specific inhibitor of mTOR.[132] It is known that sirolimus reduces cell growth, rate of cell-cycle progression and cell proliferation.[134] Sirolimus was shown to decrease tubular cell proliferation in non-cystic as well as cystic tubules in polycystic Han:SPRD rats. Moreover, sirolimus inhibited cyst formation and renal enlargement. This study also demonstrated the ability of sirolimus to delay the onset of renal dysfunction.[135]

Another study suggested that the reduction in renal cysts with sirolimus involved apoptosis and luminal shedding of cyst-lining epithelial cells in mutant rat models.[127] Inhibition of mTOR has been shown to arrest cells in the G1 phase of the cell cycle and to induce apoptosis.[136-144] A retrospective study of ADPKD patients who had received a renal transplant demonstrated that sirolimus treatment as an immunosuppressive regimen significantly decreased renal volumes of the cystic kidneys.[127,145]

Considering the safety and effectiveness of long-term administration of sirolimus in adults and children with ADPKD, further clinical and experimental studies should be encouraged.

8. Ras Inhibition

Recently, several studies have supported the participation of Ras proteins in the pathogenesis of PKD. It has been observed that the expression of H-ras and c-Ki-ras is markedly increased in *cpk* mice and human ADPKD renal cells.[146] The proteins, which are encoded by Ras gene, belong to the group of GTP binding proteins with endogenous GTPase activity. They play a major role in triggering cellular signalling cascades.[147] Post-translational modifications of Ras are important because of their requirement for plasma membrane attachment. One of the most crucial modifications is the attachment of a farensyl group to a C-terminal cysteine by the enzyme farensyl-transferase. This enzyme recognises a carboxyl terminal CAAX motif (where C is cysteine, A is often an aliphatic amino acid and X is any amino acid). Previous studies have shown the efficacy of lovastatin, an HMG-CoA reductase inhibitor, which inhibits farensyl synthesis and Ras farensylation in the treatment of PKD. Several studies have been conducted to further evaluate the effects of farensyl-transferase inhibitors on PKD.[148-150] As discussed in section 5, an increase in cAMP, as occurs in PKD, can either stimulate or inhibit MAPK/ERK signalling and cell proliferation. Activation of the EGFR and ErbB2 might have a role in the Ras/MAPK signalling process in the PCK rat model.[151] Activation of Ras may trigger a protein kinase cascade, which involves Raf and MEK. Therefore, kinase inhibitors may be valuable targets for interventions. Recently, PD-98059, a MEK inhibitor, has been shown to decrease cell proliferation in ADPKD-derived epithelial cells.[152,153]

9. Apoptosis

Cellular proliferation and apoptosis are major features of PKD.[154,155] Increased levels of apoptosis have been observed in human ADPKD and all

experimental models of PKD.[156-158] A recent study described the role of PC-1 in the regulation of apoptosis during spontaneous tubulogensis in Madin-Darby canine kidney cells.[159] Apoptosis occurs in cystic epithelium and non-cystic tubules of preuraemic human PKD, and is associated with a progressive loss of renal function.[156] In Han:SPRD PKD rats, apoptosis has been detected at an early stage of the disease in both heterozygous and homozygous models. There are two major apoptotic pathways, intrinsic and extrinsic, both of which are mediated by caspases.[160] The caspases are a family of intracellular cysteine proteases that cleave the substrates after an aspartate residue.[161] In the mitochondrial or intrinsic pathway, the balance of the pro- and anti-apoptotic Bcl-2 family proteins and caspase-2 determine cytochrome C release from mitochondria. Binding of cytochrome C to apoptosis protease-activating factor-1 activates caspase-9. Activation of caspase-9 then activates procaspase-3 and procaspase-7. In the extrinsic pathway, binding of a ligand to its death receptor recruits an adaptor protein, which in turn recruits procaspase-8, which then also activates procaspase-3 and -7. The caspase family consists of 14 members, named from 1 to 14. The caspase family can be divided into subfamilies based on their specificity and function.[162] Caspase-1 plays a major role in inflammatory processes, especially in ischaemic acute renal failure in mice.[163,164] No increase in proinflammatory caspase-1 has been reported in Han:SPRD rats.[165] Several studies have demonstrated the importance of caspase-3 in apoptosis in PKD in animal models and humans.[165,166]

Caspase-2 is a recently discovered member of this family. Activation of caspase-2 is essential for permeabilisation of mitochondria and release of cytochrome C.[167] Caspase-2 activity is increased in Cy/Cy rat kidneys due to translational regulation or increased stability of the caspase-2 protein.[168]

Caspase-3 decreases the contact between surrounding cells, reorganises the cytoskeleton, inhibits DNA replication, interrupts splicing and disintegrates the cells into apoptotic bodies. Caspase-3 blocks DNA repair by cleavage of the DNA repair enzyme, polyadenosine diphosphate-ribose polymerase (PARP), an enzyme that is an extremely sensitive indicator for DNA damage. PARP cleavage during apoptosis has also been inhibited by caspase-3 inhibitors.[166,169] Therefore, PARP inhibitors might be a potent protective strategy in ADPKD patients. Administration of aminobenzamide, a PARP inhibitor, has been shown to protect against apoptosis secondary to cerebral ischaemia. PARP knockout mice also have less apoptosis secondary to cerebral ischaemia.[170] Regulation of caspase-3 is post-transcriptional and could involve translational control or changes in protein turnover.[168]

Both intrinsic and extrinsic pathways downstream of the caspase-8 and caspase-9 also activate caspase-7. Although caspase-7 is an effector caspase that is very similar to caspase-3 in terms of substrate specificity, it may be more specific for apoptosis than caspase-3.[171] An increased level of these two caspases, as mediators of apoptosis in ADPKD, has been reported.[168]

The initiator caspase-8 belongs to the intrinsic pathway, whereas caspase-9 plays a major role in the extrinsic pathway. A recent study demonstrated the increased activity of these two caspases in Cy/Cy rats.[168] The same study suggests that the caspase pathways may not be important in the initiation of cystogenesis among Cy/+ rats, but later increased caspase activity may contribute to decreased renal function.[168]

It has been found that caspase-3 activity is under control of a family of proteins named Bcl-2. This family consists of anti- and pro-apoptotic proteins. Pro-apoptotic proteins include Bcl-2-associated X protein (Bax) and Bcl-2-associated death promoter (Bad), whereas Bcl-2 and Bcl-X_L proteins show anti-apoptotic effects.[158] It has been shown that Bcl-2-deficient mice may have a higher rate of apoptosis and manifest severe PKD.[172,173] Caspase-3 cleaves Bcl-X_L and converts it to a cytodestructive protein, which promotes apoptosis and increases the mitochondrial release of cytochrome C.[174] This process has been demonstrated in Cy/Cy rats, which have decreased expression of Bcl-X_L and increased expression of Bcl-2 at 2 weeks of age. No change of pro-apoptotic Bax and Bad proteins was detected at this age in Cy/+ and Cy/Cy rats. A decrease in Bcl-X_L enhances the activity of caspase-3 and perturbs the balance between pro- and anti-apoptotic Bcl-2 family members. This results in increased apoptosis in polycystic Han:SPRD rat models.[158] The studies

on different models of PKD rats indicate the presence of apoptosis and the correlation with cyst formation.[168] Administration of the pan-caspase inhibitor IDN-8050, which inhibits all caspases, significantly reduced the kidney enlargement, and both cyst volume and density in Cy/+ rats. IDN-8050 also reduced the increase in BUN in this rat model. Decreased apoptosis was accompanied by a decrease in cell proliferation in cystic and non-cystic tubules and attenuated renal function loss.[175] Considering the potential therapeutic effects of caspase inhibitors, more animal studies and clinical trials in polycystic kidneys are needed.[168]

10. Somatostatin

Cyst enlargement due to cell proliferation is one of the major pathogenetic factors in ADPKD.[176] Transepithelial fluid secretion into cysts results in their expansion.[177,178] Cystic fluid secretion is slightly higher than reabsorption, thus leading to fluid accumulation in cysts. This fluid movement appears to be secondary to chloride transport.[179] It has been demonstrated that the cell proliferation and fluid secretion is under the control of cAMP. Recent studies have shown that reduction of cAMP level by VPV2R antagonists has an inhibitory effect on renal cyst formation and thus renal volume enlargement.[180-182] There are five receptors for somatostatin, which bind to somatostatin or somatostatin ana-

Table I. Summary of existing drugs and potential experimental agents in treatment of polycystic kidney disease

System	Drug	Mechanism	Physiological effects	Trial results/status
RAAS	Enalapril	ACE-inhibitor	↑ RFP ↑ GFR ↓ F.F ↓ Alb/Cr (M>F)	LVH reversal ↓ Albuminuria
Vasopressin	Mozavaptan (rat) Tolvaptan (human)	V_2-receptor antagonist	↓ cAMP ↓ Ras ↓ ERK	↓ Disease progression ↓ Kidney weight ↓ BUN NA in fibropolycystic liver disease
	Water	AVP suppression	↓ cAMP	↓ Cyst growth ↓ Kidney weight ↑ Renal function
Endothelin	Bosentan	$ET_{A/B}$ receptor antagonist	↓ MAP ↑ GFR ↑ RPF	Acute treatment (rat)
	Darusentan LU 224332	$ET_{A/B}$ receptor antagonists (especially ET_A)	↑ Cyst volume ↑ Cell proliferation	↑ Kidney weight (not recommended)
Calcium channel	Verapamil BAPTA-AM	Calcium channel antagonist	↑ cAMP	
mTOR	Sirolimus Temsirolimus Everolimus	mTOR inhibitor	↓ Cell proliferation ↓ Cyst volume ↓ Renal volume	Phase I and II clinical trials
MEK	PD-098059	MEK inhibitor	↓ Cell proliferation	
Caspase	IDN-8050	Pan-caspase inhibitor	↓ Cyst volume ↓ Kidney size	Reduction in BUN (rat)
Somatostatin	Octreotide	Somatostatin receptor type 2 agonist	↓ cAMP ↑ PLC ↑ Phospholipase ↓ Cyst growth	6mo clinical trial

AVP = arginine vasopressin; **BUN** = blood urea nitrogen; **cAMP** = cyclic adenosine monophosphate; **ERK** = extracellular signal-regulated kinase; **ET** = endothelin; **F.F** = filtration fraction; **GFR** = glomerular filtration rate; **LVH** = left ventricular hypertrophy; **MAP** = mean arterial pressure; **MEK** = mitogen extracellular kinase; **mTOR** = mammalian target of rapamycin; **NA** = data not available; **PLC** = phospholipase C; **RAAS** = renin-angiotensin-aldosterone system; **RPF** = renal plasma flow; **V_2** = vasopressin 2; ↑ indicates increase; ↓ indicates decrease.

logues with different affinities.[183] Detection of the somatostatin receptor type 2 in kidney and its inhibitory effect on cAMP and stimulatory ability on phosphatase and phospholipase C, suggests a potential effect of somatostatin on cyst fluid secretion and enlargement in ADPKD patients.[184] The inhibition of cyst growth in an ADPKD patient with pituitary adenoma, who was treated with octreotide (a somatostatin analogue) for 2 years, led to the hypothesis of a potential benefit of this agent in ADPKD.[185] Recently, a randomised, crossover, placebo-controlled trial has been conducted to evaluate the benefits and adverse effects of 6-months' treatment with slow-release octreotide or placebo in ADPKD. Enrolled ADPKD patients manifested mild to moderate renal insufficiency without any other kidney complications. There was a 60% reduction in cyst volume increase detected in the somatostatin group, whereas no effect was seen with placebo. Although somatostatin may decrease the GFR in healthy individuals[186,187] and patients with type I diabetes mellitus or liver cirrhosis, [188,189] no significant effect of slow-release octreotide on GFR in these ADPKD patients was observed.[185] Thus, additional randomised clinical trials in ADPKD patients to examine inhibition by somatostatins may be indicated.

11. Conclusion

In conclusion, clinical history evaluation of the PKD families and better education of patients and their physicians, results, at least in part, to earlier diagnosis and potential treatment of PKD patients. It has been proposed (HALT PKD study[190]) that both an ACE inhibitor and an ARB will have better protective effects on cardiovascular and renal systems than an ACE inhibitor alone in ADPKD patients. VPV2R antagonists are fairly safe agents and may decrease renal disease progression in ADPKD. As a result of the effects of vasopressin suppression, water intake may decrease cystogenesis and thus kidney weights. Bosentan, an endothelin receptor antagonist, will increase the GFR and decrease the mean arterial pressure. Several classes of new agents or existing drugs may offer additional benefits for ADPKD treatment by decreasing cell proliferation and cyst growth; these include MEK inhibitors, caspase inhibitors, EGFR antagonists and sirolimus. Moreover, short-term therapy with somatostatins has shown renoprotective effects in ADPKD patients. Several of these agents are currently in phase II or III efficacy and safety trials, but more experimental and clinical trials seem to be necessary. Table I summarises the drugs and experimental agents that have potential for the future treatment of PKD.

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