

# The Role of the Mammalian Target Of Rapamycin (mTOR) in Renal Disease

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## ABSTRACT

The mammalian target of rapamycin (mTOR) is a serine/threonine kinase that plays a pivotal role in mediating cell size and mass, proliferation, and survival. mTOR has also emerged as an important modulator of several forms of renal disease. mTOR is activated after acute kidney injury and contributes to renal regeneration and repair. Inhibition of mTOR with rapamycin delays recovery of renal function after acute kidney injury. Activation of mTOR within the kidney also occurs in animal models of diabetic nephropathy and other causes of progressive kidney disease. Rapamycin ameliorates several key mechanisms believed to mediate changes associated with the progressive loss of GFR in chronic kidney disease. These include glomerular hypertrophy, intrarenal inflammation, and interstitial fibrosis. mTOR also plays an important role in mediating cyst formation and enlargement in autosomal dominant polycystic kidney disease. Inhibition of mTOR by rapamycin or one of its analogues represents a potentially novel treatment for autosomal dominant polycystic kidney disease. Finally, inhibitors of mTOR improve survival in patients with metastatic renal cell carcinoma.

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Rapamycin (also known as sirolimus) was isolated from a soil bacterium in 1975.<sup>1,2</sup> The discovery of rapamycin led to the identification and cloning of mammalian target of rapamycin (mTOR), a serine/threonine kinase, in 1994.<sup>3,4</sup> Rapamycin is a potent, specific inhibitor of mTOR and does not inhibit any kinase other than mTOR.<sup>1,2</sup> Because of its high specificity for mTOR, rapamycin has been very useful in establishing the role of mTOR in cell biology and in the pathogenesis of disease.<sup>3,4</sup> Although initially isolated as an antifungal agent, rapamycin was later found to have potent immunosuppressive effects and has been used for many years as a component of antirejection therapy for recipients of organ transplants.<sup>1,2,5</sup>

After diffusing into cells, rapamycin forms a complex with FK506-binding

protein 12 (FKBP-12), an intracellular protein. The rapamycin–FKBP-12 complex then binds and inhibits mTOR.<sup>3,4</sup> mTOR is a component of two distinct signaling complexes known as mTOR complex 1 (mTORC1) and mTORC2. These complexes contain two different scaffolding proteins, raptor and rictor, respectively (Figure 1).<sup>6–8</sup> These scaffolding proteins, by interacting with distinct downstream targets, connect mTOR to different signaling pathways. As a result, mTORC1 and mTORC2 have discrete functional roles (Figure 1).<sup>6,7,9</sup> Activation of mTORC1 by growth factors and amino acids stimulates cell growth (*i.e.*, an increase in cell size and mass) and cell proliferation. Activation of mTORC2 contributes to the regulation of cell polarity and the actin cy-

toskeleton (Figure 1).<sup>6,7,9</sup> The upstream regulators of mTORC2 are as yet unknown (Figure 1).<sup>6,7,9</sup> Rapamycin inhibits mTORC1 by preventing the interaction of mTOR with raptor. Importantly, rapamycin has no effect on the activity of mTORC2.<sup>3,6,8,10,11</sup>

## UPSTREAM REGULATION OF mTORC1

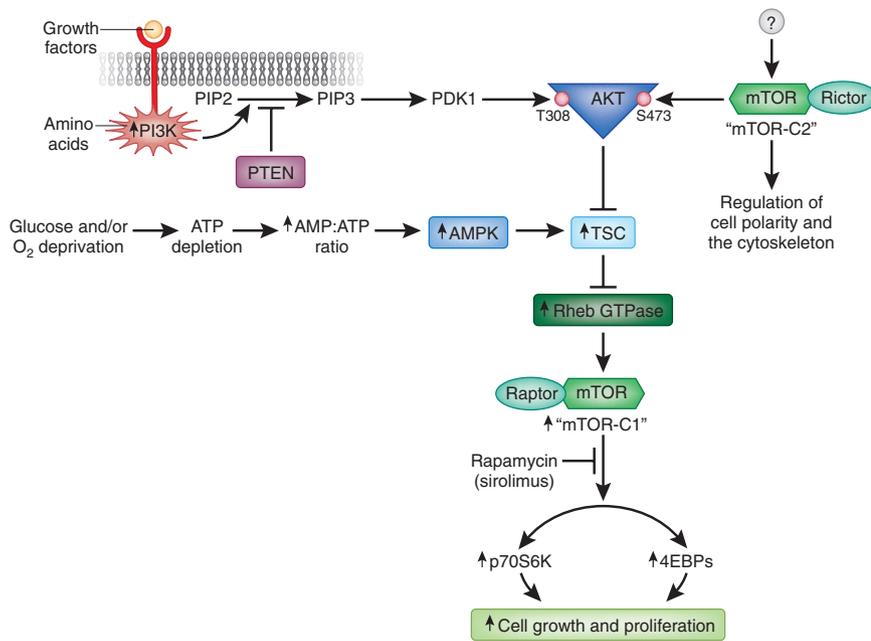
mTORC1 serves as a sensor and integrator of the availability of multiple stimuli and factors necessary for cell growth and proliferation. These include growth factors such as IGF-1<sup>12,13</sup> and EGF<sup>14,15</sup> as well as nutrients such as amino acids,<sup>13,16,17</sup> glucose, and oxygen.<sup>18–20</sup>

Activation of mTORC1 begins with activation of the lipid kinase phosphatidylinositol 3-kinase (PI3K; Figure 1).<sup>21</sup> PI3K phosphorylates the membrane-associated phospholipid phosphatidylinositol-4,5-bisphosphate [PtdIns(4,5)P<sub>2</sub>] (PIP<sub>2</sub>) to yield PtdIns(3,4,5)P<sub>3</sub> (PIP<sub>3</sub>).<sup>21,22</sup> The activity of PI3K is opposed by a phosphatase, phosphatase and tensin homolog on chromosome 10 (PTEN), which dephosphorylates PIP<sub>3</sub> back to PIP<sub>2</sub>.<sup>21,22</sup>

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**Figure 1.** mTOR is a component of two major intracellular signaling complexes: mTORC1 and mTORC2. These complexes contain two different scaffolding proteins (raptor and rictor, respectively) that “connect” them to different downstream targets. As a result, these complexes have different functional roles. mTORC1 stimulates cell growth and proliferation, whereas mTORC2 regulates cell polarity and the cytoskeleton. mTORC1 is activated by growth factors and amino acids, which activate PI3K, a lipid kinase. PI3K then phosphorylates PIP<sub>2</sub> to yield PIP<sub>3</sub>. PIP<sub>3</sub> phosphorylates and activates Akt through an intermediary kinase, 3-phosphoinositide-dependent kinase-1 (PDK1). The activity of PI3K is negatively regulated by PTEN, a phosphatase that dephosphorylates PIP<sub>3</sub> back to PIP<sub>2</sub>. Once activated, Akt phosphorylates and inhibits the TSC. TSC negatively regulates mTORC1 by inhibiting Rheb, a small cytoplasmic GTPase and activator of mTORC1. AMPK, which is activated by any form of cell stress that decreases cell ATP stores and increases the AMP:ATP ratio, inhibits mTOR by phosphorylating and activating TSC2. Akt is also phosphorylated by mTORC2, although phosphorylation of Akt by mTORC2 is not necessary for the activation mTORC1. Once activated, mTORC1 phosphorylates p70S6K and 4EBP, leading to the translation of mRNAs and the synthesis of proteins necessary for cell growth and cell-cycle progression. The upstream modulators of mTORC2 are as yet unknown.

PIP<sub>3</sub> activates a 3-phosphoinositide-dependent kinase-1, which in turn activates Akt (also known as protein kinase B) by phosphorylating amino acid residue Thr<sup>308</sup>.<sup>3,21</sup> Akt then activates mTORC1 through a cascade of downstream intermediates.<sup>3,13,23,24</sup> These include the tuberous sclerosis complex (TSC), a dimer composed of TSC1 (hamartin) and TSC2 (tuberin), and Rheb, a Ras family GTPase that directly activates mTOR.<sup>3,13,25</sup> TSC2 has GTPase-activating protein activity, which inhibits Rheb activity, whereas TSC1 binds to and is necessary for TSC2 function.<sup>3,25–28</sup> Akt activates mTOR by phosphorylating and inhibiting TSC2, thereby releasing

Rheb from the inhibitory effects of TSC.<sup>3,4,29,30</sup>

Akt, in addition to being phosphorylated by 3-phosphoinositide-dependent kinase-1 at Thr<sup>308</sup>, can be phosphorylated by mTORC2 at Ser<sup>473</sup>.<sup>31–33</sup> However, the phosphorylation of AKT by mTORC2 is not necessary for the activation mTORC1 but instead is necessary for Akt to phosphorylate a distinct subset of downstream targets that promote cell survival.<sup>32–34</sup>

Growth factors and amino acids activate Akt and mTOR through PI3K (Figure 1). Growth factors use the class IA PI3K,<sup>21</sup> whereas amino acids stimulate the class III PI3K.<sup>17</sup> A deficiency of glu-

cose and/or oxygen, by reducing cell energy stores, inhibits mTOR through a PI3K-independent pathway. A fall in cell ATP stores is a potent stimulus for the activation of 5'-adenosine monophosphate-activated protein kinase (AMPK), a serine/threonine kinase<sup>18–20</sup> that inhibits mTOR by phosphorylating and activating TSC.<sup>3,24,35</sup>

### Downstream Effectors of mTORC1

Cell division results in the formation of two daughter cells that are approximately half the size of a normal cell. Before reentering the cell cycle, these cells must double in size (mass). mTORC1 promotes efficient and sustained cellular proliferation by tightly coupling increases in cell size with progression through G<sub>0</sub>/1 of the cell cycle.<sup>3,4,36–39</sup> These effects of mTORC1 are mediated by stimulating the translation of mRNA and the synthesis of proteins necessary for mediating increases in cell size and for promoting progression of the early stages (G<sub>0</sub>/1) of the cell cycle (Figure 1).<sup>3,4,36–39</sup> The translation of mRNA involves an initiation phase and an elongation phase. The initiation phase mediates the association of ribosomal subunits with mRNA, whereas amino acids are sequentially added to the nascent peptide chain during the elongation phase in accordance with mRNA-determined codon sequences.<sup>39,40</sup>

The best characterized downstream targets of mTORC1 are the 4E-binding proteins (4EBP) and the 70-kD ribosomal S6 kinases (p70S6K).<sup>3,39</sup> The 4EBP are a family of translation repressor proteins. When unphosphorylated, these proteins bind to and inhibit the activity of the eukaryotic translation initiation factor 4E (eIF4E).<sup>38,39</sup> Activation of mTOR leads to the phosphorylation of 4EBP.<sup>38,39</sup> Once phosphorylated, 4EBP can no longer bind to and inhibit eIF4E, which then becomes free to initiate mRNA translation.<sup>3,4,38,39</sup> mTOR also phosphorylates and activates p70S6K, a kinase that enhances the translation and synthesis of proteins essential to ribosomal function and the elongation phase of translation.<sup>41</sup> Thus, phosphorylation of the 4EBP and p70S6K by mTOR acts

in concert to promote the initiation and elongation phases of mRNA translation.<sup>3,39</sup> More detailed information regarding the regulation of mRNA translation by mTOR can be found in other review articles.<sup>38,41,42</sup> In this review, we focus exclusively on the role of mTORC1 in renal disease and use the generic term “mTOR” to refer to the effects of mTORC1.

## ROLE OF mTOR IN ACUTE KIDNEY INJURY

Complete restoration of renal morphology and function can occur after acute kidney injury (AKI) induced by ischemic or toxic injury.<sup>43,44</sup> Renal regeneration after acute tubular injury depends, in part, on the ability of the remaining, viable tubular cells to proliferate and restore the injured tubular epithelium.<sup>43–46</sup> The widely recognized immunosuppressive properties of rapamycin are due largely to inhibition of mTOR-mediated proliferation and clonal expansion of T cells.<sup>5,47</sup> However, mTOR is a ubiquitous kinase, and its inhibition by rapamycin also blocks the proliferation of virtually all cells types, including cells within the kidney.<sup>2,48</sup>

Our group has demonstrated that mTOR plays an important role in mediating the process of regeneration and recovery after experimental AKI.<sup>48,49</sup> mTOR activity is low or absent in the normal kidney but increases markedly after ischemia-reperfusion injury.<sup>48</sup> In addition, inhibition of mTOR by rapamycin delays renal recovery and repair.<sup>48</sup> This effect of rapamycin is due to the dual effects of inhibition of proliferation and induction of apoptosis of tubular cells.<sup>48,49</sup> Notably, although rapamycin delays recovery of glomerular filtration rate after AKI by approximately 2 to 3 d, full recovery of renal function still ultimately occurs, despite continued treatment with rapamycin.<sup>49</sup> Studies with cultured renal tubular cells suggest that the transient nature of the effect of rapamycin on the process of recovery after AKI is due, at least in part, to an acquired resistance of renal tubular cells to the effects

of rapamycin on cell growth and proliferation.<sup>49</sup>

Because mTOR is activated by growth factors and amino acids and is inhibited by ATP depletion (Figure 1), mTOR activity is probably highly suppressed during the ischemic period of ischemia/reperfusion injury, when the availability of growth factors, amino acids, and cell ATP all are likely to be reduced. The mechanisms responsible for the marked activation of mTOR during the reperfusion period after ischemia/reperfusion injury remain to be elucidated.<sup>48,49</sup> However, we speculate this represents a rebound phenomenon occurring in response to the sudden availability of growth factors, amino acids, and cellular ATP after a period of profound deficiency.

The clinical relevance of our findings in rats became evident when later studies of human renal transplant recipients showed that rapamycin causes and/or exacerbates delayed graft function.<sup>50–55</sup> Delayed graft function, which is due to ischemic tubular injury during the peritransplantation period, is an important clinical problem, occurring in approximately 30% of cadaveric and 10% of living-related renal transplants. Since recognition of the adverse effects of rapamycin on recovery from delayed graft function, it has become routine to delay administration of rapamycin until the transplanted kidney is functional and temporarily to discontinue administration of rapamycin in patients who have renal transplants and develop AKI.<sup>50–55</sup>

## ROLE OF mTOR IN CHRONIC KIDNEY DISEASE

Substantial and persuasive evidence exists that the mTOR pathway plays an important role in the mechanisms underlying the progression of chronic kidney disease (CKD) caused by diabetes and other causes. This evidence has been obtained from a number of studies using animal models, in which inhibition of mTOR by rapamycin markedly ameliorates the interstitial inflammation,

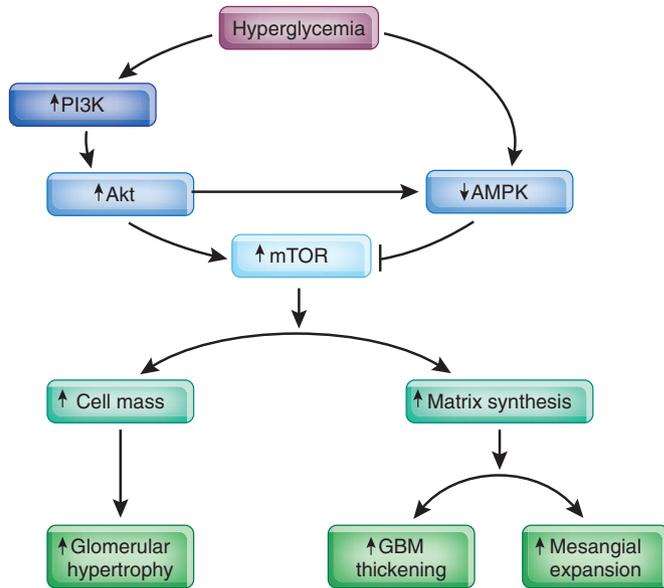
fibrosis, and loss of renal function associated with CKD.

## Diabetic Nephropathy

Diabetic nephropathy (DN) is the leading cause of ESRD in the United States and Western Europe.<sup>56,57</sup> Its characteristic morphologic changes include glomerular hypertrophy, basement membrane thickening, and the accumulation of mesangial matrix.<sup>56–58</sup> In addition, DN is associated with progressive tubulointerstitial injury, inflammation, and fibrosis.<sup>56–58</sup>

Renal enlargement, one of the first structural changes in DN, is due to the hypertrophy of existing glomerular and tubular cells rather than to cellular proliferation.<sup>56,57,59,60</sup> A number of studies have shown that activation of mTOR plays a pivotal role in physiologic and pathologic forms of hypertrophy in the kidney and other organs, including the renal hypertrophy characteristic of DN.<sup>11,61</sup> The pathogenic importance of glomerular hypertrophy is that it may contribute to podocyte injury and the progressive loss of renal function in DN and in other forms of CKD.<sup>59,60</sup> In addition to its role in glomerular hypertrophy, mTOR-dependent changes increase the synthesis of matrix proteins that contributes to basement membrane thickening and the accumulation of mesangial matrix characteristic of DN.<sup>40,62,63</sup> Activation of mTOR in diabetes is due, at least in part, to the effects of hyperglycemia. Hyperglycemia increases mTOR activity by the combined effects of Akt activation and AMPK inhibition (Figure 2).<sup>40,62,64–69</sup>

The importance of mTOR in mediating the renal changes associated with DN has been demonstrated *in vivo* by several studies evaluating the effect of rapamycin on the course of DN in rats with streptozotocin-induced diabetes.<sup>70–72</sup> This model of DN is associated with early activation of mTOR within the kidney.<sup>70–72</sup> Rapamycin not only reduces mTOR activity in this model, but also ameliorates the glomerular changes characteristic of DN, including hypertrophy, basement membrane thickening, and mesangial matrix accumula-



**Figure 2.** Hyperglycemia stimulates mTOR. mTOR is activated within the kidney in DN. Hyperglycemia activates PI3K and Akt and inhibits AMPK. The activation of Akt and inhibition of AMPK lead to activation of mTORC1. Activation of mTORC1 contributes to the renal changes characteristic of DN, including glomerular hypertrophy, glomerular basement membrane (GBM) thickening, and the accumulation of mesangial matrix.

tion.<sup>70–72</sup> Importantly, these effects of rapamycin were also associated with a reduction in albuminuria.<sup>70–72</sup>

Rapamycin markedly inhibited the influx of inflammatory cells, predominantly lymphocytes and macrophages, associated with DN.<sup>70–72</sup> This effect is likely attributable to rapamycin-induced inhibition of the proliferation and clonal expansion of B and T lymphocytes.<sup>3,4</sup> Rapamycin also ameliorates the release of proinflammatory cytokines and chemokines within the kidney, such as monocyte chemoattractant protein-1, RANTES, IL-8, and fractaline, which exacerbate the inflammatory process in DN (Figure 3).<sup>70–72</sup>

Interstitial fibrosis is another important feature of DN. While the precise pathways and mediators responsible for the pro-fibrotic effects of mTOR in DN and other forms of CKD have not been fully elucidated, several potential mechanisms can be envisioned (Figure 3). mTOR stimulates the proliferation of fibroblasts and their synthesis of collagen.<sup>73–75</sup> In addition, mTOR increases the expression of profibrotic cytokines, such as TGF- $\beta$ 1 and connective tissue growth factor, which play important

roles in mediating the tubulointerstitial changes associated with DN and other forms of progressive CKD.<sup>70–72</sup> Finally, mTOR may play a role in epithelial-to-mesenchymal transition (EMT). EMT is a process in which tubular epithelial cells undergo a phenotypic change into fibroblasts. These fibroblasts then migrate through the tubular basement membrane and into the interstitium.<sup>76–80</sup> A role for mTOR in EMT is suggested by studies showing that rapamycin inhibits EMT in some animal models of studies of DN and other forms of CKD.<sup>72,81,82</sup>

### Role of mTOR in Nondiabetic Forms of CKD

Rapamycin also slows the progression of renal fibrosis and delays the onset of renal failure in experimental models of CKD due to causes other than diabetes. Many of the same events that promote progression of diabetic CKD are also important in nondiabetic forms of CKD. These include glomerular hypertrophy, an increased expression of proinflammatory and profibrotic cytokines, infiltration of the interstitium by inflammatory cells, and renal fibrosis.<sup>76,80,83</sup>

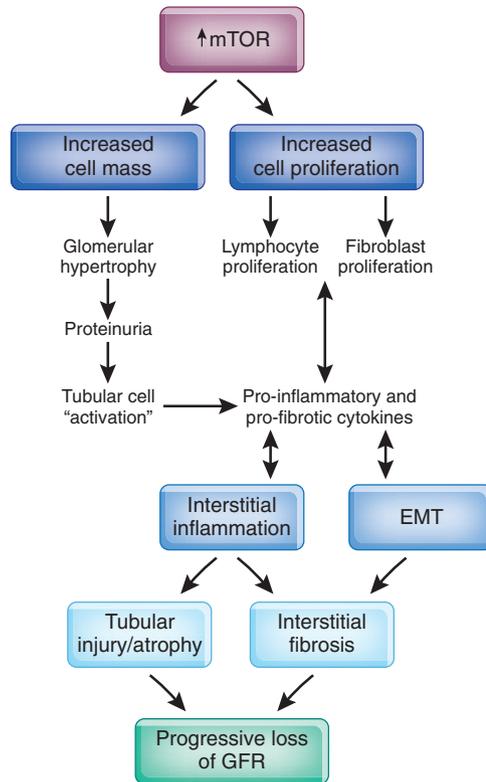
In a rat model of progressive membranous nephropathy (Heymann nephritis), inhibition of mTOR with rapamycin ameliorated glomerular hypertrophy, decreased the renal expression of proinflammatory and profibrotic cytokines, and retarded the development of tubulointerstitial inflammation and fibrosis.<sup>84</sup> Similar findings were reported in animal models of CKD from other causes, including reduced renal mass from five-sixths nephrectomy,<sup>82</sup> renal obstruction after ureteral ligation,<sup>81,85</sup> and chronic mesangioproliferative glomerulonephritis induced by anti-Thy1 antibody.<sup>73,86</sup>

In summary, mTOR plays an important role in the progression of CKD in a variety of animal models. Available evidence suggests a hypothetical schema to explain the beneficial effects of rapamycin on the progression of CKD (Figure 3). This model highlights five major effects of rapamycin: Reduction of glomerular hypertrophy, decreased production of proinflammatory and profibrotic cytokines, amelioration of interstitial inflammation, reduced fibroblast proliferation, and inhibition of EMT. Despite the appeal of this relatively simple model, however, additional research is needed to elucidate more fully the connection between mTOR and progressive CKD.

### mTOR AND AUTOSOMAL DOMINANT POLYCYSTIC KIDNEY DISEASE

Autosomal dominant polycystic kidney disease (ADPKD) is one of the most common human monogenic diseases, with an incidence of 1:400 to 1:1000.<sup>58</sup> It is characterized by the development and gradual enlargement of multiple fluid-filled cysts within both kidneys. These cysts encroach on and destroy normal adjacent nephrons.<sup>58</sup> ADPKD typically presents clinically in the second or third decade of life with hypertension, hematuria, and/or proteinuria, followed by progressive renal failure. Although the rate of progression to ESRD is highly variable, most patients with ADPKD reach ESRD by the fifth decade of life.

The majority of cases of ADPKD are



**Figure 3.** Activation of mTORC1 in CKD plays a major role in mediating the glomerular hypertrophy associated with the loss of functioning nephrons in CKD. The increased intracapillary pressures and flows associated with adaptive glomerular hypertrophy ultimately lead to podocyte injury and proteinuria. Increased mTORC1 activity in CKD also promotes interstitial inflammation by promoting the proliferation of lymphocytes and interstitial fibroblasts. Fibroblasts produce the connective tissue necessary for interstitial fibrosis. mTORC1 activation also leads indirectly to the release of proinflammatory and profibrotic cytokines, which are derived from multiple sources, including tubular cells (activated by proteinuria), inflammatory cells, and fibroblasts. These cytokines result in a positive-feedback loop that increases inflammation and fibrosis. Some cytokines are also necessary for EMT, which further promotes interstitial fibrosis. Injury to tubules caused by inflammatory cells promotes tubular injury, atrophy, and “dropout.” In addition, the deposition of fibrous tissue within the renal interstitium reduces local blood flow, causing areas of hypoxia that lead to further loss of functioning nephrons.

due to mutations in either the *PKD1* or the *PKD2* genes,<sup>87–89</sup> which cause abnormalities of polycystin 1 (PC1) and PC2 proteins, respectively.<sup>87–89</sup> Mutations in *PKD1* account for  $\geq 85\%$  of cases of ADPKD.<sup>87–89</sup> The pathogenesis of cyst development in ADPKD is a process of growing complexity. A number of abnormalities have been described in the phenotype of tubular cells lining the cysts in ADPKD. These include increased proliferation, increased apoptosis, abnormalities of protein sorting and polarity, and disorganization of the underlying extracellular matrix.<sup>90–92</sup>

Current management of ADPKD is

limited to the treatment of the complications of hypertension and renal failure<sup>93</sup>; however, ongoing elucidation of the mechanisms underlying cyst formation has led to the development of several promising new therapies. These include nonpeptide vasopressin 2 receptor antagonists,<sup>94–97</sup> inhibitors of the receptors for EGF and vascular endothelial growth factor (VEGF),<sup>93,98</sup> and inhibitors of *c-myc* expression.<sup>99–101</sup> All of these modalities inhibit disease progression in experimental models of PKD.<sup>92–99</sup>

A role for mTOR in the pathogenesis of ADPKD was first suggested in studies by Edelman and colleagues,<sup>102</sup> who dem-

onstrated that rapamycin slowed cyst formation in the Han:SPRD rat model of PKD. These findings have been confirmed by other investigators in the same model.<sup>103</sup> Subsequently, Shillingford *et al.*<sup>104</sup> provided substantial evidence that mTOR contributes to the pathogenesis of human ADPKD. These investigators demonstrated the cytoplasmic tail of PC1 interacts with and inhibits mTOR. They also found that loss of function of PC1 in ADPKD leads to the marked activation of mTOR within the epithelial cells of renal cysts in both mouse and human cystic disease.<sup>104</sup> In addition, they found that inhibition of mTOR with rapamycin not only slowed cyst enlargement in murine models of PKD but also slowed the increase in size of native kidneys of humans with ADPKD who had received a renal transplant.<sup>104</sup> There is as yet no information regarding the role, if any, of mTOR in the pathogenesis of ADPKD as a result of mutations in the *PKD2* gene.

Evidence that mTOR plays an important role in the pathogenesis of ADPKD has spurred several studies of humans that examine the effect of rapamycin on the progression of ADPKD.<sup>105,106</sup> Three clinical trials are currently under way, one at the Cleveland Clinic (a Phase I/II study of 30 patients), one at the Mario Negri Institute in Milan (a Phase II study of 16 patients), and one at Zurich University (a Phase II study of 100 patients).<sup>106</sup>

## mTOR AND RENAL CELL CARCINOMA

Renal cell carcinoma (RCC) accounts for 2 to 3% of all adult malignancies.<sup>107–109</sup> Surgery is curative in approximately one third of patients with RCC in whom the tumor is localized to the kidney. However, approximately 30% of all patients with RCC have metastases at the time of presentation and an additional 30% of patients develop metastatic disease during the follow-up period.<sup>110</sup> Metastatic RCC has a poor prognosis because it is highly resistant to conventional forms of chemother-

apy. Until recently, high-dosage IL-2 was the only approved therapy for RCC<sup>111</sup>. However, novel therapeutic approaches for metastatic RCC that target angiogenesis have been developed.<sup>108,111–113</sup>

Interest in targeting angiogenesis arose from the observed link between inactivation of the *von Hippel-Lindau* (*VHL*) tumor suppressor gene and predisposition to RCC. Malignant transformation of RCC is driven by loss-of-function mutations of the *VHL* gene, leading to increased expression of hypoxia-inducible factor (HIF), a transcription factor that plays a central role in the adaptation to hypoxia (Figure 4).<sup>108,112–115</sup> In general, tumor growth is characterized by an initial phase of rapid

proliferation, which then slows as malignant cells outstrip their blood supply and become hypoxic.<sup>116</sup> Adaptations to hypoxia, which include alterations in cell metabolism and neovascularization (angiogenesis), are necessary for continued tumor growth. HIF, which is an important stimulus to angiogenesis, consists of two subunits: HIF-1 $\alpha$  and HIF-1 $\beta$ . Expression of HIF-1 $\alpha$  is tightly coordinated with oxygen availability through the ubiquitin-proteasome pathway.<sup>117</sup>

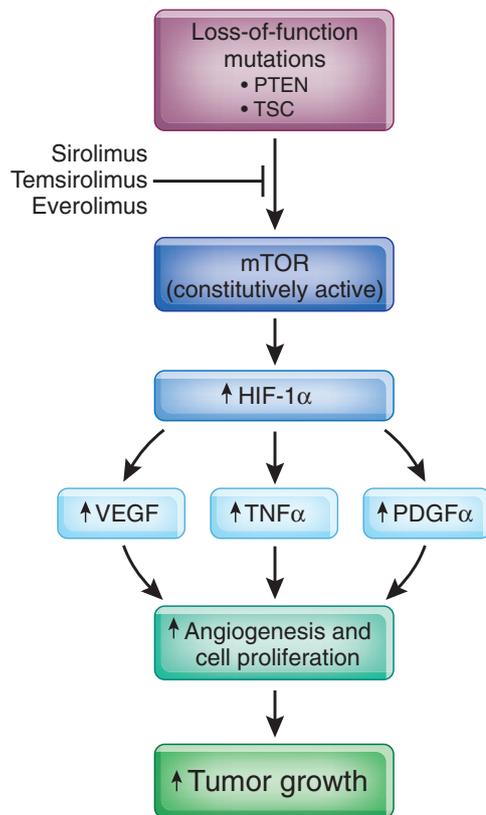
The *VHL* product controls HIF expression in response to oxygen availability.<sup>117</sup> During normoxia, HIF-1 $\alpha$  binds to VHL, which marks HIF-1 $\alpha$  for ubiquitin-mediated degradation. Under hypoxic conditions, there is reduced bind-

ing of HIF-1 $\alpha$  to VHL and consequently decreased degradation such that cellular levels of HIF-1 $\alpha$  increase. Loss-of-function mutations of *VHL* lead to increased HIF-mediated expression of VEGF, PDGF- $\alpha$ , and TGF- $\alpha$ , all of which stimulate tumor angiogenesis and proliferation (Figure 4).<sup>111</sup> Several novel therapeutic agents, such as sunitinib and sorafenib, have been developed for the treatment of metastatic RCC.<sup>107–109,118</sup> These agents inhibit VEGF and PDGF-mediated angiogenesis by inhibiting their tyrosine kinase receptors (VEGF-R and PDGF-R, respectively; Figure 4).<sup>107–109,118</sup>

In addition, mTOR plays a critical role in the pathogenesis of RCC. Genetic mutations that lead to constitutive increases in mTOR activity increase the incidence of metastatic RCC. For example, loss-of-function mutations of *PTEN*, a negative regulator of mTOR through the PI3K/Akt pathway (Figure 1), are found in approximately 5% of patients with RCC (Figure 4).<sup>119</sup> Also, in patients with tuberous sclerosis, loss-of-function mutations of genes encoding either *TSC1* or *TSC2* lead to the inactivation of the TSC, a negative regulator of mTOR (Figure 1). Patients with tuberous sclerosis are predisposed to the development of RCC.<sup>120,121</sup>

Inhibitors of mTOR show substantial promise for the treatment of patients with metastatic RCC and underlie the importance of mTOR in the pathogenesis of RCC. Temsirolimus, an analogue of rapamycin that is administered intravenously, has been approved by the Food and Drug Administration for the treatment of metastatic RCC.<sup>111,122</sup> In addition, preliminary data from Phase II studies suggest that everolimus, an oral derivative of rapamycin, is beneficial in some patients with metastatic RCC.<sup>107–109,111</sup>

Part of the therapeutic benefit of mTOR inhibition in RCC is likely due to inhibition of cell growth and proliferation (Figure 4). However, mTOR increases the expression of HIF by mechanisms that remain to be elucidated. Part of the efficacy of mTOR inhibitors in RCC is related to a reduction in HIF-mediated angiogenesis (Figure 4).<sup>121,123</sup>



**Figure 4.** An increase in the constitutive activity of mTORC1 promotes the growth and invasiveness of many aggressive tumors, including metastatic RCC. This is due to several mechanisms. First, increased activity of mTORC1 promotes cell growth and proliferation. In addition, mTORC1 increases the cellular levels of HIF-1 $\alpha$ , which in turn stimulates the production of proangiogenic factors such as VEGF, PDGF- $\alpha$ , and TNF- $\alpha$ . Increased angiogenesis is necessary to meet the increasing oxygen requirements of growing tumors. In some patients with RCC, activation of mTORC1 is mediated by loss-of-function mutations of the phosphatase PTEN, which negatively regulates mTORC1 through the upstream PI3K/Akt pathway. Also, patients with tuberous sclerosis are predisposed to the development of RCC because of loss-of-function mutations of the TSC, which interferes with the negative regulation of mTORC1 activity by TSC.

## CONCLUSIONS

The discovery of mTOR has led to the elucidation of a complex signaling pathway that plays a pivotal role in cell growth, proliferation, and survival. Numerous studies have recognized that mTOR plays an important role in many renal diseases. Activation of mTOR plays an important role in mediating renal recovery and repair after AKI, and inhibition of mTOR delays renal recovery after AKI.

Interestingly, whereas activation of mTOR plays an adaptive role in AKI, its activation in many other renal diseases has been shown to be deleterious. Provocative studies in animal models suggested that the mTOR signaling pathway is activated in diabetic and in nondiabetic forms of CKD and that rapamycin and its analogues ameliorate the progression of renal failure in these models. In addition, the inappropriate activation of mTOR in ADPKD contributes to the progression of renal failure by increasing the formation and enlargement of cysts. Finally, mTOR is an important pathogenic factor in some patients with RCC.

Our increased understanding of the complex mechanisms that regulate the mTOR pathway has led, during a relatively short period of time, to the development of novel forms of treatment for a number of renal diseases. Rapamycin has been used for many years as an immunosuppressant in recipients of renal transplants. More recently, inhibition of mTOR, using analogues of rapamycin, benefit patients with metastatic RCC. The potential therapeutic role of mTOR inhibition in patients with ADPKD is being evaluated in ongoing clinical trials. Clinical studies are also needed to determine whether inhibitors of mTOR slow progression of renal failure in DN and other forms of CKD in humans for which no specific forms of therapy are currently available.

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## DISCLOSURES

None.

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