[Cell Cycle 5:21, 2425-2429, 1 November 2006]; ©2006 Landes Bioscience

Extra View

Regulation of mTOR by Polycystin-1

Is Polycystic Kidney Disease a Case of Futile Repair?

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Original manuscript submitted: 09/06/06
Revised manuscript submitted: 09/13/06
Manuscript accepted: 09/13/06

Previously published online as a *Cell Cycle* E-publication:
http://www.landesbioscience.com/journals/cc/abstract.php?id=3408

KEY WORDS

Polycystic kidney disease, polycystin, mTOR, rapamycin, tuberous sclerosis complex, tuberin, kidney injury and repair

ACKNOWLEDGEMENTS

I sincerely appreciate the dedication, enthusiasm and hard work of the present and past members of my laboratory. Supported by a grant from the NIH (DK62338).

ABSTRACT

Recent work has uncovered a functional link between polycystin-1 (PC1), the protein affected in autosomal-dominant polycystic kidney disease (ADPKD) and tuberin, the protein affected in tuberous sclerosis complex (TSC). These data suggest that PC1 functions by inducing the formation of a complex with tuberin and the Ser/Thr kinase mTOR thereby inhibiting mTOR activity. In normal, adult kidney, mTOR is inactive. However, it is activated in response to insults and required for proliferative and hypertrophic repair processes. We propose a model in which the PC1-tuberin-mTOR complex functions to sense renal insults, possibly by ciliary mechanotransduction, and regulates the activity of mTOR to trigger a formal repair program. In ADPKD, defects in PC1 would lead to constitutive activation of mTOR, and the affected cells would be engaged in a permanent state of futile repair leading to the formation and growth of renal cysts. The mTOR inhibitor rapamycin has proven highly effective in preventing and even reversing cyst growth in rodent models of polycystic kidney disease resulting in preservation of renal function. mTOR inhibitors, already in clinical use as immunosuppressants, may therefore be promising for future therapeutic approaches for ADPKD.

POLYCYSTIC KIDNEY DISEASE

ADPKD is considered to be the most common life-threatening genetic disease. The most significant defect in ADPKD is the development and growth of thousands of cysts in both kidneys in a progressive manner. This results in replacement of the normal renal tissue and significant overall growth of the organs. Eventually, most patients experience renal failure which mandates life-long hemodialysis or kidney transplantation. There is currently no available treatment to slow or prevent the onset of renal failure in ADPKD. For excellent reviews on ADPKD and its pathogenesis, (see refs. 1–3).

POLYCYSTINS AND CILIA FUNCTION

A major obstacle towards designing treatments for ADPKD has been that the molecular mechanisms leading to the pathogenesis have remained poorly understood. Mutations in two genes, PKD1 and PKD2, are the root cause of ADPKD, with PKD1 mutations accounting for 85% of the cases. Since the discovery of the PKD genes over a decade ago, a wealth of information has implicated their protein products, polycystin-1 and -2, in an impressive variety of mechanisms including calcium channel activity, regulation of heterotrimeric G proteins, wnt-signaling, STAT activity and many others.[4] Despite this, it is still unclear what the exact function of the polycystins is and why their disruption leads to renal cyst growth.

Polycystin-2 (PC2), also called TRPP2, is a calcium-permeable, non-selective cation channel of the TRP channel family.[5] Polycystin-1 (PC1), is an integral membrane protein with a very large extracytoplasmic domain, eleven transmembrane domains and a C-terminal cytoplasmic tail whose domain structure suggests a potential surface receptor function. Both polycystin-1 and -2 have been found to localize—among other places—to the primary cilia of renal epithelial cells. These are non-motile extensions of the apical plasma membrane which protrude into the lumen of the renal tubules. A recent development has been the discovery that primary cilia act as mechano-sensors that bend in response to lumenal fluid flow resulting in a transient rise in the intracellular calcium concentration.[6,7] Renal epithelial cells that are null for PC1 lack this flow response[8] suggesting that PC1 is involved in ciliary mechanotransduction. Our group has recently reported a new mechanism of PC1 function that links ciliary mechanosensation to changes in gene

expression via flow-regulated proteolytic cleavage of the cytoplasmic tail of PC1, its nuclear translocation and stimulation of the transcriptional activity of STAT6.[9] Furthermore, several other proteins whose mutation leads to renal cystic diseases in humans or animals have been found to localize to renal cilia.[1,10,11] This has led to the current thinking that loss of cilia function somehow causes a proliferative response in renal epithelial cells leading to cyst formation. The functions of most of these cilia proteins are still poorly understood. It is clear, however, that these cilia proteins must play very diverse roles because they include a great variety of proteins including ion channels, microtubule motors and proteins implicated in intra-flagellar transport. Since the disruption of any of these diverse proteins leads to renal cyst formation it appears plausible that their functions eventually converge on a common pathway that is central to the observed changes in proliferation, apoptosis and differentiation in renal cysts. If this hypothesis is correct, then such a central pathway may be an excellent target for therapeutic intervention.

REGULATION OF MTOR BY PC1 AND TUBERIN

In a recent paper,[12] we have reported another novel function of PC1 and the identification of what could be such a common pathway that is dysregulated in ADPKD and other renal cystic diseases. These findings suggest that PC1 regulates the activity of the Ser/Thr kinase mTOR via the regulatory protein tuberin. Tuberin, the product of the TSC2 gene, is mutated in the autosomal-dominant disease TSC, which is characterized by benign tumors in multiple organs and developmental abnormalities. TSC is about 10-times less common than ADPKD. For excellent recent reviews on the pathogenesis of TSC, see.[13-15] A possible functional linkage between tuberin and PC1 had been considered for the following reasons.

(1) Most TSC patients have renal lesions that consist of epithelial cysts in addition to mesenchymal angiomyolipomas. Furthermore, rats with a germline inactivation of the tuberin gene exhibit renal cysts.[16]

(2) The TSC2 (tuberin) and PKD1 (PC1) genes lie immediately adjacent to each other on the human genome in a tail-to-tail orientation separated by only ~60 nucleotides. These two genes exhibit well-conserved synteny and their close proximity is observed in mammals, birds and fish.[17] While this synteny in itself may be a coincidence, it could also suggest a functional linkage or at least that the expression of both genes may be coregulated.

(3) Patients in which both the PKD1 and TSC2 genes are affected by a larger deletion exhibit polycystic kidney disease of much earlier onset and severity than patients with PKD1 mutations alone.[18] This may suggest that PC1 and tuberin cooperate functionally.

(4) Cells that are null for tuberin exhibit a defect in the trafficking of PC1 to the plasma membrane which again suggests functional linkage.[16]

We have now reported evidence for a physical interaction—direct or indirect—between PC1 and tuberin, and for the regulation of tuberin's activity by PC1.[12] What is known about the function of tuberin? Tuberin has emerged as a critical regulator of mTOR activity.[19-21] mTOR controls the translation of certain messages by phosphorylating regulators of translation initiation and ribosomal function, most notably 4E-BP1 and the p70-S6-kinase (S6K). mTOR activity stimulates cell growth and proliferation and plays a role in the regulation of differentiation. In contrast, mTOR inhibition can result in apoptosis. mTOR activity requires the GTP-bound form of the small G-protein rheb. The main function of tuberin appears to be to regulate the GTP/GDP status of rheb by virtue of its GAP domain. The GAP activity of tuberin depends on it being in a complex with hamartin, the product of the TSC1 gene. The tuberin-hamartin complex therefore acts as an inhibitor of mTOR. Disruption of the tuberin-hamartin complex, e.g., after phosphorylation of tuberin by Akt in response to growth factor signaling, leads to relief of this inhibition and therefore mTOR activation.

Our biochemical and in vivo evidence suggests that tuberin interacts, directly or indirectly, with the C-terminal cytoplasmic tail of PC1. Furthermore, our results suggest that also mTOR interacts with the PC1 tail. This has led us to hypothesize that PC1 induces proximity between tuberin and mTOR by the formation of a novel complex whose function it is to regulate mTOR activity (see model figure). If correct, then the disruption of PC1 should lead to dysregulation of mTOR. This was tested in two systems: ADPKD patients, most of which have PC1 defects, and a novel mouse model in which a floxed PKD1 gene is conditionally inactivated leading to polycystic kidney disease. In both cases, cyst-lining epithelial cells exhibited high mTOR activity as evidenced by the phosphorylation status of mTOR and its downstream targets. Furthermore, two additional mouse models of polycystic kidney disease exhibited high mTOR activity even though the affected genes in these mice are unrelated to PC1. In one model (orpk-rescue), the protein polaris, implicated in intra-flagellar/cilia transport, is affected. In the other model, the protein MAL (implicated in apical membrane trafficking) is overexpressed.

If the aberrant activation of mTOR is important for the development and/or growth of renal cysts, then inhibition of mTOR should be expected to lead to inhibition of cyst growth. This prediction could be tested because a very effective and specific inhibitor of mTOR is available. mTOR is an acronym for "mammalian target of rapamycin". Rapamycin, also called sirolimus, inhibits mTOR activity and is in clinical use as an immunosuppressive drug, mainly for kidney transplant patients.[22] Treatment of two polycystic mouse models led to striking results. Treatment of the slowly-progressing orpk-rescue model for one month resulted in a dramatic reduction of cyst and overall kidney sizes. MRI imaging of animals during treatment revealed that not only was rapamycin able to prevent further renal growth but it even resulted in a significant regression of already enlarged kidneys. This regression appeared to be due to the selective induction of apoptosis in cyst-lining epithelial cells. Equally impressive was the effect of rapamycin on the aggressive, fast-progressing bpk polycystic mouse model that exhibits a defect in a protein called bicaudal-C. Treatment with rapamycin from postnatal day 7 to 21 resulted in significant reduction of the massively enlarged cystic kidneys. Moreover, kidney function, which starts to fail around postnatal day 21 in untreated animals, was preserved by rapamycin. Since inhibition of mTOR alone had these dramatic beneficial effects this suggests that aberrant mTOR activity is a major driving force of renal cyst growth.

Could rapamycin be an option for the treatment of ADPKD patients? As a first attempt at addressing this question, we made use of the fact that many ADPKD patients undergo kidney transplantation after their advancement to end-stage renal disease. Rapamycin is used as an immunosuppressant in some of these transplant patients. Furthermore, the transplanted kidneys are frequently added to ADPKD patients while the polycystic kidneys are left in place. Therefore, we were able to identify a small group of ADPKD patients who were treated with rapamycin and whose kidneys were imaged by CT scans at the beginning and approximately two years after

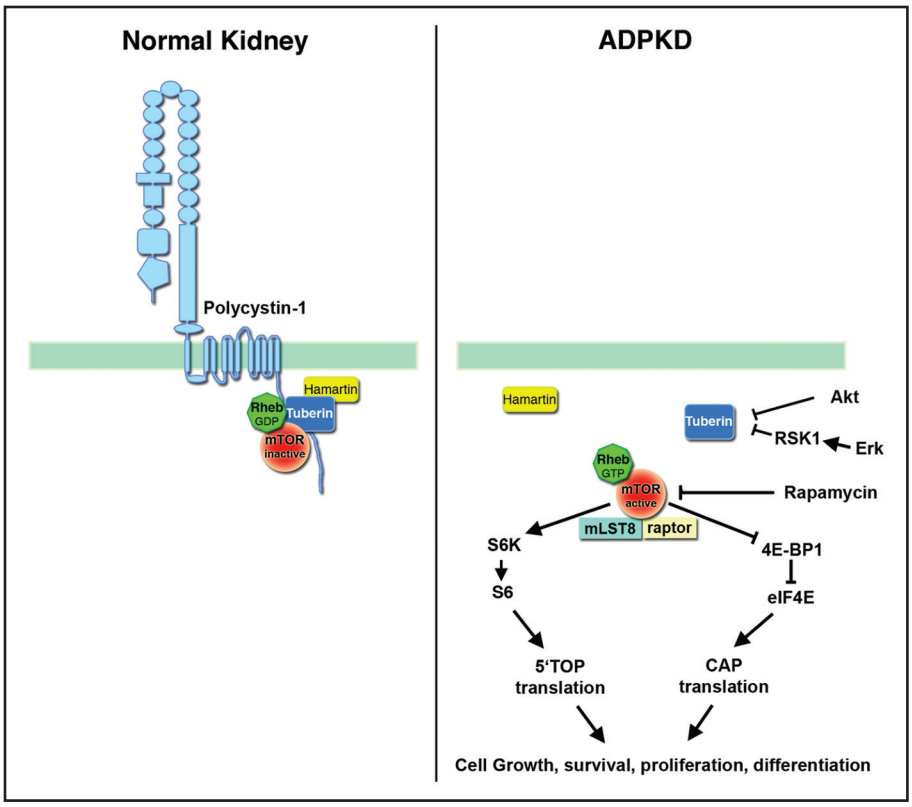

Figure 1. Working model of the regulation of mTOR activity by PC1 and the defect in ADPKD.

the transplantation. In these patients, the volume of the polycystic kidneys decreased by 25%. In contrast, a non-rapamycin treated control group showed no significant change in their renal volumes. These results are highly encouraging but we have to keep in mind that this was a very small, retrospective study that should be repeated with larger patient numbers.

In two other independent studies, beneficial effects of rapamycin were also reported on a polycystic rat model.[23,24] Rapamycin treatment of the Han:SPRD rat model resulted in significant reduction of cyst and renal sizes and preservation of renal function. The gene affected in this model is still unknown. However, these results, together with ours make it likely that mTOR is indeed at a converging point of pathways originating from multiple genotypes that lead to renal cyst growth. To summarize, manipulation of the genes for the following diverse variety of proteins lead to renal cysts that have either been shown to exhibit aberrantly high mTOR activity and/or respond to mTOR inhibition: PC1, tuberin, polaris, bicaudal-C, MAL, and the Han:SPRD gene. Therefore, it appears likely that renal cystic diseases caused by other genotypes will also exhibit aberrant mTOR activation.

A MODEL FOR THE REGULATION OF MTOR BY PC1

Our results are consistent with the working model shown in Figure 1. We suggest that an important function of PC1 is to inhibit mTOR by assembling a complex with tuberin and mTOR. Complex formation would depend on the membrane-proximal domain of the PC1 cytoplasmic tail. Since tuberin is known to interact with rheb via its GAP domain[25,26] and since rheb has been shown to bind directly to mTOR irrespective of the guanine nucleotide status of rheb and independent of tuberin,[27] we hypothesize that rheb will also be part of the PC1-tuberin-mTOR complex. Because rheb would be in the GDP-bound state, mTOR in this complex would remain inactive. Since hamartin is known to complex with tuberin and is believed to be required for tuberin function, we would expect that hamartin would also be part of the PC1-tuberin-mTOR complex. The detailed characterization of this complex is an important task ahead.

One function of the complex formation between PC1 and tuberin may also be to sequester tuberin so that it is unavailable as a substrate for kinases that inactivate it such as Akt and RSK1. In ADPKD, a defect in complex formation may expose tuberin to these kinases which themselves are activated by growth factor signaling. In effect, this would hyper-sensitize epithelial cells in ADPKD to growth factors that activate the PI3-kinase or Erk pathways, and lead to further activation of mTOR (see model). EGF-receptor signaling leading to Erk activation has been well documented in human renal cystic diseases and animal models.[28] Inhibition of EGF-receptor/Erk signaling suppresses renal cystic disease in some but not all animal models.[29-31] Activation of Erk signaling may be an autocrine response involving epithelial growth factor secretion and apical mistargeting of the EGF-receptor[28] and/or induced by stretching of the cystic epithelium due to lumenal fluid pressure.[32,33]

DOES THE PC1-TUBERIN-MTOR COMPLEX PLAY A ROLE IN REPAIR RESPONSES TO RENAL INJURY?

We found that renal epithelial cells in growing kidneys during postnatal development exhibit high mTOR activity (Larson, Weimbs, unpublished). However, it is clear that mTOR becomes largely or completely inactive in normal adult renal epithelial cells. As we have reported, normal human and mouse kidneys do not express significant levels of phospho-mTOR (Ser-2448, considered an indicator of active mTOR) or the downstream targets phospho-S6K (Thr-389) or phospho-S6 (Ser-235/236). This is consistent with results by ref. 34 who found no detectable levels of phospho-S6K (Thr-389) in normal rat kidney. mTOR inhibition also has no apparent effect on normal kidneys. Treatment of control mice with rapamycin for one month caused no apparent renal effect or change in the total renal volume.[12] Rapamycin treatment also has no effect on the glomerular filtration rate or apoptosis in rat kidney.[34] Even high doses of rapamycin do not cause any morphological changes in rat kidney or have any significant effect on renal function (DiJoseph, 1992). In humans, rapamycin shows no significant nephrotoxicity.[35] All this indicates that mTOR activity is neither present nor needed in the normal adult kidney. Therefore, the normal function of the PC1-tuberin-mTOR complex would be expected to keep mTOR inactive. But why go through all this trouble just to turn off a kinase? There are several reported conditions under which mTOR is activated in renal epithelial cells in response to insults. This would suggest that the PC1-tuberin-mTOR complex can sense these insults and provide the appropriate regulatory response.

Acute renal failure is a serious condition with a very high mortality rate that can be mimicked experimentally by stopping the renal blood supply for some time followed by reperfusion. Under these conditions, tubular cell death occurs leading to impairment of renal function. In contrast to many other organs, the kidney has the remarkable capacity to regenerate itself in a process that involves the partial de-differentiation of surviving epithelial cells, proliferation to replace lost cells and finally redifferentiation to reform functional nephrons.[36,37] Lieberthal et al. reported that the level of phospho-S6K (Thr 412) is dramatically increased in rats during kidney repair after ischemia/reperfusion (Lieberthal, 2001). Furthermore, rapamycin treatment of the animals markedly impaired the recovery of the ischemic kidneys due to inhibition of proliferation and induction of apoptosis. This clearly suggests that mTOR activity is needed as part of a proliferative repair program.

Another way to injure a kidney in animal models is to obstruct the ureter. This leads to the stop of tubular fluid flow while blood supply to the kidney is maintained. Renal epithelial cells respond to this insult by starting a process that could be considered a repair program in overdrive. The kidneys increase in size due to severe tubular dilation and, in a longer-term process, many cells de-differentiate to such a degree that they become fibroblastic. This epithelial-to-mesenchymal transition leads to the excessive production of extracellular matrix and renal fibrosis which is thought to play a role in the progression of chronic kidney disease.[38] Interestingly, the renal response to ureteral obstruction can be blunted by treating the animals with rapamycin[39] which suggests again that mTOR activity is essential for this proliferative response to injury.

Compensatory renal hypertrophy is another trick up the kidney's sleeve to respond to problems. Loss of functioning nephrons results in the growth of remaining tissue as a compensatory measure to restore working capacity. In animal models, removal of one kidney leads to substantial growth of the remaining kidney. In contrast to recovery from ischemic injury, however, this does not involve proliferation but only an increase in the size of renal epithelial cells. Chen et al. found that compensatory renal hypertrophy involves dramatic upregulation of mTOR activity in the kidney. Rapamycin treatment prevents this process.[40] Similar effects of rapamycin on renal hypertrophy associated with proteinuria or renal mass reduction were reported by another group.[41] A possibly related process may be the renal hypertrophy that is induced under diabetic conditions and can lead to diabetic nephropathy. Again, renal epithelial cells increase in size in the absence of proliferation. It is unclear whether this is triggered by the increased workload of the kidney or by the over-abundance of glucose. Recent studies reported a strong increase in mTOR activity as an early response to the onset of diabetes in rodent models.[42,43] Rapamycin treatment again prevented the renal hypertrophy in these cases.

The overall conclusion from these observations is that mTOR is normally inactive in the kidney unless it is needed in response to insults. In that case, mTOR activation plays an essential role in a proliferative (ischemic injury or ureteral obstruction) or hypertrophic (compensatory hypertrophy or diabetes) response. It is currently unknown by what mechanism mTOR is activated under any of these circumstances. It is tempting to hypothesize that the PC1-tuberin-mTOR complex plays a crucial role. Since PC1 has been implicated in ciliary mechanosensation, an exciting possibility might be that renal injuries can be sensed by changes in tubular fluid flow. During ischemic injury and ureteral obstruction, fluid flow essentially ceases. In contrast, in the conditions that lead to compensatory renal hypertrophy and in diabetes, tubular fluid flow is expected to increase. Interestingly, we and others have shown that PC1 can undergo proteolytic cleavage that releases its C-terminal cytoplasmic tail from the membrane, leading to nuclear translocation.[9,44] Chauvet et al. have shown that this cleavage is triggered during ureteral obstruction.[44] Therefore, a possible mechanism that may link tubular fluid flow and mTOR activation would be that the proteolytic cleavage of the PC1 tail results in disruption of the PC1-tuberin-mTOR complex and therefore mTOR activation. This exciting idea remains to be tested.

IS ADPKD A DISEASE OF FUTILE RENAL REPAIR?

How does this all tie together with the growth of cysts in ADPKD? Since mTOR is normally activated in the kidney only in response to insults we suggest that the high activity of mTOR in ADPKD may result from the aberrant activation of a proliferative renal repair program. Indeed, ADPKD has many features in common with a kidney that is responding to ischemic damage or ureteral obstruction. In all of these cases, renal epithelial cells undergo de-differentiation and become proliferative. Fibrosis and excessive deposition of extracellular matrix are common to all. Tubule dilation occurs after ureteral obstruction just as it does in ADPKD. Importantly, all of these conditions can be blunted by mTOR inhibition with rapamycin. If ciliary mechanosensation and the PC1-tuberin-mTOR complex are indeed the trigger for a proliferative renal repair program, then this would suggest the following scenario. A single renal epithelial cell in an ADPKD patient has just undergone a second-hit somatic mutation affecting its remaining PKD1 allele. From this moment on, the cell will lack functional PC1 and will be unable to assemble the PC1-tuberin-mTOR complex that normally silences mTOR. The cell will be under the false "impression" that tubular fluid flow has stopped. It will do what it is normally supposed to do under these conditions: it de-differentiates and proliferates in an attempt to respond to the insult, only that there is no insult. This "repair hypothesis" is appealing because it explains why so many different genetic defects all lead to the same outcome, namely renal cyst formation. If any protein that plays a role in ciliary mechanosensation is mutated, we would expect that consequently the same formal cellular response program is activated. This program is aimed at injury repair and involves mTOR as a key player. Clearly, much work lies ahead to test this unifying model.

DO MTOR INHIBITORS HOLD PROMISE FOR THE TREATMENT OF ADPKD?

For an excellent recent discussion of emerging possibilities for therapeutic approaches in ADPKD, see.[45] Rapamycin treatment is clearly very effective in rat and mouse models of PKD. It results in the inhibition of renal and cyst growth, and in the preservation of renal function. Our animal data even showed that rapamycin causes a reduction in the volume of already enlarged kidneys[12] which may suggest that even more advanced patients may benefit. This decrease in renal mass appears to be due to the selective induction of apoptosis in cyst-lining epithelial cells. Therefore, while the anti-proliferative effect of rapamycin appears to prevent cyst growth and perhaps the formation of new cysts, the pro-apoptotic effect appears to eliminate existing cysts. The induction of apoptosis by rapamycin has been reported in many cell types including cultured mouse proximal tubule cells[34] and renal tumor cells in rapamycin-treated, tuberin-mutant Eker rats.[46]

Clinical trials to test the potential beneficial effect of mTOR inhibitors on ADPKD patients are currently in the early or planning phases.[45] The immuno-suppressive effect of rapamycin may be expected to be the most concerning possible complication. However, in contrast to immuno-suppressed transplant patients, treatment in ADPKD patients could be interrupted without difficulties should a complication occur. It is currently unclear whether the treatment regimen that is used for immunosuppression is anywhere near optimal for the treatment of ADPKD. Lower doses may be sufficient which would eliminate concerns of effects of immunosuppression. Alternatively, higher doses may be required to prevent cyst growth or reduce renal mass. Doses used on PKD animal models ranged from 0.2 mg/kg/d intraperitoneally[23] and 0.2 mg/kg/d orally[24] on a rat model to 1.67–5 mg/kg/d intraperitoneally on mouse models.[12] Especially the latter regimens significantly exceed the doses administered to renal transplant patients (typically 5 mg/d orally). Furthermore, the genetic defects in all of these animal models differ from the PKD1 or PKD2 defects in human ADPKD patients. This illustrates that further animal experiments, especially using models with PKD1/2 genotypes, are urgently needed to establish the best treatment regimen and to guide clinical trials. For example, it is conceivable that short-term, intermittent treatment with very high doses may be ideal because this may reduce the cyst burden in a short burst by induction of apoptosis, analogous to our results from animal experiments,[12] and may allow for prolonged recovery phases free of treatment.

The discovery of a link between PC1 and mTOR activity and the mTOR-dependence of renal cyst growth have led to some cautious optimism that mTOR inhibitors may become the first effective treatment option for the vast number of people suffering from ADPKD. The fact that mTOR inhibitors are already utilized clinically with a track-record of long-term use should help to accelerate the quest to test their potential.

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