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REVIEW ARTICLE

THE ROLE OF mTOR INHIBITION IN THE CONTROL OF AUTOSOMAL DOMINANT POLYCYSTIC KIDNEY DISEASE

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SUMMARY

Autosomal dominant polycystic kidney disease (ADPKD), the most common type of genetic kidney disease, affects more than 12 million people worldwide. Effective therapies for ADPKD are desperately needed. Persistent progress has been made in the understanding of processes that are responsible for renal cyst formation and progression of ADPKD. Cellular pathways that involve polycystins, intracellular calcium and cAMP regulation, and the serine/threonine-protein kinase mTOR (mammalian target of rapamycin) pathway have been characterized. The understanding of the ADPKD phenotype at the cellular level and the encouraging results in experimental models of polycystic kidney disease have laid the foundation for the development of clinical trials and potentially effective targeted treatments. This review addresses the current knowledge about the pathogenesis of the disease and the overwhelming evidence in support of mTOR as a common molecular pathway for cystogenesis. The recent advances and challenges in understanding the role of mTOR inhibitors in controlling ADPKD in animal models and clinical trials will be discussed.

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INTRODUCTION

Polycystic kidney diseases (PKDs) are a large family of inherited disorders characterized by the formation and growth of renal cysts, often leading to end-stage renal disease (ESRD). The two major forms of PKDs can be transmitted as autosomal dominant or autosomal recessive traits.

Autosomal dominant polycystic kidney disease (ADPKD) occurs in more than 12 million people worldwide (1) with an estimated prevalence rate of 1:400 to 1:1,000 individuals (2). It is characterized by the development of fluid-filled cysts in the kidneys, liver, pancreas and seminal vesicles (3) and accounts for up to 6% of the new ESRD patients in Europe (4). ADPKD is a systemic disorder with cardiovascular manifestations including cardiac valve abnormalities, cardiac hypertrophy and intracranial aneurysms. Intestinal diverticuli and abdominal wall hernia are also common. Complications associated with the development and enlargement of cysts arise long before renal function begins to diminish. Nearly all patients with ADPKD early in life experience one or more severe symptoms attributed to the enlargement of their kidneys. These include hypertension, abdominal pain, gross and microscopic hematuria, nephrolithiasis and urinary tract infections (3). The disease manifests in adulthood and renal insufficiency is usually not detected until the fifth or sixth decade of life.

Autosomal recessive polycystic kidney disease (ARPKD) is a less frequent childhood disease with an incidence of 1:20,000. ARPKD is characterized by cystic kidneys and congenital hepatic fibrosis with a high level of mortality in affected newborns (5). Most cases manifest in utero or at birth with renal enlargement and biliary dysgenesis. A smaller number of patients display later onset of disease with portal hypertension or cholangitis (6).

This review will mainly focus on ADPKD and will summarize the pathobiology of the disease and the overwhelming evidence in support of serine/threonine-protein kinase mTOR (mammalian target of rapamycin) as a common molecular pathway of cystogenesis. The recent advances and challenges in understanding the role of mTOR inhibition in controlling ADPKD in animal models and clinical trials will be discussed.

MOLECULAR GENETICS OF ADPKD

ADPKD is genetically heterogeneous with two disease loci identified. Approximately 85% of all ADPKD cases are caused by mutations in the *PKD1* gene, encoding for polycystin-1, which maps to human chromosome 16p13.3 (7-10). More than 270 different *PKD1* mutations have been described (11), which are predicted to produce truncated protein and are unique to a single family, although missense mutations have also been identified. The *PKD2* gene, which accounts for around the remaining 15% of cases, encodes for polycystin-2 and is located on chromosome 4q21 (12, 13). Nearly 70 different mutations have been described for the *PKD2* gene (11). While genotype/phenotype correlations in *PKD1* suggest that mutations in the 5′ portion of the gene are associated with a more severe phenotype, no obvious correlations have been found in the *PKD2* gene (14). Clinical presentation of the *PKD1* and *PKD2* mutations is very similar, although the disease phenotype in the latter is milder. Typically, individuals with *PKD2* display later mean age at diagnosis, hypertension and ESRD (15, 16). Indeed, the mean age of ESRD is 54 and 74 years, respectively, for *PKD1* and *PKD2* patients (17). The greater severity of *PKD1* is due to the development of more cysts at an early age and not to faster cyst growth (18). Large intrafamiliar variability is also seen in age of onset, rate of cystic disease progression and extrarenal manifestations, highlighting a role for gene modifier effects as well as environmental modifying factors. Evidence that the heterogeneity in renal disease progression in ADPKD siblings is significantly greater than that found in monozygotic twins lends support for the existence of modifier genes influencing renal survival and accounting for a significant part of the variability in ADPKD (19).

Clinical studies of candidate modifying genes in ADPKD selected because of association with worse prognosis in other chronic renal diseases have been mostly disappointing. Functional polymorphisms in the angiotensin-converting enzyme (*ACE*), transforming growth factor beta-1 (*TGFB1*), bradykinin receptors B_1 and B_2 (*BDKRB1* and *BDKRB2*), epidermal growth factor receptor (*EGFR*) and endothelin ET_A receptor (*EDNRA*) genes and of the regulatory region of the vascular endothelial growth factor (*VEGF*) gene did not have a significant effect on ADPKD disease progression (20-23). Although so far studies of candidate loci have been mostly negative, the identification of modifying genes influencing the severity of ADPKD remains important for better understanding the molecular pathogenesis and providing new insights into potential therapies. Along these lines, in the *cpk* mouse, a model of ADPKD, the first putative modifier of renal disease severity in PKD has been mapped to chromosome 4 as the kinesin family member 12 gene (*Kif12*). Interestingly, mutations in hepatocyte nuclear factor 1-beta (HNF-1β), which regulates *Kif12* gene transcription, cause a syndrome characterized by cystic disease of the kidney or renal dysplasia and diabetes (24). Recently, mutations in the oral-facial-digital syndrome 1 (*OFD1*) gene have also been suggested to play a role in renal cystic disease, at least in animal models (25), and could be an additional gene modifier accounting for intrafamilial heterogeneity of PKD.

ADPKD PATHOGENESIS

Patients with ADPKD carry a mutant copy of either the *PKD1* or *PKD2* gene in every cell. However, cyst formation is focal in nature. This led to the "two-hit hypothesis" for cyst development (26), such that in the cells of the renal tubule cysts form only within the nephron segments where function of the *PKD1* or *PKD2* gene inherited from the parent who does not carry the mutation is also compromised. Thus, an inherited mutation in one allele and a subsequent somatic mutation in the other allele converge to set in motion complex intracellular molecular processes that ultimately lead to aberrant proliferation of tubular wall and cystogenesis (3). This hypothesis received experimental support through identification of somatic mutations in a subset of kidney cysts (27-29). However, further studies suggest that other pathogenetic mechanisms may trigger cyst formation, such as reduced expression of the normal *PKD1* allele below a critical level due to genetic or environmental factors (30).

Hyperplasia of tubular epithelial cells in a tiny fraction of the nephrons promotes the formation of a saccular cyst of the tubular wall (31). Progressive expansion eventually causes the emerging cyst to separate from the parent tubule, leaving an isolated sac that fills with fluid by transepithelial secretion. The continued proliferation of the mural epithelium of isolated cysts and the transepithelial secretion of NaCl and water into the lumen eventually sustain the relentless growth of the cysts (32). The progressive enlargement of the cyst leads to crowding of adjacent nephrons, ultimately injuring normal functioning parenchyma, and in time, to loss of renal function (33-37). The formation and growth of cysts in ADPKD is accompanied by increased proliferation and apoptosis of cyst-lining epithelia, dysregulation of cell/matrix interactions and transformation of the absorptive epithelial phenotype to a secretory phenotype (38-40).

Epidermal growth factor (EGF), TGF-α and EGFR promote tubular epithelial cell proliferation and contribute to cyst formation. Renal expression of EGF is downregulated in *cpk* mice and Han:SPRD rats, models of PKD (41-43), while expression of TGF-α is increased in models of rapidly progressive PKD disease (44). Moreover, overexpression of TGF-α in transgenic animals leads to renal cyst formation (45). Cyst-lining epithelia also produce large amounts of soluble extracellular matrix-associated proteins such as TGF-β and periostin (46), which actively contribute to epithelial cell proliferation and cyst growth. Furthermore, there is evidence that increased tubular cell proliferation is accompanied by enhanced apoptosis in PKD which is also essential for cystogenesis. Deletion of the antiapoptotic *Bcl2* and *AP-2beta* genes and overexpression of the proapoptotic *Myc* gene in mice results in renal cyst formation (47).

In normal conditions, tubular epithelial cells beyond the loop of Henle secrete and reabsorb solutes and fluid, with absorptive flux overwhelming secretory flux. In the early stage of ADPKD, when cysts are still attached to the parent tubule, cystic fluid is derived from glomerular filtrate. When cysts separate from the tubule of origin, they continue to expand through a transepithelial chloride secretion mechanism (34). In ADPKD, chloride enters cells via the basolateral Na-K-Cl cotransporter, driven by low intracellular sodium concentration generated by basolateral Na^+/K^+-ATPase, and accumulates in the cytoplasm. The chloride channel CFTR (cystic fibrosis transmembrane conductance regulator) in the apical membrane mediates the movement of chloride into the cystic lumen. Chloride secretion drives sodium into the cystic cavity through paracellular mechanisms, causing movement of water through aqua-

porins, down transepithelial potential and osmotic gradients (34). Thus, in cyst-lining epithelia the secretory phenotype overshadows the absorptive phenotype, eventually leading to fluid accumulation in the cystic lumen and cyst growth.

INTRACELLULAR SIGNALING PATHWAYS IN ADPKD

In early 2000 it was discovered that defects in the structure and function of primary cilium, a microtubule-containing organelle that projects from the surface of most eukaryotic cells (48), underlie multiple human diseases with diverse phenotypes, including PKD. Increasing experimental evidence suggests that polycystins are localized in primary cilia, in addition to other specialized structures such as focal adhesions and adherens complexes (49). An initial link between ciliary dysfunction and PKD came from studies in the nematode *Caenorhabditis elegans* (50). They showed that mutations in the *lov-1* and *pkd-2* genes of *C. elegans*, which are closely related to human polycystins, were associated with mechanosensation defects of ciliated sensory neurons (50). These findings prompted interest in the role of cilia in mammalian kidneys, specifically those with ADPKD. Inactivation of kinesin-like protein KIF3A, a subunit of kinesin II essential for cilia formation, specifically in renal tubular epithelial cells, inhibited renal ciliogenesis and resulted in the development of PKD (51). Moreover, in normal renal epithelial MDCK cells bending of cilia increased intracellular calcium influx followed by calcium release from intracellular stores (52). It has also been suggested that polycystin-1 may function as a sensor of ciliary bending, while polycystin-2, a nonselective cation channel capable of transporting calcium ions, transduces the mechanical signal into a calcium response (53). Since changes in intracellular calcium concentration are known to regulate multiple cellular functions, including cell cycle and ion transport, ciliary and polycystin-1/polycystin-2 complex function has been linked to cell proliferation, cell death, sodium absorption and potassium secretion in the collecting duct (54, 55). In ADPKD-derived cells with mutated *PKD1*, the ciliary mechanosensation of fluid-flow shear stress by polycystins is absent (53). Thus, disruption of the polycystin complex associated with cilia could lead to dysregulation of the cell cycle and proliferation, resulting in cystic disease (56, 57).

Important players in this cell proliferation cascade, largely controlled by calcium influx, are 3′-5′-cyclic adenosine monophosphate (cAMP) and mTOR. In the past decade, studies using in vitro cultured cells from ADPKD cysts and animal models of PKD have suggested a final common pathway for cystogenesis, involving a major role for cAMP-stimulated signaling pathways in controlling both the rate of epithelial cell growth and fluid secretion in cysts (58, 59). In contrast to inhibition of normal kidney epithelial cells, cAMP was able to induce proliferation of ADPKD-derived cystic epithelial cells through activation of the B-Raf/MEK/ERK pathway (60). This shift from conventional cAMP-induced inhibition of growth to the cAMP-induced stimulation of growth in cystic epithelia was mediated by decreased intracellular calcium levels secondary to dysfunction of mutated polycystins (61). Moreover, cAMP stimulates CFTR-driven chloride and fluid secretion (62). Increased levels of cAMP and expression of cAMP-dependent genes are a common finding in the kidneys of mice and rats with PKD (60, 63-67). Besides alterations in intracellular calcium, high circulating vasopressin levels through binding to upregulated vasopressin V_2 receptors of the basolateral membrane of tubular epithelial cells (62), and forskolin, a potent adenylyl cyclase agonist isolated within the cyst fluid (68), may contribute to the increased cAMP levels. Together these observations point to an important role of abnormally high cAMP in the PKD renal tubular epithelial cells in stimulating cell proliferation and fluid secretion, causing cyst enlargement and eventually contributing to the development and progression of PKD.

Proliferation of ADPKD cells may be further enhanced by the intracellular pathway stimulated by EGF-like factors present in cyst fluid and insulin-like growth factor I (IGF-I) in cystic tissues, as well as by activation of mTOR.

DYSREGULATION OF mTOR SIGNALING IN ADPKD

Another change consistently found in PKD cells is activation of mTOR, an enzyme that coordinates cell growth, cell cycle progression and proliferation (69). Within mammalian cells, mTOR forms two distinct protein complexes, mTORC1 and mTORC2. mTORC1 is made up of mTOR and regulatory-associated protein of mTOR (raptor) and is sensitive to inhibition by rapamycin (Fig. 1). The defining unit of mTORC2 is rapamycin-insensitive companion of mTOR (hAVO3), which is not sensitive to rapamycin (70, 71). Unlike the regulation of mTORC1, little is known about the regulation of mTORC2.

The mTOR–raptor complex promotes cell growth by at least two mechanisms that involve phosphorylation of regulators of translation initiation and ribosomal function (Fig. 1). Indeed, the mTOR–raptor complex phosphorylates ribosomal protein S6 kinase beta-1 (P70S6K1), thereby augmenting protein translation and ribosomal biogenesis (72). In addition, mTORC1 inactivates by phosphorylation the eukaryotic translation initiation factor 4E-binding protein (4E-BP1), dissociating 4E-BP1 from eukaryotic translation initiation factor 4E (eIF4E), promoting cap-dependent messenger RNA (mRNA) translation (73) (Fig. 1). Eventually, increased P70S6K1 and eIF4E act independently to promote cell proliferation by stimulating the translation of mRNA and the synthesis of proteins necessary for mediating increases in cell size and sustaining progression of the early stage ($G_{0/1}$) of the cell cycle (69, 74-78). Activated phospho-mTOR and P70S6K1 are induced in cyst-lining epithelial cells in cysts from mouse and human kidneys (79). Moreover, P70S6K1 is increased in Han:SPRD rat kidney with PKD (80).

Tissue turnover involves the balance of cell growth/division and apoptosis. Beside proteins targeting mTOR to ultimately sustain cell proliferation, mTOR-binding proteins that regulate apoptosis have also been identified (81). The proline-rich AKT1 substrate 1 binds mTORC1. Deficiency of proline-rich AKT1 substrate 1 prevents cell apoptosis. On the contrary, proline-rich protein 5-like binds specifically to mTORC2 and mediates apoptosis. Thus, mTOR is a key component of intracellular signaling for cell growth and death, the disturbance of which promotes abnormalities of tubular epithelial cells lining the cysts.

Since mTORC1 serves as an integrator of multiple stimuli necessary for cell growth and proliferation, a possible functional link with the ciliary mechanosensor polycystin-1 was suggested (82). In vitro studies demonstrated that the *N*-terminal cytoplasmic domain of polycystin-1 co-localizes and interacts with tuberin protein (79)

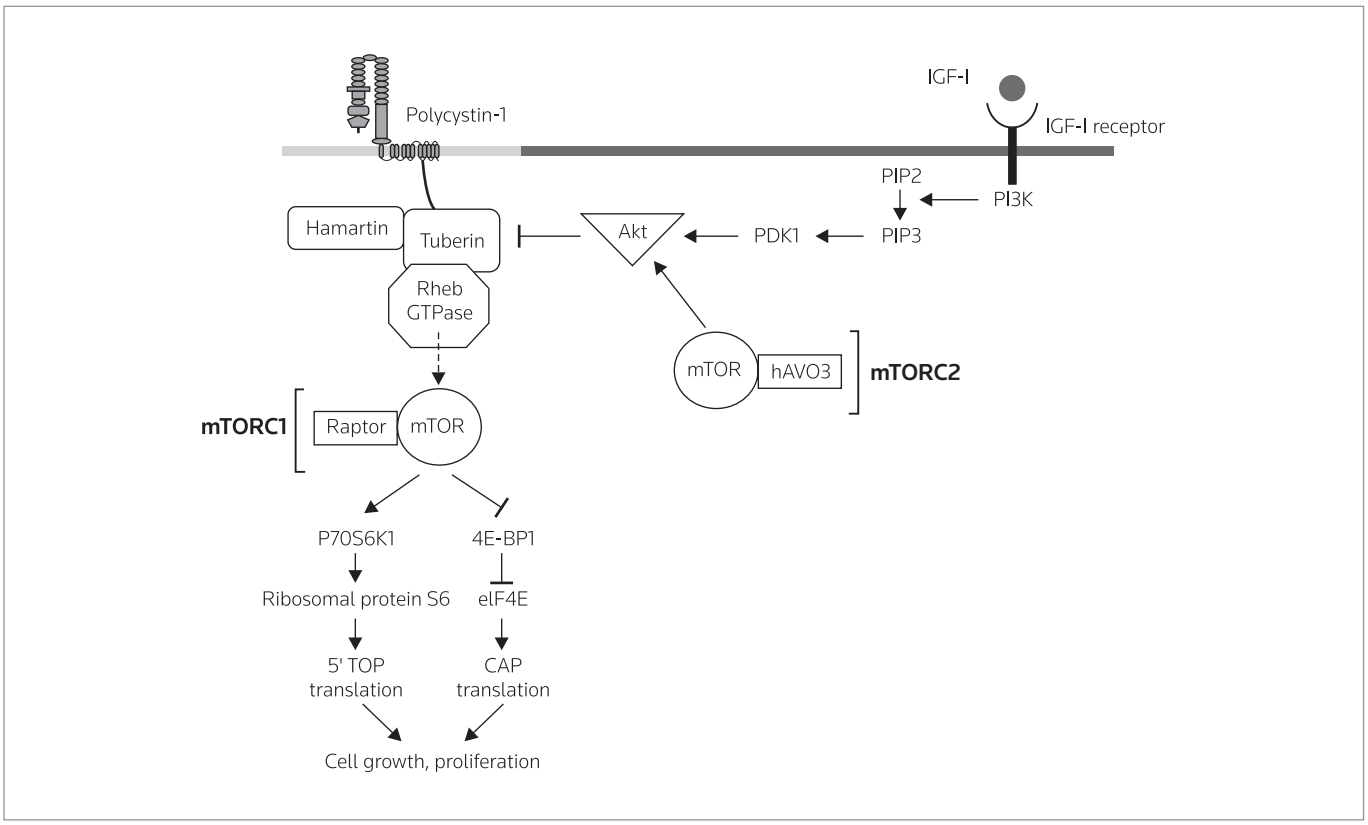

Figure 1. The serine/threonine-protein kinase mTOR (mammalian target of rapamycin) signaling pathway. In normal kidney tubular cells, polycystin-1 inhibits mTORC1 by assembling a complex with tuberin through the membrane-proximal domain of the polycystin-1 cytoplasmic tail. Defects in polycystin-1 in autosomal dominant polycystic kidney disease persistently promote aberrant mTORC1 activation and signaling. mTORC1 is also activated by growth factors such as insulin-like growth factor I (IGF-I), which activates phosphatidylinositol-4,5-bisphosphate 3-kinase (PI3K). PI3K phosphorylates the lipid phosphatidylinositol-4,5-bisphosphate (PIP_2) to phosphatidylinositol-3,4,5-triphosphate (PIP_3), which activates protein kinase B (Akt) through an intermediary kinase, 3-phosphoinositide-dependent protein kinase 1 (PDK1). Once activated, Akt phosphorylates and inactivates tuberin. Tuberin negatively regulates mTORC1 by inhibiting the Rheb GTPase, an activator of mTORC1. mTORC1 is a complex that is made up of mTOR and regulatory-associated protein of mTOR (raptor) and is sensitive to inhibition by rapamycin. mTOR phosphorylates both ribosomal protein P70S6K1 and eukaryotic translation initiation factor 4E-binding protein (4E-BP1) via independent pathways, resulting in activation of P70S6K1 and inactivation of 4E-BP1. The increased P70S6K1 and eukaryotic translation initiation factor 4E (eIF4E) act independently to promote cell growth and proliferation by stimulating the translation of messenger RNA (5′ TOP and CAP translation, respectively). mTOR is also a component of the other major intracellular mTORC2, which includes the the rapamycin-insensitive companion of mTOR (hAVO3). mTORC2 phosphorylates Akt.

responsible for the tuberous sclerosis complex (TSC). Interestingly, the *PKD1* gene lies adjacent to the major gene for tuberous sclerosis, *TSC2*, which encodes the tuberin protein (83). Patients with contiguous *PKD1-TSC2* gene syndrome develop a severe form of PKD. A functional link between polycystin-1 and tuberin was further established by the finding that tuberin is required for membrane trafficking of polycystin-1 (84). Polycystin-1 forms a complex with tuberin which ultimately regulates the activity of mTORC1 (79). Indeed, the TSC, composed of hamartin and tuberin, and the Ras/Rheb-related GTPase (Ras homolog enriched in brain) forms a cascade of downstream intermediates that leads to mTORC1 activation (74, 85, 86). Tuberin is a GTPase-activating protein that inhibits Rheb activity, whereas hamartin binds to and is necessary for tuberin function (74, 86). In turn, tuberin is known to be phosphorylated by ERK (mitogen-activated protein kinase) and Akt (also known as protein kinase B), with a resulting dissociation from the tuberin–hamartin complex. This dissociation interrupts the tuberin–hamartin complex-mediated Rheb inhibition, leading to increased mTORC1 signaling (87, 88) (Fig. 1). Thus, these observations led to the hypothesis that defects in polycystin-1 in ADPKD persistently promote aberrant mTORC1 activation and signaling (79).

It is also possible that signaling through other cell membrane receptors could lead to stimulation of the mTORC1 pathway, which is likely to be a late stage of the cystogenesis cascade. There is evidence that IGF-I, by binding to its receptor, is a major regulator in the mTORC1 pathway via signaling to phosphatidylinositol-4,5-bisphosphate 3-kinase (PI3K), Akt and mTOR (69).

Increase in IGF-I mRNA levels in the kidneys in the *pcy* mouse model of PKD (43) and in IGF-I protein in Han:SPRD rats (89) has been

reported. In addition, the amount of phospho-AKT in cystic *Pkd1*$^{-/-}$ mouse kidneys was more than in wild-type kidneys (90).

Thus, if mTORC1 is such a converging point in PKD cells, it would be worthwhile as a possible drug target for the treatment of renal cystic disorders.

mTOR INHIBITION IN PKD ANIMAL MODELS

Animal models of PKD have been critical in supporting studies of disease pathogenesis and testing potential therapies. Several preclinical PKD models have been described, some with spontaneous mutations and others generated through chemical/genetic manipulations (91). Mutations in these models occurred in different genes, including *Pkd1* and *Pkd2*. However, no single model fully recapitulates all aspects of human PKD pathogenesis, but each represents a facet of human disease. For example, cystic disease in the Han:SPRD rat and *pcy* mouse is slowly progressive, with cyst formation similar to ADPKD. The *jck* mouse model is characterized by the development of cysts in multiple nephron segments and, despite the autosomal recessive mode of inheritance, resembles human ADPKD phenotypically (63).

Understanding the role of mTORC1 in the molecular mechanisms of cyst formation and growth has led to the testing of mTOR inhibitors as a novel potential therapeutic strategy for ADPKD.

Sirolimus (originally referred to as rapamycin) is a macrocyclic lactone that is derived from *Streptomyces hygroscopicus* and exerts antiproliferative and growth-inhibitory effects, as well as antifibrotic effects, by inhibition of mTORC1 (92, 93). This drug, as well as other mTOR inhibitors such as everolimus, has been used in kidney transplant recipients as part of maintenance immunosuppressive therapy (94), and more recently as an antitumor agent (95, 96) and in drug-eluting stents to prevent coronary artery stenosis (97, 98).

Edelstein and colleagues (98) first demonstrated that sirolimus slowed cyst formation in the Han:SPRD rat model of PKD. Sirolimus treatment decreased proliferation in cystic and noncystic tubules, markedly inhibited renal enlargement and cystogenesis, and prevented the loss of kidney function in these PKD rats. These findings have been confirmed by other investigators in the same PKD model (80). Similarly, everolimus given orally for 5 weeks to Han:SPRD rats markedly reduced cyst volume density and ameliorated renal function (99).

Treatment of *ozpk*-rescue and *bpk* mouse models with the mTOR-inhibiting sirolimus also showed effective inhibition of PKD (79). In particular, *bpk* mice that were treated with sirolimus from day 7 postpartum for a period of 14 days demonstrated significantly smaller cyst sizes, an improved renal cystic index and normalization of renal function compared to untreated controls.

Previous studies with mTOR inhibitors used rodent PKD models with mutations in genes that encode proteins (polaris, bicaudal-C, SamCystin and folliculin) with poorly understood function and no known functional link to polycystin-1 (79, 80, 98-102). This limitation has been recently overcome in a new mouse model that results from conditional inactivation of the *Pkd1* gene, which replicates characteristic features of human PKD, including aberrant mTOR activation, epithelial proliferation and apoptosis, and progressive fibrosis (103). Of note, treatment with sirolimus was highly effective in this murine model of PKD. It reduced cyst growth, inhibited epithelial cell proliferation, increased apoptosis of cyst-lining cells, inhibited fibrosis and preserved renal function.

All of these were short-term studies of mTOR inhibitors in animal models of PKD. More recently, long-term sirolimus treatment in Han:SPRD rats with PKD (1-12 months of age) resulted in normalization of kidney volume, renal function, blood pressure and heart weight (102). The only side effect of sirolimus treatment was a slight decrease in body weight, which would support the feasibility of the use of long-term mTOR inhibitors in PKD patients.

Along these lines, there is also evidence that in Han:SPRD rats pulse everolimus treatment at an early age effectively controlled cyst growth and ameliorated kidney function, similarly to a continuous mTOR inhibitor treatment regimen (104).

Together, these animal studies point to a prominent role of the mTOR pathway in cyst growth and progression of PKD and have prompted the initiation of several clinical studies to examine the effectiveness of mTOR inhibition in patients affected by ADPKD.

mTOR INHIBITION IN ADPKD PATIENTS

Current treatment of patients with ADPKD is directed towards reducing morbidity and mortality due to the complications of the disease. At present, blood pressure control remains the major treatment modality for ADPKD. However, during the last few years our enhanced understanding of the PKD phenotype at a cellular level and the encouraging results in experimental models of PKD have offered the opportunity to test more targeted treatments in clinical trials. There are now 20 clinical trials for children and adults with PKD, with agents including somatostatin analogues, the vasopressin V_2 receptor antagonist tolvaptan, renin–angiotensin system inhibitors, statins and mTOR inhibitors (http://www.clinicaltrials.gov). Some preliminary results of clinical studies with somatostatin show promise for patients with ADPKD who are in desperate need of effective therapies (105-108).

Evidence that mTORC1 plays an important role in the pathogenesis of ADPKD has spurred several studies in humans that examine the effect of mTOR inhibitors on the progression of ADPKD. A retrospective study of patients who progressed to ESRD because of ADPKD and received a renal transplant, showed that patients who were given sirolimus immunosuppression had a 24% decrease in size of their native polycystic kidneys on computed tomography (CT) scan compared to patients receiving other immunosuppressants (79). In a similar retrospective study in renal transplant recipients, the size of liver cysts, in addition to the size of native kidneys, also decreased while patients were on mTOR inhibitor therapy, but did not change appreciably with other immunosuppressive drugs (109). Collectively, these observations offered the rationale for prospective clinical trials with mTOR inhibitors in patients with ADPKD and the results of these studies have recently become available.

We first reported the results of one of these clinical trials, the SIRENA study, a randomized, crossover study designed to assess the risk:benefit profile of sirolimus in a cohort of adults with ADPKD (110). The trial randomly allocated 21 patients with ADPKD and a glomerular filtration rate (GFR) ≥ 40 mL/min/1.73 m^2 to 6 months of

treatment with sirolimus added to conventional therapy, followed by 6 months of conventional therapy or to 6 months of conventional therapy followed by 6 months of sirolimus plus conventional therapy. In total, 15 patients completed the study. Six months of sirolimus treatment halted the growth of renal cysts and increased the volume of apparently healthy kidney parenchyma, as measured in serial contrast-enhanced CT images. By contrast, conventional therapy alone was associated with an increase in cyst volume and no change in parenchyma volume. The availability of a sensitive and reproducible technique such as spiral CT allowing for precise volumetric evaluations of different kidney tissue components and intrapatient comparisons in the setting of a cross-sectional design increased the power of the analysis and allowed the achievement of robust findings, despite the relatively small sample size and short follow-up (111). No serious adverse events occurred during the study period, and although aphthous stomatitis complicated sirolimus treatment in 10 of the 15 patients, it was effectively controlled with a mouthwash. No changes in GFR were observed with either treatment. This proof-of-concept study provided clear-cut evidence that in patients with ADPKD 6 months of sirolimus therapy limits renal cyst enlargement with acceptable adverse effects.

Reports of two large clinical trials on potential renoprotection afforded by mTOR inhibitors in patients with ADPKD have recently been published in the *New England Journal of Medicine* (112, 113). The double-blind study by Waltz et al. (112) was performed in 433 patients with ADPKD with normal renal function to moderate dysfunction (GFR > 30 mL/min/1.73 m^2). They were randomized to receive either placebo or the mTOR inhibitor everolimus during 2 years of follow-up. Compared to placebo, everolimus slowed the increase of total kidney volume, as measured by magnetic resonance imaging (MRI). However, this effect was transient, since the difference in the change in total kidney volume between the two groups was statistically significant at 1 year, but not at the end of the 2-year follow-up. A similar trend was documented for the increase of cyst volume over the 2 years. Although the beneficial effect of everolimus in slowing kidney enlargement and cyst growth was not statistically long-lasting, the mean volume increase for total kidney and cyst during the entire 2-year follow-up was numerically lower in the mTOR inhibitor group than in the placebo group. Thus, together these findings are encouraging for the capability of everolimus to limit the growth of kidneys and cysts in patients with ADPKD. In the complementary Swiss trial, Serra et al. (113) randomly assigned 100 ADPKD patients with normal or mildly impaired renal function (GFR ≥ 70 mL/min/1.73 m^2) to placebo or sirolimus for 18 months. Serial MRI was performed to monitor total kidney and cyst volume growth. In contrast to the study by Waltz et al. (112), the Swiss trial found that treatment with sirolimus did not slow kidney growth, as documented by a median increase of total kidney volume over the 18-month period of 97 and 99 mL, respectively, in the placebo and sirolimus groups. Both trials also showed that mTOR inhibition had no beneficial effect on renal function decline, as evaluated by estimated GFR, and that changes in total kidney volume and cyst volume did not correlate with GFR decline.

Overall, the results of the two trials are puzzling, since they are discordant with those of preclinical studies in animal models of PKD that consistently showed the beneficial effect of mTOR inhibitors on kidney size and renal function. However, these clinical studies can be of help to highlight methodological limitations that could have contributed to the conflicting results and eventually should be taken into account for the design of future interventional trials in ADPKD patients. The lack of statistically long-lasting beneficial effects of everolimus on the renal volume increase in the study by Waltz et al. (112) might reflect the fact that approximately one-third of patients did not complete the study. This factor may have contributed to lower the power of the trial to detect persistently significant differences between the placebo and everolimus groups with regard to total kidney and cyst volumes. The failure of the Swiss trial (113) to slow renal growth may be due to the relatively low target dose of sirolimus (2 mg/day) set by the protocol and the fact that the mean sirolimus dose actually given to the patients (1.4-1.6 mg/day) was 25% lower than intended because of dose-limiting side effects. Indeed, the average sirolimus dose normalized by mean patient body weight was approximately 0.020 mg/kg. Of interest, receiver operating characteristic (ROC) curve analysis of a previous study (110) allowed the identification a double cut-off threshold of sirolimus dosage (0.049 mg/kg body weight) that predicted reduction or reversal of total cyst volume growth. Therefore, it is possible that the dose of sirolimus used in the Swiss trial was inadequate to achieve mTOR inhibition at the cyst level (114). This underlines the need of carefully choosing the target dose of mTOR inhibitors in ADPKD trials that may anticipate efficacy in slowing renal growth.

Furthermore, there are more general methodological considerations that could explain the lack of benefit of mTOR inhibitors on the secondary outcomes of both studies, namely renal function decline and correlation between changes in total kidney volume and cyst volume with GFR decline. The loss of renal function in patients with ADPKD can remain undetected for several decades as a consequence of the ability of surviving nephrons to increase GFR in the face of the advancing renal structural damage (3). Thus, a follow-up longer than the 2-year period of these two trials should be considered when assessing the impact of novel treatments on changes in GFR in patients with ADPKD with normal or near normal renal function upon study enrollment.

Estimated GFR, as in the two trials, is not a suitable tool to assess renal function decline, since available prediction equations for GFR estimation do not reliably reflect GFR as directly measured by clearance of exogenous tracers (115). Along these lines, we have recently found in patients with ADPKD that evaluation of GFR by both aMDRD (abbreviated Modification of Diet in Renal Disease) and CKD-EPI (Chronic Kidney Disease Epidemiology Collaboration) prediction equations, used respectively by Waltz et al. and Serra et al., led to a large error in GFR estimation as compared with a true measured GFR by a gold standard method with plasma clearance of iohexol (Gaspari, F., personal communication). Indeed, our patients, with renal function similar to that considered in the two ADPKD clinical trials, showed a large error in GFR estimation ranging from −39 to 49%.

Failure by Waltz et al. and Serra et al. to show a correlation between changes in total kidney volume and GFR decline was also not unexpected. Indeed, evidence is available that, in patients with ADPKD, changes in kidney or cyst volume do not correlate with measured GFR. By contrast, such a correlation has been documented for the renal intermediate volume, corresponding to hypoenhanced regions

of extracystic kidney tissue in contrast-enhanced CT images, which cannot be evaluated by MRI. It has also been shown that the ratio between renal intermediate volume over parenchymal volume, but not total kidney or cyst volume, strongly correlated with the rate of GFR decline (111). The risk of possible acute nephrotoxicity of repeated spiral CT procedures as a result of contrast agent injection, particularly for patients with low GFR, can be minimized by adequate fluid infusion pre- and postimage acquisition. These findings suggest that total kidney volume and cyst volume are not adequate surrogate markers of renal function in patients with ADPKD. Thus, follow-up of ADPKD patients would require a finer quantification of tissue volume beyond solely kidney and cyst measurements.

CONCLUSIONS

The discovery of mTOR dependence of renal cyst growth has led to some cautious optimism that mTOR inhibitors may become an effective treatment option for the vast number of patients suffering from ADPKD. Failure to achieve clear-cut and consistent answers to this quest by the clinical trials so far completed is mainly due to methodological pitfalls in some of these studies, and the nephrology community should avoid the false perception that mTOR inhibitors are of no value for patients with ADPKD. There is no question, however, that better surrogate indexes for assessing ADPKD progression and novel targets for development and efficacy evaluation of therapeutic strategies are needed.

Future clinical trials with mTOR inhibitors should also require careful patient monitoring to ensure long-term tolerability of the therapy. Nevertheless, given the complexity of the cystic disease process, it seems likely that a combination strategy with other novel drugs will be required for maximal therapeutic benefit.

DISCLOSURES

The authors state no conflicts of interest.

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