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Mammalian target of rapamycin and the kidney. II. Pathophysiology and therapeutic implications

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Lieberthal W, Levine JS. Mammalian target of rapamycin and the kidney. II. Pathophysiology and therapeutic implications. *Am J Physiol Renal Physiol* 303: F180–F191, 2012. First published April 11, 2012; doi:10.1152/ajprenal.00015.2012.— The mTOR pathway plays an important role in a number of common renal diseases, including acute kidney injury (AKI), diabetic nephropathy (DN), and polycystic kidney diseases (PKD). The activity of mTOR complex 1 (mTORC1) is necessary for renal regeneration and repair after AKI, and inhibition of mTORC1 by rapamycin has been shown to delay recovery from ischemic AKI in animal studies, and to prolong delayed graft function in humans who have received a kidney transplant. For this reason, administration of rapamycin should be delayed or discontinued in patients with AKI until full recovery of renal function has occurred. On the other hand, inappropriately high mTORC1 activity contributes to the progression of the metabolic syndrome, the development of type 2 diabetes, and the pathogenesis of DN. In addition, chronic hyperactivity of mTORC1, and possibly also mTORC2, contributes to cyst formation and enlargement in a number of forms of PKD. Inhibition of mTOR, using either rapamycin (which inhibits predominantly mTORC1) or "catalytic" inhibitors (which effectively inhibit both mTORC1 and mTORC2), provide exciting possibilities for novel forms of treatment of DN and PKD. In this second part of the review, we will examine the role of mTOR in the pathophysiology of DN and PKD, as well as the potential utility of currently available and newly developed inhibitors of mTOR to slow the progression of DN and/or PKD.

acute kidney injury; diabetic nephropathy; polycystic kidney disease

THIS IS THE SECOND PART OF a two-part review. In the first part (90), we described the molecular events involved in the regulation and downstream effects of the mammalian target of rapamycin (mTOR). Briefly, mTOR is a serine/threonine kinase that provides the catalytic activity for two distinct multiprotein complexes, known as mTOR complex 1 (mTORC1) and 2 (mTORC2). Activation of mTORC1 promotes both cell growth (increased cellular mass or size) and cell proliferation (increased cell number). mTORC1 acts as a metabolic "sensor," ensuring that conditions are optimal for growth and proliferation. Its activity is tightly regulated by the availability of amino acids, growth factors, and energy stores. The effects of mTORC2 are different from those of mTORC1 and include modulation of cell survival, cell polarity, cytoskeletal organization, and activity of the aldosterone-sensitive sodium channel (90). In this review (part II), we discuss the role of mTORC1 and mTORC2 in three important renal diseases: acute kidney injury (AKI), diabetic nephropathy (DN), and polycystic kidney disease (PKD).

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AKI

AKI, a common clinical problem caused by acute ischemic or toxic injury to the kidney, is associated with substantial morbidity and mortality (25). Unlike the heart and brain, the kidney is capable of almost complete recovery after acute injury (13). Renal recovery after AKI requires the proliferation of viable tubular cells to replace cells lost by apoptosis or necrosis during AKI (13, 91) (Fig. 1). Renal recovery also entails the recovery of sublethally injured cells, with restoration of their normal polarity and ability for unidirectional transport (20, 138) (Fig. 1). mTOR has the potential to affect recovery from AKI in several ways. First, mTORC1 stimulates mitochondrial activity and ATP production (80, 135), thereby aiding in the generation and maintenance of renal energy stores necessary for recovery (80, 135). In addition, mTORC1, through its role in the regulation of cell growth and proliferation (176), contributes vitally to the repopulation of renal tubular cells (Fig. 1).

Our group has studied the role of mTOR in a rat model of ischemic-reperfusion injury (IRI). We found that mTORC1 activity, as assessed by the degree of phosphorylation of its downstream substrate S6 kinase (S6K), while low or absent in the normal rat kidney, is increased markedly after IRI (89). Notably, inhibition of mTORC1 with rapamycin delayed renal

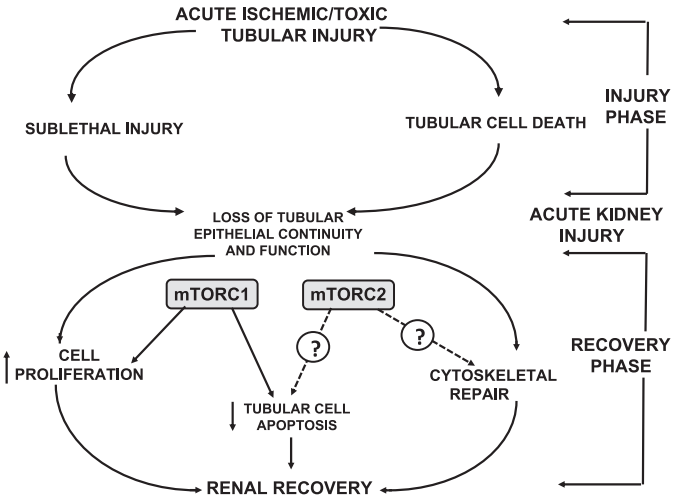

Fig. 1. Potential role of mammalian target of rapamycin (mTOR) in repair and recovery of renal function after ischemic or toxic acute kidney injury (AKI). The tubular epithelium is the major target of injury in AKI. Tubular cells, if exposed to toxins or depleted of ATP, die by apoptosis or necrosis. Both forms of cell death result in discontinuity of the tubular lumen, permitting the "back-leak" of glomerular filtrate from the tubular lumen into the interstitium. Less severe forms of injury can cause sublethal changes through disruption of the actin cytoskeleton. Cytoskeletal disorganization, in turn, leads to a loss of cell polarity as well as a loss of cell-cell and cell-matrix adhesion, with a loss of epithelial unidirectional transport. Together, these effects result in desquamation of sublethally injured cells and further loss of epithelial continuity and function. Desquamated viable and dead cells form intratubular casts, which can further reduce glomerular filtration by obstruction of nephrons. Available evidence suggests that recovery of renal function after AKI requires proliferation of the remaining viable tubular cells. Activation of mTOR complex 1 (mTORC1) plays an important role in recovery after AKI by inhibiting further apoptotic death and by promoting the regeneration of tubular cells. While a role for mTOR complex 2 (mTORC2) in AKI has yet to be established, its known importance in regulation of the cytoskeleton, cell polarity, and aspects of tubular transport suggests that mTORC2 may also contribute to recovery from AKI, especially sublethally injured cells.

recovery, as assessed by measurement of the glomerular filtration rate (GFR) (89). This effect of rapamycin was mediated, at least in part, through both decreased tubular cell proliferation and increased tubular cell death by apoptosis (89). Although rapamycin delayed recovery of renal function after AKI, full recovery of renal function eventually still occurred, despite continued rapamycin treatment (88). The ultimate recovery of renal function, even in the face of continued mTORC1 inhibition, may be explained by the recent observation that rapamycin does not fully and durably inhibit the stimulatory effect of mTORC1 on cell proliferation (discussed in detail below).

The effects of rapamycin on cell survival and apoptosis during IRI are complex, as is the relationship between mTOR and the prosurvival kinase Akt. As discussed in the first part of this review (90), activation of Akt is mediated by phosphorylation at two major sites, Thr^{308} and Ser^{473}. Phosphorylation at Thr^{308} is a major upstream event leading to activation of mTORC1 and is positively regulated by phosphatidylinositol 3-kinase (PI3K) (90). In contrast, phosphorylation at Ser^{473} lies downstream of mTOR and is accomplished predominantly by mTORC2 (90). Phosphorylation at these two sites mediates different Akt-dependent effects (90). Renal IRI activates Akt by inducing phosphorylation at both Thr^{308} and Ser^{437}. Moreover, activation of Akt by IRI has been shown to ameliorate tubular cell apoptosis (11, 62, 111, 128, 132, 154). Our finding that rapamycin increases apoptosis after IRI is consistent with a role for mTORC1 in this prosurvival effect of Akt on renal tubular cells (89). The possibility that mTORC2-mediated phosphorylation at Ser^{437} contributes to the prosurvival activity of Akt during IRI, although likely, has not been directly studied (Fig. 1).

In addition to potential effects on cell survival, mTORC2 could promote recovery from IRI in other ways. Through its effects on cytoskeletal organization and solute transport (90), mTORC2 could contribute vitally to the recovery of sublethally injured cells (2, 176). Potentially beneficial effects may include the restitution of cell polarity, the reestablishment of tight junctions and cell-cell adhesion, and restoration of the capacity for unidirectional transport (74, 75) (Fig. 1).

Our findings that rapamycin delays recovery in an experimental model of IRI (88, 89) have been confirmed in humans with AKI. In recipients of renal transplants, administration of rapamycin can cause and/or exacerbate delayed graft function (42, 47, 51, 94, 99, 141). Following recognition of the adverse effects of rapamycin in the peritransplant period, it has become routine practice to delay usage of rapamycin until the transplanted kidney is functional. In addition, it would seem advisable to discontinue rapamycin in all patients during episodes of AKI.

In addition to its effects on renal recovery after AKI, rapamycin may also modulate the kidney's response to injury through effects on autophagy (68). As described in the first part of this review (90), mTORC1 is an important upstream inhibitor of autophagy. Through its inhibition of mTORC1, rapamycin could potentially stimulate autophagy (90). A detailed discussion of the role of autophagy in the pathogenesis of AKI is beyond the scope of this review but is discussed in detail elsewhere (68). It is worth noting that autophagy has been shown to be stimulated in proximal tubular cells during AKI, despite activation of mTORC1 (68). While the impact of autophagy on cell fate during AKI remains somewhat controversial, the current balance of evidence suggests that increases in autophagic flux may provide tubular cells with an essential source of nutrients and energy during AKI and thereby protect tubular cells from apoptosis (68). However, it is not yet known whether rapamycin, by inhibiting mTORC1, increases autophagic flux above levels seen during AKI.

DN

DN, which affects ~40% of patients with diabetes mellitus, presents initially with microalbuminuria. Proteinuria increases gradually in magnitude until development of the nephrotic syndrome (3, 36). The onset of proteinuria is followed by hypertension and a loss of renal function, which progresses inexorably to end-stage renal disease (ESRD). A characteristic early feature of DN is renal enlargement, due to both glomerular and tubular hypertrophy (66). Later glomerular changes include basement membrane thickening and mesangial matrix accumulation, culminating in global glomerulosclerosis (3, 36). In addition, tubulointerstitial inflammation and tubular "drop out" progress ultimately to renal fibrosis and ESRD. Current approaches to slowing the course of DN include rigorous control of blood glucose and blood pressure. Despite these interventions, DN remains the leading cause of ESRD in adults in the United States and Western Europe (3).

Abundant evidence supports a role for mTOR in the pathogenesis of DN. Overactivity of mTOR by glomerular podocytes occurs early in the course of DN, in both animal models and humans (46, 72, 92, 102). Moreover, in animal models, inhibition of mTOR with rapamycin slows progression of DN (73, 92, 102, 160, 165). While the role of mTOR seems beyond dispute, the mechanism by which mTOR is activated, especially in patients with type 2 diabetes, is less clear.

Potential mechanisms of mTOR activation in type 2 diabetes. In patients with type 2 diabetes, activation of mTOR probably begins well before the development of overt hyperglycemia (Fig. 2). A major contributing factor is dietary, through excessive caloric intake. Chronic ingestion of excess nutrients has been shown to induce a sustained activation of mTOR (31, 101, 112, 149), with subsequent development of obesity and insulin resistance (58, 60, 148).

Obesity in the setting of nutrient excess and mTOR overactivity occurs in part through stimulation of peroxisome proliferator-activated receptor-γ (PPAR-γ), a transcription factor crucial for adipogenesis (26, 78). Both mTORC1 (78, 172) and mTORC2 (104, 142) promote deposition of fat, not only in white adipose tissue but also in liver and muscle. These events are all forerunners of type 2 diabetes (112, 149). Consistent with these notions, hyperlipidemia, characterized by increases in both LDL cholesterol and triglycerides, is a well-recognized side effect of chronic rapamycin administration and has been attributed to impaired deposition of lipids in adipose tissue and to increased hepatic synthesis of triglycerides (67, 103).

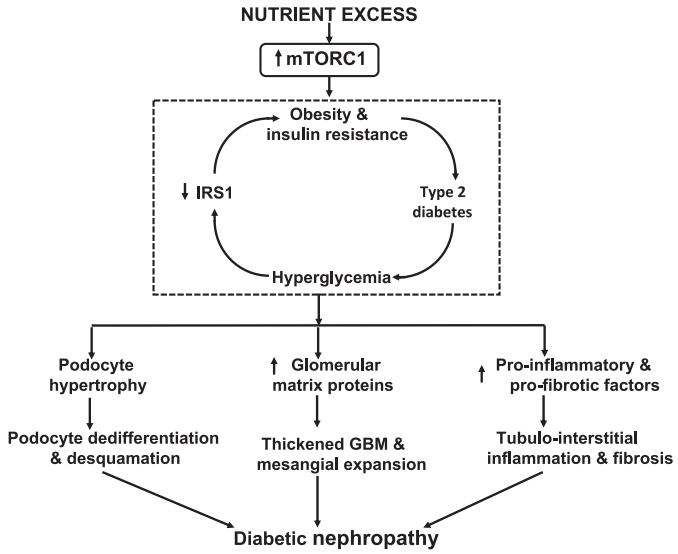

Fig. 2. Role of mTORC1 in development of the metabolic syndrome, type 2 diabetes, and diabetic nephropathy (DN). The majority (>80%) of patients with type 2 diabetes are obese. The metabolic syndrome, characterized by obesity and insulin resistance, predisposes to type 2 diabetes. DN develops in ~40% of patients with diabetes. Chronic overactivity of mTORC1, induced by an excessive intake of dietary nutrients, precedes occurrence of the metabolic syndrome and plays an important role in the development of obesity, insulin resistance, and ultimately type 2 diabetes. Once diabetes develops, mTORC1 activity is further increased by hyperglycemia. Nutrient excess and hyperglycemia inhibit AMP-activated protein kinase (AMPK), releasing mTORC1 from inhibition by AMPK. mTORC1 overactivity is also an important mediator of events within the kidney that lead to DN. mTORC1, acting via S6 kinase 1, induces hypertrophy of glomerular and tubular cells, leading to renal enlargement early in DN. While initially adaptive, glomerular hypertrophy eventually contributes to podocyte injury, with resulting dedifferentiation and desquamation from the glomerular basement membrane (GBM). mTORC1 overactivity also promotes the synthesis of glomerular matrix proteins, leading to GBM thickening and expansion of the mesangial matrix expansion. mTORC1 also promotes tubulointerstitial inflammation and fibrosis through enhanced secretion of proinflammatory and profibrotic factors. Together, these effects lead ultimately to glomerulosclerosis and interstitial fibrosis. While evidence exists for the activation of mTORC2 in animal models of diabetes, the role of mTORC2 in the pathogenesis of DN remains uncertain.

Insulin resistance is an additional consequence of nutrient excess and mTOR overactivity. Under conditions of chronic insulin stimulation, negative feedback loops come into play and partially uncouple the insulin receptor from its downstream effectors, thereby attenuating insulin-dependent signal transduction (175). A critical mediator of this negative feedback is insulin receptor substrate-1 (IRS-1), a protein that acts as an adaptor to connect the insulin receptor with its many downstream pathways. mTOR inhibits IRS-1 in at least two ways. First, phosphorylation of IRS-1 by mTORC1, an event mediated by physical interaction between IRS-1 and regulatory-associated protein of mTOR (Raptor), suppresses IRS-1-dependent signaling (148, 150). Second, sustained activation of mTOR triggers the proteosomal degradation of IRS-1 through both rapamycin-sensitive (60) and -insensitive pathways (58).

Once diabetes develops, hyperglycemia contributes further to the activation of mTOR. Studies in cultured glomerular and proximal tubular epithelial cells have shown that hyperglycemia activates mTORC1, as manifested by phosphorylation of S6K1/2 and 4E-BP1 (84, 98). These events are mediated, in part, through inhibition of AMPK (84), as well as activation of PI3K and Akt (98). Overactivity of PI3K and Akt has also been observed in cortical homogenates from the kidneys of *db/db* mice, a murine model of obesity and type 2 diabetes (38).

mTOR and diabetic glomerulosclerosis. The major glomerular manifestations of DN are cellular hypertrophy, glomerular basement membrane (GBM) thickening, and mesangial matrix accumulation. Increased mTOR activity plays a role in all three events, culminating ultimately in global glomerulosclerosis (Fig. 2). Renal cortical tissue from type 1 diabetic rats and type 2 diabetic *db/db* mice showed increased phosphorylation of S6K1/2 and 4E-BP as well as increased capacity for protein synthesis, each of these events coinciding with the onset of renal hypertrophy and mesangial matrix accumulation (97).

Renal enlargement and glomerular hypertrophy occur as a compensatory response to the loss of nephrons, which occurs in virtually all forms of chronic kidney disease (CKD) (36, 66, 73). In the case of DN, renal enlargement and glomerular hypertrophy also precede nephron loss and occur in association with an increased GFR (36, 66, 73). While initially adaptive, glomerular hypertrophy eventually contributes to podocyte injury. Such injury is now believed to play a primary role in the development of proteinuria and glomerulosclerosis, leading to a progressive loss of renal function (65, 66). A role for mTORC1 in the pathogenesis of DN was initially suggested by studies showing that rapamycin substantially attenuated renal hypertrophy in animal models of DN (127). A subsequent study in S6K1-deficient mice provided the first genetic evidence that mTORC1 plays a central role in the renal hypertrophy in DN and that these effects of mTORC1 are mediated by S6K1. This study also showed for the first time that DN is associated with activation of S6K1, but not S6K2 (24).

Although it has been known for many years that, in patients with diabetes, glomerular hypertrophy precedes the irreversible

structural changes of glomerulosclerosis, it is only recently that podocyte hypertrophy has been identified as a prominent and early feature of DN (63). Two recent studies, both of which determined the effects of podocyte-specific genetic deletion of critical components of the mTORC1 signaling pathway, provide exciting new insights into the role of mTOR in podocyte function and the development of DN (46, 73).

In the study by Godel et al. (46), mice with a podocyte-specific deletion of both alleles of the gene expressing Raptor developed proteinuria within a few weeks of birth, followed by progressive glomerulosclerosis. Interestingly, in mice lacking only one podocyte-specific Raptor allele, induction of diabetes with streptozotocin led to substantially less severe proteinuria and glomerular disease than in wild-type controls. The results of this study suggest that, while the total absence of Raptor abolishes podocyte integrity and function, genetic interventions that reduce the expression of Raptor (and therefore mTORC1 activity) can protect mice from DN (46).

In a complementary study, Inoki et al. (73) studied mice with a podocyte-specific conditional ablation of TSC1. Since TSC is a negative regulator of mTORC1 (see Fig. 2*A* in Ref. 90), genetic ablation of TSC1 increased mTORC1 activity by podocytes. Ablation of podocyte-specific TSC1 in nondiabetic mice led to the development of glomerular disease with many features of DN (73). Changes included podocyte loss, GBM thickening, mesangial expansion, and proteinuria. Increased podocyte-specific mTORC1 activity in nondiabetic mice led also to mislocalization of slit diaphragm proteins and induction of epithelial-mesenchymal transformation. Moreover, in spontaneously diabetic mice, genetic reduction of podocyte-specific mTORC1 activity by single-allele deletion of Raptor suppressed the development of DN (73). Thus, in two distinct models of diabetes, podocyte-specific reduction of mTORC1 activity protected mice from DN, while in nondiabetic mice upregulation of mTORC1 activity produced a glomerular disease closely resembling DN (40, 46, 73).

Together, these studies provide strong genetic evidence that activation of mTORC1 in podocytes is a critical early step in the development of DN and that reduction of mTORC1 activity represents a logical therapeutic strategy to slow or prevent DN (40, 46, 73) (Fig. 2). In support of this notion, inhibition of mTORC1 with rapamycin ameliorated renal hypertrophy and diminished hyperphosphorylation of S6K1/2 and 4E-BP1 in cortical homogenates from hyperglycemic *db/db* mice (133). These events correlated with rapamycin-mediated inhibition of mRNA translation of the matrix protein laminin-β1 (133).

mTOR and tubulointerstital fibrosis. DN is also characterized by tubulointerstitial inflammation and fibrosis. In addition to its beneficial effects on the glomerulus, rapamycin also reduces interstitial inflammation and fibrosis in many animal models of DN. Improvement is associated with the diminished release of proinflammatory cytokines, such as monocyte chemoattractant protein-1, as well as of profibrotic cytokines, such as transforming growth factor-β1 and connective tissue growth factor (15, 92, 160, 165) (Fig. 2). Importantly, these beneficial effects of rapamycin occur independently of changes in blood glucose or blood pressure (92, 102, 160, 165).

Potential problems in the use of rapamycin for human DN. While interventions to suppress mTORC1 activity hold promise for slowing the progression of DN in humans, the use of rapamycin-like agents for this purpose carries two potential risks. First is the recent recognition that mTORC1, acting via S6K1, plays an important role in promoting pancreatic β cell mass and function (41, 115). Administration of rapamycin to diabetic mice worsened hyperglycemia through impaired insulin production (41). The second potential risk relates to the perplexing observation that administration of rapamycin to humans can induce proteinuria, often in the nephrotic range. This phenomenon was initially described in renal transplant recipients with chronic allograft nephropathy, in whom calcineurin inhibitors were replaced with rapamycin (87, 126, 134). Although proteinuria was ascribed at first to calcineurin inhibitor withdrawal, severe proteinuria has subsequently been observed with the use of rapamycin in recipients of islet or bone marrow transplants (86). There have also been occasional reports of proteinuria and focal glomerulosclerosis in nontransplant patients receiving rapamycin without other immunosuppressive therapy (85).

Nondiabetic Forms of Chronic Glomerular Disease

Activation of mTOR within the kidney has also been observed in several animal models of nondiabetic progressive chronic glomerular disease. Moreover, these studies also showed a beneficial effect of rapamycin, or its analog evrolimus. As in DN, inhibition of mTOR resulted in amelioration of glomerular hypertrophy, decreased proteinuria, and a reduction in interstitial inflammation and fibrosis (12, 23, 33, 35, 57, 82, 93, 125, 159).

PKD

PKD is a heterogeneous group of inherited disorders characterized by the development and progressive enlargement of fluid-filled cysts in the parenchyma of both kidneys. Loss of renal function in PKD, in general, is the result of cystic growth leading to a gradual but inexorable loss of renal function. Damage occurs through distortion of the normal renal architecture, interference with intrarenal blood flow, compression of adjacent normal tubules, and ultimately replacement of normal renal tissue by fluid-filled cysts (56, 158). Clinically, the most common forms of PKD are autosomal dominant PKD (AD-PKD), autosomal recessive PKD (AR-PKD), and nephronophthisis (NPHP).

Clinical features of AD-PKD, AR-PKD, and NPHP. AD-PKD is one of the most common monogenic disorders, with an estimated prevalence of 1 in 400 to 1 in 1,000 individuals (56, 158). There are two known types of AD-PKD, caused by loss-of-function mutations in either the *PKD1* or *PKD2* genes, encoding polycystin 1 (PC1) (69, 131) or polycystin 2 (PC2), respectively (81, 100). Mutations of *PKD1* account for the majority (~85%) of cases of AD-PKD, with mutations in *PKD2* accounting for the rest (147). Patients with either form of AD-PKD typically present in adulthood. Major clinical manifestations at presentation are microscopic hematuria and hypertension, followed later by progressive loss of renal function. Cysts form not only in the kidneys but also in the liver and pancreas. In addition, these patients have a fivefold higher prevalence of intracranial aneurysms than the general population (56). Cardiac defects, predominantly valvular, may also occur (56). Patients with mutations of *PKD1* typically have a more aggressive course, with ESRD occurring ~20 yr earlier than in patients with mutations of *PKD2* (61). AD-PKD ac-

counts for ~5% of all patients who develop ESRD in the United States and Europe.

AR-PKD is an autosomal recessive disorder caused by mutations in the *PKHD1* gene, which encodes the protein fibrocystin (also known as polyductin) (108, 155). AR-PKD is far less common than AD-PKD, affecting 1 in 20,000 live births. Patients typically present as neonates or in early childhood, although milder forms of the disease can present for the first time in late childhood or adolescence (1, 52). Renal involvement is characterized by markedly enlarged kidneys, hypertension, and loss of renal function. Renal replacement therapy is required in up to a third of patients (1, 144).

NPHP is an autosomal recessive disorder characterized by the development of cysts in the renal medulla and at the corticomedullary junction. Interstitial fibrosis and loss of renal function typically lead to ESRD and the need for renal replacement therapy during adolescence (70, 161). The majority of cases of NPHP (50–85%) have been associated with mutations in the *NPHP1* gene, which encodes the protein nephrocystin. Mutations of the *NPHP2* gene, which encodes the protein inversin, are responsible for an infantile form of NPHP that progresses to ESRD before age 5 (144).

Genetic and molecular mechanisms of cyst formation. Although AD-PKD is inherited in an autosomal dominant manner, analysis of epithelial cells from renal or hepatic cysts indicates that cystogenesis requires mutation of both alleles, leading to homologous loss of function of either *PKD1* or *PKD2* (114, 119, 157). This is known as the "two-hit" model of cystogenesis. The somatic "second hit" is believed to involve a single cell, which proliferates clonally, generating an out-pouching that eventually becomes a cyst (114, 119, 157). In some cysts, the second hit does not lead to homologous inactivation of a single *PKD* locus, but rather leads to inactivation of a single allele at each *PKD* locus. In such cases, a patient is heterozygous at both *PKD* loci, having a normal and an abnormal copy of each *PKD* gene. This too can result in AD-PKD, suggesting that trans-heterozygosity of the two genes is sufficient for cyst formation (157). These findings have led to a "threshold" model of cystogenesis, in which combined expression of PC1 and PC2 must fall below a critical threshold for cystogenesis to occur (8).

The cells lining the renal and hepatic cysts in AD-PKD have a number of characteristic features. First, they are larger than normal tubular epithelial cells (49). As mTOR is the major determinant of mammalian cell size (176), this feature alone suggests an important role for mTOR overactivity in cystogenesis (49). Second, cystic epithelial cells are hyperproliferative, a process that contributes to the expansion of cysts over time (49, 117, 145). Third, cystic cells undergo apoptosis at an increased rate (117, 143, 162). The concurrence of increased rates of cell birth and death is a consistent observation in human and animal forms of AD-PKD. Fourth, the basement membrane on which cystic cells sit is abnormally thickened, a consequence of abnormal matrix deposition (18). Finally, cystic cells secrete fluid into the cyst lumen, a cAMP-driven process that is an important contributor to cyst enlargement (49, 96, 162, 164).

Role of excessive mTOR activity. The evidence linking excessive mTOR activity to cystogenesis in PKD is extensive (113). Data derive from four major sources. The first is genetic. In both humans and animals, loss of function of the TSC complex is associated with the development of renal cysts. Since the TSC complex is an important upstream inhibitor of mTOR activity (see Fig. 2, *A* and *B*, in Ref. 90), these data imply that excessive mTOR activity can promote cystogenesis. For example, rats with a germline inactivation of one allele of the *TSC2* locus developed severe early bilateral polycystic kidney disease (79). Mice with a tubular cell-specific deletion of *TSC1* also developed severe cystic disease (174). Moreover, the epithelial cells lining these cysts had elevated mTORC1 activity (174). In humans, loss-of function mutations of *TSC1* or *TSC2* produce a syndrome called tuberous sclerosis, which is characterized by multiple benign tumors, such as angiomyolipomas and cysts, in the kidney and other organs (17, 30, 129). Finally, a rare syndrome in humans, caused by a large deletion on chromosome 16 encompassing both the *TSC2* and the adjacent *PKD1* loci, is characterized by an unusually severe form of infantile AD-PKD, with ESRD occurring by the second decade of life (14, 130). A synergistic interaction between deletions of *TSC2* and *PKD1* on renal cyst formation is consistent with the hypothesis that the proteins encoded by these genes act within a common signaling pathway to promote cystogenesis.

The second source of data linking mTOR overactivity to PKD is biochemical. Increased phosphorylation of the downstream targets of mTORC1, in particular S6K1/2 and the 4E-BPs, has been observed consistently in human AD-PKD (19, 32, 139). Increased phosphorylation of S6K1/2 and 4E-BPs has also been seen in animal models, including the Han:SPRD rat (a nonorthologous model of PKD) (145, 151, 152, 169) and mice with mutations of *PKD1* (139, 140) or *PKD2* (170). Some animal models of PKD also show evidence of increased activity of mTORC2, as indicated by increased phosphorylation of Akt at Ser^{473} in cystic epithelial cells (4, 122, 140, 170). Fewer data are available regarding the role of mTOR in AR-PKD. In one study, cystic epithelial cells from children with AR-PKD showed strong staining for fibrocystin, phospho-Thr^{308} of Akt, and phospho-Ser^{2448} of mTOR in cystic cells compared with normal tubular cells from control kidneys (39).

The third source of data is pharmacological. In phase I/II trials in humans with tuberous sclerosis, rapamycin has been shown to reduce the size of renal tumors, such as angiomyolipomas (129). In addition, in multiple studies using a variety of animal models of PKD, rapamycin or its analogs has been shown to slow cyst progression. Beneficial effects have been seen in Han:SPRD rats (145, 152, 169, 173) and in mice with inactivating mutations of *PKD1* (139, 140) or *PKD2* (170). In PCY mice, which have a mutation of one of the genes responsible for NHPH, rapamycin did not affect initial cyst development, but was effective later on in reducing the rates of cyst enlargement, kidney fibrosis, and renal functional loss (45, 123). However, the effects of rapamycin have not been uniformly beneficial, as rapamycin was ineffective in altering cyst progression in PCK rats (124), a nongenetic model of AR-PKD.

The final piece of evidence favoring a pathogenetic role for mTOR in PKD comes from two small retrospective studies of patients with AD-PKD, who received rapamycin as immunosuppressive therapy following renal transplantation and were reported to have a reduction in the volumes of their native polycystic livers and kidneys (121, 139).

Ciliary dysfunction in PKD. The primary (or nonmotile) cilium is a sensory organelle protruding from the surface of virtually all vertebrate cells. It originates from a cytoplasmic structure known as the "basal body" (Fig. 3). Compared with motile cilia, each cell typically has only one primary cilium. In renal tubular cells, primary cilia are located on the apical surface and protrude into the lumen. Primary cilia act as sensors of a wide range of mechanical and chemical events, including intratubular flow rate, osmolality, and various hormonal stimuli (64). In turn, ciliary responses modulate critical cellular events, including growth, proliferation, polarity, and tubulogenesis (64). Importantly, ciliary dysfunction has emerged as a common factor underlying the pathogenesis of multiple forms of renal cystic disease (44).

The proteins mutated in PKD all localize to the primary cilium (Fig. 3), where they associate with other well-characterized ciliary proteins such as polaris and cystin (156, 166). This includes PC1 and PC2 in AD-PKD, fibrocystin in AR-PKD, and inversin in NPHP. PC1 and PC2 both function within the same mechanotransduction pathway. In response to physiological fluid flow, they mediate increased entry of calcium into the cell (106, 120). Loss of function of PC1 or PC2 may lead to PKD, in part, because of an impaired ability of cells to sense mechanical and chemical cues that serve to limit cell growth, cell proliferation, and tubulogenesis.

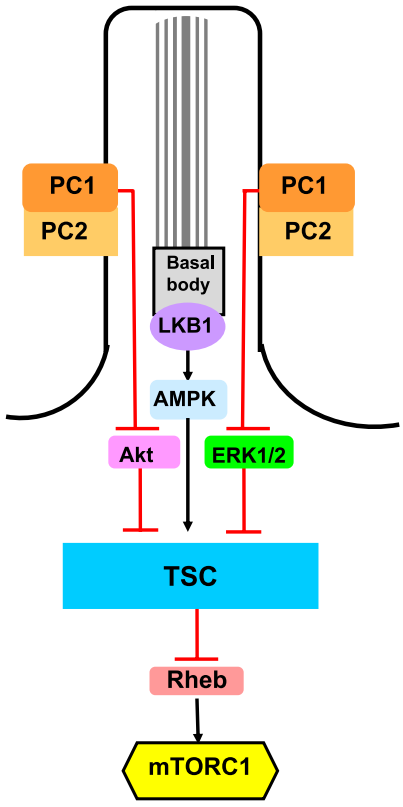

Fig. 3. Regulation of mTORC1 by polycystin 1 (PC1) and polycystin 2 (PC2). Considerable evidence links overactivity of mTORC1 within tubular epithelial cells to the abnormal cystogenesis of patients with autosomal dominant polycystic kidney disease (AD-PKD). There are 2 known types of AD-PKD, caused by loss-of-function mutations in either the *PKD1* or the *PKD2* gene, encoding PC1 or PC2, respectively. PC1 and PC2 form a complex within the basal bodies of primary cilia, sensory organelles protruding from the surface of virtually all vertebrate cells. In its native (nonmutated) form, PC1 inhibits the activity of mTORC1 via a number of pathways. Bending of primary cilia activates liver kinase B1 (LKB1), which like PC1/PC2 is localized to the basal bodies of primary cilia. LKB1 activates AMPK, which then activates TSC, a negative regulator of mTORC1. PC1 also inhibits mTORC1 via inhibition of ERK1/2 and Akt. In their active states, ERK1/2 and Akt stimulate mTORC1 by releasing mTORC1 from TSC-mediated inhibition. While LKB1 is activated by ciliary events, it is not yet clear whether inhibition of ERK1/2 and Akt by PC1 is also associated with ciliary events. Some evidence exists that PC1 may also activate mTORC2, although little is known about the role of mTORC2 in AD-PKD. In addition, only a few studies have addressed the role of PC2 in modulating the activity of mTORC1 and mTORC2.

Although the signaling pathways are not yet fully elucidated, primary cilia contribute to the regulation of mTOR activity. TSC1 (59) and TSC2 (79) both localize to the basal body of primary cilia. In addition, Boehlke et al. (7) have shown that flow-induced bending of primary cilia inhibited mTORC1 activity and reduced cell size (7). These events were mediated by LKB1, a kinase also localized to the basal body of primary cilia. As previously discussed, LKB1 inhibits mTORC1 through activation of AMPK (77) (Fig. 3).

Polycystins, cystogenesis, and mTOR. PC1 is a large (~500 kDa) plasma membrane receptor with a short C-terminal intracellular tail, multiple transmembrane domains, and an extensive N-terminal extracellular domain (69, 107). PC2 is an integral membrane protein that acts as a calcium channel in the plasma membrane, the endoplasmic reticulum, or both (48, 55, 81, 100). PC1 and PC2 interact via their cytoplasmic domains to form a dimeric complex (9, 55, 118, 163, 168). Activity of this complex is thought to be essential for preventing renal cystogenesis. The fact that mutation of either protein alone leads to loss of channel activity (9, 55, 118, 168) most likely accounts for the similar phenotypes of patients with AD-PKD1 and AD-PKD2 (9). Substantial evidence exists for an important role of PC1 in inhibiting cell growth, proliferation, and apoptosis (10, 34). Through its role in cell-cell and cell-matrix interactions, PC1 is thought to promote tubulogenesis and inhibit cystogenesis (10, 69, 71).

Our understanding of the mechanisms by which mutations in PC1 and/or PC2 lead to aberrant mTOR signaling remains fragmentary (Fig. 3). Available evidence suggests that nonmutated PC1 inhibits mTOR via TSC2, with which PC1 interacts directly (139). This interaction increases the activity of TSC2, resulting in inhibition of mTOR (32). Two distinct mechanisms, both entailing abrogation of an inhibitory influence, mediate the stimulatory effect of PC1 on TSC2. First, PC1 prevents ERK1/2-mediated phosphorylation and inhibition of TSC2 (34, 95) (Fig. 3). PC1 also protects TSC2 from inhibition by Akt (32) (Fig. 3). Normally, phosphorylation of TSC2 by Akt leads to translocation of TSC2 from the cell membrane and cytosolic sequestration by 14-3-3 proteins (16). Interaction with PC1 maintains TSC2 at the cell membrane, where it can continue to inhibit the mTOR pathway (32).

PC1 may also activate mTORC2. Stable expression of PC1 protected cultured renal tubular cells from apoptosis through increased phoshorylation of Akt at both Thr^{308} and Ser^{473} (6). Activation of mTORC2 by PC1 seems likely, since mTORC2 is the major kinase known to phosphorylate Akt at Ser^{473} (see Fig. 2*A* in Ref. 90), although other kinases such as DNA-dependent kinase may also phosphorylate Akt at this site (110). Modulation by PC1 of tubular cell migration and cytoskeletal organization also depended in part on phosphorylation of Akt at Ser^{473} (5).

Clinical trials of rapamycin and its analogs in AD-PKD. Currently, therapy for AD-PKD is largely supportive. No single specific form of therapy has yet been shown to prevent or slow the decline in renal function. Based on the substantial evidence linking aberrant mTOR activity to cystogenesis (9, 140, 156, 168), several trials have been performed of the efficacy of rapamycin (Sirolimus), or its analog everolimus (Afinitor), on the course of AD-PKD. Interpretation of these studies is complicated by two features of the disease. First, cyst enlargement and loss of renal function are very slow processes, typically occurring over many decades. Second, there is tremendous variability in the rate of disease progression. While some patients reach ESRD by the third or fourth decade of life, others never reach ESRD (29, 56, 158). Human studies of PKD are complicated by two further issues. One is that by the time a cyst is radiologically evident, it is probably years to decades old, the cells having undergone multiple doublings. The other is uncertainty as to whether AD-PKD has a "point of no return" beyond which progression is unresponsive to any form of therapy. The National Institutes of Health-sponsored "Consortium for Radiologic Imaging Studies of PKD" (CRISP) laid the groundwork for designing meaningful clinical trials in AD-PKD (22, 50). CRISP showed that cyst size and total kidney volume not only are continuously increasing quantifiable processes but also are reliable end points for human studies (22, 50).

To date, three major studies of the efficacy of rapamycin in AD-PKD have been published. The "Sirolimus Treatment in Patients with Autosomal Dominant Polycystic Disease: Efficacy and Safety (SIRENA)" study was a small randomized crossover study ($n = 15$) that compared conventional treatment to rapamycin plus conventional treatment over a 6-mo period (116). There was no difference between the treatment groups in the absolute increase in total kidney volume. However, total cyst volume was stable on rapamycin, whereas it increased on conventional therapy. Correspondingly, noncystic parenchymal volume increased on rapamycin, whereas it was stable on conventional therapy. The authors concluded that rapamycin inhibits cyst growth and increases noncystic parenchymal volume in patients with AD-PKD (116).

In contrast, two other studies failed to show a beneficial effect of rapamycin in AD-PKD. The first of these studies was a 2-yr multicenter, double-blind, placebo-controlled trial in which 433 patients with AD-PKD and reduced renal function (estimated GFR between 30 and 89 ml/min/1.73 m^2) were randomized to receive either everolimus (Certican), an analog of rapamycin, or a placebo (153). During the first year of the trial, total kidney volume increased at a slower rate in the everolimus-treated than placebo-treated group. However, by the end of the second year, the difference between the groups was no longer statistically significant. As opposed to the SIRENA trial, noncystic parenchymal volume increased to a greater extent with a placebo than everolimus (153). The third study, an open-label, single-center study of 100 patients, randomized to receive either rapamycin or conventional care for 18 mo, also showed no effect of rapamycin on total kidney volume (136).

These studies are limited by their small numbers and short duration. In the largest of the studies (153), the inclusion of many patients with advanced CKD (stages III and IV) may have precluded finding a benefit of mTOR inhibition on renal function. Overall, the results of these studies are inconclusive.

The Future of mTOR Inhibition in the Treatment of PKD and Other Diseases

Rapamycin and its analogs. Rapamycin has several characteristics that may limit its efficacy as a treatment for diseases driven by mTOR overactivity. It has become clear that rapamycin does not block all the downstream effects of mTORC1 with equal effectiveness. For example, rapamycin inhibits S6K1/2 more effectively than the 4E-BPs (27). While inhibition of S6K1/2 persists for the duration of rapamycin treatment, phosphorylation of 4E-BP1 is restored within a few hours and eventually becomes rapamycin resistant (27). In addition, rapamycin is far more effective in inhibiting mTORC1 than mTORC2. Although high doses of rapamycin given for prolonged periods can block mTORC2 in some cell lines, inhibition of mTORC2 remains less than that of mTORC1.

An additional concern relates to the effect of rapamycin on feedback loops that attenuate mTORC1 activation in response to continuous stimulation by growth factors. For example, as discussed above, sustained activation of S6K1/2 leads to a diminution of the cellular effects of insulin and IGF-1 through suppression of the activity of IRS-1 (60, 137, 148). mTORC1-mediated feedback also suppresses ERK1/2- (21) and platelet-derived growth factor (PDGF)-dependent pathways (21, 171). In some cancer cells, through inhibition of mTORC1 and abrogation of these feedback loops, rapamycin has led to a marked upregulation of PI3K-dependent events, including pro-survival and proliferative signals though Akt and other members of AGC family of kinases (109, 176). The extent to which rapamycin has this effect in noncancer cells has not been examined.

Catalytic Inhibitors of mTOR and Dual PI3K-mTOR Inhibitors

These and other concerns about the use of rapamycin and its analogs have led to the development of a new class of mTOR inhibitors known as ATP-competitive or "catalytic" inhibitors (28, 37, 43, 146, 167). Unlike rapamycin, which interferes with the assembly of mTORC1, these agents directly inhibit the kinase activity of mTOR. Not only do these new agents durably and effectively prevent phosphorylation of S6K1/2 and 4E-BP1, the two major downstream effectors of mTORC1, but they also inhibit mTORC2-mediated events. Preclinical data in mice suggest that these agents are well tolerated (54, 105). Their efficacy and toxicity in humans remain to be defined.

Similarities between the catalytic domains of mTOR and class I PI3Ks have led to the design of so-called "dual" inhibitors, which block the catalytic activity of both mTOR and PI3K (53). Dual inhibition of PI3K and mTOR has a potential advantage over catalytic mTOR inhibitors, since dual inhibition would prevent activation of Akt by both PI3K and mTORC2. To date, use of these dual inhibitors has been limited by toxicity and a narrow therapeutic range (76).

Summary and Future Directions

The mTOR kinase has emerged as a critical regulator of many fundamental cellular processes, including growth, proliferation, autophagy, and aging. mTOR lies at the center of a vast signaling network, within which it serves two integrated

roles. The first is to "sense" the availability of nutrients and energy within individual cells. The second is to alter the activity of downstream metabolic pathways, so their activity is appropriate to the prevailing environment. Nutrient deficiency inhibits the activity of mTORC1, leading to conservation of energy stores, inhibition of biosynthetic pathways, and stimulation of autophagy. Nutrient availability has the opposite effect, stimulating mTORC1 and driving anabolism and energy consumption.

mTOR kinase has also emerged as an important player in the pathogenesis of multiple diseases. These include the metabolic syndrome, type 2 diabetes mellitus, and several types of autoimmune diseases, neurological disorders, and cancer. While the focus of our review has been on the role of mTOR in three specific renal diseases, AKI, DN, and PKD, rapamycin and its analogs are likely to prove useful in the treatment of other chronic progressive kidney diseases, such as human immunodeficiency virus nephropathy (83).

The promise of rapamycin and its analogs as a treatment for DN has been dampened by the recognition that this approach can have serious adverse effects in humans. For example, inhibition of mTORC1 reduces pancreatic β cell mass and insulin production, effects that are clearly undesirable in diabetic patients. In addition, while in animals rapamycin ameliorates proteinuria and slows the progression of chronic glomerular disease, both diabetic and nondiabetic, it is clear that in humans, for unclear reasons, rapamycin may cause or worsen proteinuria and glomerular injury. If mTOR inhibitors are to be used in the treatment of DN and other renal diseases, these mysteries need to be unraveled, not only to plan rationally when in the course of disease to initiate treatment but also to choose the right type of mTOR inhibitor, offering the greatest efficacy with the fewest side effects.

Overall, despite these misgivings and mysteries, the future looks bright, both in terms of our understanding of the complex network of signaling events at which mTOR lies in the center and in our ability to treat patients with diseases in which this network is perturbed.

NOTE ADDED IN PROOF

Until recently, as discussed in this review, it has been believed that rapamycin predominantly inhibits mTORC1. However, a recent study by Lamming and associates (Lamming DW, Ye L, Katajisto P, Goncalves MD, Saitoh M. Stevens DM, Davis JG, Salmon AB, Richardson A, Ahima RS, Guertin DA, Sabatini DM, Bauer JA. Rapamycin-induced insulin resistance is mediated by mTORC2 loss and uncoupled from longevity. *Science* 335: 1638–1643, 2012) shows conclusively that rapamycin inhibits both complexes. Rapamycin inhibits mTORC2 by disrupting the mTORC2 complex, rather than through interference with its assembly (as suggested by earlier work). Lamming et al. also demonstrate that the downstream effects of rapamycin on mTORC1 and mTORC2 can be dissociated. Whereas rapamycin-mediated inhibition of mTORC1 activity promotes longevity, its disruption of mTORC2 induces insulin resistance and impaired glucose homeostasis.

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