mTOR Signaling in Kidney Diseases
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Abstract
The mammalian target of rapamycin (mTOR), a serine/threonine protein kinase, is crucial in regulating cell growth, metabolism, proliferation, and survival. Under physiologic conditions, mTOR signaling maintains podocyte and tubular cell homeostasis. In AKI, activation of mTOR signaling in tubular cells and interstitial fibroblasts promotes renal regeneration and repair. However, constitutive activation of mTOR signaling in kidneys results in the initiation and progression of glomerular hypertrophy, interstitial fibrosis, polycystic kidney disease, and renal cell carcinoma. Here, we summarize the recent studies about mTOR signaling in renal physiology and injury, and discuss the possibility of its use as a therapeutic target for kidney diseases.

KIDNEY360 1: 1319–1327, 2020. doi: https://doi.org/10.34067/KID.0003782020

Introduction
In the 1970s, Streptomyces hygroscopicus was isolated from a Rapa Nui (Easter Island) soil sample, and the antibiotic compound rapamycin was later isolated from this bacterium (1). A series of subsequent studies discovered that this compound has antifungal, immunosuppressive, and antitumor properties (2). In the early 1990s, researchers identified target of rapamycin 1 (TOR1) and TOR2 as mediators of the toxic effect of rapamycin on yeast. Later, the mammalian TOR (mTOR) was identified in mammals (3). Over the following decades, mTOR inhibitors have been approved for the treatment of host rejection, renal cell carcinoma (RCC), and pancreatic cancer.

The mTOR pathway regulates cell growth, proliferation, survival, and metabolism (4). Dysregulation of mTOR signaling disrupts renal cell homeostasis and results in kidney diseases, such as AKI (5,6), kidney fibrosis (7,8), glomerular disease (9,10), polycystic kidney disease (PKD) (11,12), and renal cancer (13). Notably, the efficiency of mTOR inhibitors (rapamycin and its analogues) has been demonstrated in many types of kidney disease (14–16). Here, we will give a brief review of the mTOR signaling pathway, the pivotal roles of mTOR in kidney diseases, and targeting mTOR signaling as a therapeutic strategy for kidney disorders.

Architecture and Composition of mTOR Complexes
mTOR is a 289-kD serine/threonine protein kinase belonging to the phosphatidylinositol-3-hydroxide kinase (PI3K)–related protein kinase family that functions as a central regulator of cell growth, metabolism, proliferation, and survival. As a core component, mTOR consists of two distinct complexes: mTOR complex 1 (mTORC1) and complex 2 (mTORC2) (4) (Figure 1).

mTORC1
mTORC1 consists of five major components: mTOR, regulatory-associated protein of mTOR (Raptor), mammalian lethal with Sec13 protein 8 (mLST8), proline-rich AKT substrate 40 kD (PRAS40), and DEP domain–containing mTOR-interacting protein (Deptor). mLST8 stabilizes the kinase domain of mTOR, but is dispensable for mTORC1 signaling. Raptor is essential for mTORC1 activity because it assembles the complex and recruits the substrates of mTORC1 (17). Both PRAS40, a raptor binding protein, and Deptor act as endogenous inhibitors of mTOR kinase activity (18).

mTORC2
MTORC2 consists of mTOR, mLST8, Deptor, rapamycin-insensitive companion of mTOR (Rictor), mammalian stress-activated protein kinase-interacting protein 1 (mSIN1), and protein associated with rictor 1 or 2 (PROTOR1/2). Rictor interacts extensively with mTOR and defines mTORC2 assembly and its downstream signaling pathway, including Akt, protein kinase C, and serum- and glucocorticoid-induced kinases 1, and deletion of this protein remarkably reduces mTORC2 activity (19). Protor1/2 interacts with Rictor as the Rictor-binding subunit of mTORC2. In addition, mSIN1 is necessary for maintaining mTORC2 assembly and its capacity for phosphorylating Akt/PKB (20). Like those in mTORC1, mLST8 is dispensable for maintaining mTORC2 activity, whereas Deptor negatively regulates mTORC2 activity (18).

Regulation of mTORC1 Signaling
Growth Factors
Many growth factor pathways converge on tuberous sclerosis complex (TSC), a heterodimer comprising TSC1 (also known as hamartin) and TSC2 (also known
as tuberin), through the PI3K-Akt pathway. TSC inhibits mTORC1 signaling by acting as a guanosine triphosphatase (GTPase)–activating protein for a small GTPase known as Ras homolog enriched in brain (Rheb) (21). TSC1/2 is negatively regulated by phosphoinositide-dependent protein kinase 1, which phosphorylates Akt/PKB at threonine 308 to activate mTORC1 signaling. PTEN, a lipid phosphatase and tumor suppressor, inhibits PI3K signaling by dephosphorylating phosphatidylinositol-3,4,5-triphosphate (PIP3) in plasma membrane (22). Besides the PI3K-Akt signaling axis, some growth factors stimulate extracellular signal–regulated kinase (ERK) and p90 ribosomal S6 kinase 1 to inhibit TSC1/2 by phosphorylation and inactivation of TSC2 (23). However, it should be mentioned that certain growth factors inhibit Rheb-induced mTORC1 activation via Akt-mediated phosphorylation of PRAS40 (Figure 1).

**Energy and Oxygen Supplies**

AMP-activated protein kinase (AMPK), a sensitive indicator of cellular energy status, maintains the balance between intracellular ATP production and consumption. In a low-energy state, AMPK negatively regulates mTORC1 activity. Hypoxia is able to activate TSC1/2 to inhibit mTORC1 through the Redd1 gene. Wnt activates mTORC1 via inhibiting GSK3β. AMPK, AMP-activated protein kinase; Deptor, DEP domain–containing mTOR-interacting protein; GSK3β, glycogen synthase kinase 3β; GTPase, guanosine triphosphatase; mTORC1, mammalian target of rapamycin; mTORC1, mTORC1 complex 1; PDK1, phosphoinositide-dependent protein kinase 1; PI3K, phosphatidylinositol-3-hydroxide kinase; PRAS40, proline-rich AKT substrate 40 kDa; PROTOR1/2, protein associated with rictor 1 or 2; PTEN, phosphatase and tensin homolog; Rag, ras-related GTP-binding protein; Raptor, regulatory-associated protein of mTOR; Redd1, transcriptional regulation of DNA damage response 1; Rheb, Ras homolog enriched in brain; Rictor, rapamycin-insensitive companion of mTOR; TSC1, tuberous sclerosis complex 1; TSC2, tuberous sclerosis complex 2; Wnt, Wingless-type MMTV integration site.

**Figure 1.** The intracellular mTOR signaling cascade. mTOR is a component of two major intracellular signaling complexes: mTORC1 and mTORC2. mTORC1 is formed by mTOR, Raptor, mLST8, PRAS40, and Deptor. mTORC2 is formed by mTOR, mLST8, Deptor, Rictor, mSIN1, and PROTOR1/2. mTORC1 is activated by growth factors and amino acids through the PI3K-Akt pathway. Activated Akt inhibits TSC1/2. TSC1/2 negatively regulates mTORC1 signaling by acting as a GTPase-activating protein for Rheb. In a low-energy state, AMPK negatively regulates mTORC1 activity. Hypoxia is able to activate TSC1/2 to inhibit mTORC1 through the Redd1 gene. Wnt activates mTORC1 via inhibiting GSK3β. AMPPK, AMP-activated protein kinase; Deptor, DEP domain–containing mTOR-interacting protein; GSK3β, glycogen synthase kinase 3β; GTPase, guanosine triphosphatase; mLST8, mammalian lethal with Sec13 protein 8; mSIN1, mammalian stress-activated protein kinase-interacting protein 1; mTOR, mammalian target of rapamycin; mTORC1, mTORC1 complex 1; PDK1, phosphoinositide-dependent protein kinase 1; PI3K, phosphatidylinositol-3-hydroxide kinase; PRAS40, proline-rich AKT substrate 40 kDa; PROTOR1/2, protein associated with rictor 1 or 2; PTEN, phosphatase and tensin homolog; Rag, ras-related GTP-binding protein; Raptor, regulatory-associated protein of mTOR; Redd1, transcriptional regulation of DNA damage response 1; Rheb, Ras homolog enriched in brain; Rictor, rapamycin-insensitive companion of mTOR; TSC1, tuberous sclerosis complex 1; TSC2, tuberous sclerosis complex 2; Wnt, Wingless-type MMTV integration site.
Amino Acids

Amino acids play a pivotal role in stimulating the mTORC1 signaling activation. In 2008, two independent studies showed that Rag proteins are a family of four related small GTPases that interacts with mTORC1 in an amino acid-sensitive manner (27). With specific amino acid stimulation, RagA/B binds to GTP and RagC/D binds to GDP, which recruits mTORC1 from the cytosol to the lysosome, and stimulates mTORC1 activation (28).

Other Signals and Conditions

In addition to growth factors, oxygen levels, and amino acids described above, some other signals and cellular conditions are able to regulate mTORC1 activity. Inoki et al. (29) reported that Wnt activates mTORC1 via inhibiting GSK3 without involving β-catenin-dependent transcription. Lee et al. (30) found that IKKβ, a major downstream kinase in the TNFα signaling pathway, activates the mTORC1 pathway through interacting with and phosphorylating TSC1 at serine 487 (Ser487) and Ser511. In response to DNA damage, the p53-Sestrin1/2 upregulation activates AMPK which, in turn, phosphorylates TSC2 to inhibit mTORC1 activation (31).

Regulation of mTORC2 Signaling

In contrast to mTORC1, the activators of mTORC2 signaling are still poorly defined. Some growth factors may activate mTORC2 kinase through stimulating the PI3K pathway to phosphorylate Akt at Ser473 (32). With growth factor stimulation, PI3K may recruit mTORC2 and Akt to the plasma membrane to phosphorylate Akt at Ser473 (33). It is of note that mTORC1 could negatively regulate mTORC2 through insulin-PI3K-Akt signaling. Apart from "growth factors, AMPK may activate mTORC2 signaling under energetic stress (34) (Figure 1).

Interaction between mTORC1 and mTORC2 Signaling

Many studies have shown there is complicated communication between mTORC1 and mTORC2 in various cell types. In natural killer cells, Wang et al. (35) reported that Raptor deficiency reduces the abundance of phosphorylated Akt (Ser473). In contrast, Rictor deficiency increases the abundance of p-S6, suggesting mTORC1 sustains mTORC2 activity, whereas mTORC2 negatively regulates mTORC1 activity (35). In epithelial cells or adipocytes, S6K phosphorylates Sin1 at both T86 and T389 to inhibit mTORC2 kinase activity by dissociating Sin1 from the mTORC2 complex (36). In addition, several lines of evidence show that knocking down Raptor enhances mTORC2 activation by alleviating negative feedback loops (37). Recently, our laboratory demonstrated that ablation of Rictor in primary cultured kidney fibroblasts largely decreases S6 phosphorylation, suggesting mTORC2 may positively regulate mTORC1 signaling in kidney fibroblasts (38). Future studies are needed to gradually crack open the mystery of mTORC1 and mTORC2 crosstalk.

mTOR in Kidney Diseases

AKI

AKI is a global health problem with a high incidence of morbidity and mortality. Ischemia, nephrotoxins, and sepsis are the common causes for AKI. A number of studies reported that mTOR signaling maintains renal tubular homeostasis and inhibits tubular cell death after acute injury (5). Activation of mTOR signaling in tubular cells protects against AKI (6,39). In addition, our studies showed that activation of mTORC1 and mTORC2 signaling in kidney fibroblasts may stimulate the fibroblast to express HGF to protect against tubular cell death and AKI (38) (Figure 2).

Administration of rapamycin, an inhibitor of mTORC1, impairs tubular cell regeneration and delays the recovery of renal function after AKI (40). In addition to directly inhibiting tubular cell proliferation and regeneration, rapamycin markedly promotes the recruitment of regulatory T cells (CD4+Foxp3+ regulatory T cells) and myeloid-derived suppressor cells and strengthens their immunosuppressive activity in the ischemic kidneys (41,42). During kidney transplantation in mice, predonation of rapamycin delays tubular regeneration, exacerbates graft dysfunction, and halts post-transplantation recovery (43). In contrast, most studies found that administration of rapamycin in donors markedly attenuates the ischemia-reperfusion injury (IRI) process, decreases inflammatory mediators in situ, and improves graft function after kidney transplantation (44). In another report, administration of rapamycin at the early stage of IRI aggravates kidney dysfunction, whereas no difference is observed in the kidney function of mice treated with rapamycin at day 7 after IRI (Table 1) (45). Therefore, the mechanisms of mTOR signaling in regulating renal injury and recovery after transplantation are still not clear.

Renal Fibrosis

CKD pathologically manifests as interstitial excessive extracellular matrix deposition and kidney fibrosis. All of the renal resident cells, including fibroblasts, tubular epithelial cells, pericytes, and endothelial cells, contribute to renal fibrosis. Over the past 20 years, extensive studies uncovered the pivotal roles for mTOR signaling in fibrotic kidney diseases. Rapamycin is able to attenuate interstitial inflammation and kidney fibrosis in various types of kidney disease, including IRI, transplantation, adriamycin nephropathy, unilateral ureteral obstruction (UUO), and glomerulopathy (46,47).

mTOR signaling activation contributes to kidney fibrosis through multiple pathways. In glomerular mesangial cells, TGFβ1 stimulates miR-21 expression through the PETN-Akt-mTORC1 axis to induce mesangial cell hypertrophy and matrix expansion (48). In tubular epithelial cells, rapamycin inhibits the mTOR-promoted epithelial-to-mesenchymal transition (49). In kidney fibroblasts (NRK 49F cell line), TGFβ1 stimulates both mTORC1 and mTORC2 signaling activation in a time- and dosage-dependent manner, whereas blockade of mTOR signaling with rapamycin markedly inhibits TGFβ1-induced fibroblast activation. In mouse kidneys with UUO nephropathy, both mTORC1 and mTORC2 are activated in interstitial myofibroblasts. Activation of mTORC1 signaling in fibroblasts promotes renal interstitial fibrosis. Ablation of Rictor in fibroblasts attenuates UUO.
nephropathy in mice (7,8,50). In macrophages, mTORC2 signaling activation is indispensable for macrophage M2 polarization and UUO- or IRI-induced kidney fibrosis (51) (Figure 2).

Autophagy is a dynamic process in which damaged organelles and macromolecules are degraded. A recent study showed that mTORC1 negatively regulates autophagy through the association of the late autophagosome and formation of the TOR-autophagy spatial coupling compartment (TASCC), which promotes secretion of profibrotic factors (52). Severe kidney injury leads to increased TOR-autophagy spatial coupling compartment formation in tubular cells, increased secretion of profibrotic factors, and progression of fibrosis (53).

Although it is clear that mTOR signaling activation promotes kidney fibrosis, targeting mTOR in patients with kidney diseases should be treated with caution. Rapamycin (mTORC1 inhibitor) and PP242 (dual inhibitor of mTORC1 and mTORC2) are not cell type-specific inhibitors, so they may induce side effects during the treatment of patients with kidney diseases.

Podocytopathy

Podocytes are highly differentiated cells with numerous foot processes that cover the filtration surface area, and play a vital role in the selective permeability of the glomerular filtration barrier. The loss of podocytes leads to proteinuria, glomerular sclerosis, the progression of kidney dysfunction, and ESKD. A number of studies have shown that mTOR is crucial to maintain glomerular podocyte morphology and function (9). mTOR inhibitors (sirolimus and everolimus) may alter the integrity of the actin cytoskeleton and decrease cell adhesion to disturb podocyte function (54,55) (Table 1).

Podocyte-selective deletion of the mTOR gene results in proteinuria and end stage renal failure (56). In animal models, ablation of raptor in podocytes causes proteinuria and progressive glomerulosclerosis. Podocyte-specific ablation of Rictor results in a reduced ability to adapt to stress, suggesting that mTORCs are indispensable for glomerular homeostasis (9).

Podocyte-specific deletion of autophagy-related 5 leads to glomerulopathy in aging mice (57). In glomerular injury, aberrant activation of mTORC1 suppresses podocyte autophagy, damages podocyte function, and deteriorates diabetic kidney disease (10,58,59), whereas administration of rapamycin protects against podocyte injury (60,61). Therefore, blockade of mTOR signaling may activate autophagy to protect against podocytopathy.

In FSGS, mTORC1 target genes are largely induced in glomeruli, and deletion of one Raptor allele or low-dose rapamycin treatment retards the progression of glomerulosclerosis (62). However, in patients with FSGS, mTOR inhibitors show conflicting results, ranging from remission to deterioration of kidney dysfunction (63–65). In IgA nephropathy and lupus nephritis, mesangial cell mTORC1 activation induces the production of collagen IV, collagen I, and α-smooth muscle actin in glomeruli (66). Rapamycin may induce the level of anti-double-stranded DNA antibodies, suppressing the infiltration of inflammatory cells in models of lupus. In IgA nephropathy, rapamycin attenuates...
IgA deposition in the glomeruli to protect renal function (15,67). mTOR inhibitors have also been shown to decrease proteinuria and mesangial and endocapillary proliferation, improving immunoregulation and renal function in patients with IgA nephropathy and lupus nephritis (68–70) (Table 1).

In summary, mTOR plays an important pathogenic role in glomerular diseases. Several studies suggested that mTORC1 inhibition may reduce glomerular hypertrophy and albuminuria, and prevent the progression of glomerular diseases in animal models. However, in patients, rapamycin treatment frequently causes podocyte apoptosis, proteinuria, and FSGS (71,72). The underlying mechanisms remain obscure.

### PKD

Autosomal dominant PKD (ADPKD), due to the genetic mutation of PKD1 or PKD2, is one of the most common human monogenic diseases. mTOR has been demonstrated to play an important role in cyst formation and enlargement in PKD. Several studies showed that the cytoplasmic tail of PC1 interacts with tuberin to form a complex, functioning as the endogenous inhibitor for Rheb, a constitutive mTORC1 activator. When PC1 is mutated in tubular epithelial cells, the multiprotein complex inappropriately activates mTORC1, promoting cyst formation and PKD in patients and animal models (11). PC1 regulates mTORC1 activation by relying on ERK-mediated TSC2 phosphorylation. In the absence of PC1, ERK may phosphorylate tuberin at Ser664 to activate mTORC1 signaling (12). Metformin inhibits mTORC1 and cyst growth in mouse models of ADPKD by stimulating AMPK (73). A recent study revealed that, in Tsc1-mutant mice, Afadin (a component of cell adhesion systems) is directly phosphorylated by S6K1, which affects cell division orientation and promotes kidney cyst formation (74).

In the early 21st century, two groups demonstrated that rapamycin and sirolimus can significantly reduce cell proliferation in cystic and noncystic tubules, retard the progression of cystogenesis, and protect kidney function in Han:SPRD rats with ADPKD (75,76). A number of subsequent studies confirmed these findings (77,78). Researchers also reported that some other pathways, including PI3K, Erk, cAMP, and mTORC2, are activated in ADPKD (79–81). Shillingford et al. (11) found that rapamycin treatment largely reduces native polycystic kidney size in transplant recipients with ADPKD. Recently, there have been extensive clinical trials for mTOR inhibitors in patients with ADPKD (Table 1). In some clinical trials, mTOR inhibitors cannot significantly decrease the total kidney volume, halt polycystic kidney growth, or slow the progression of renal impairment compared with placebo groups (82–84), which may be attributed to the short follow-up period, low blood concentration in cystic tissue, and advanced disease stage in patients (82,84). However, high doses of mTOR inhibitor treatment decrease the progression of cystogenesis, halt cyst growth, and increase parenchymal volume in patients with ADPKD (85). Although mTOR inhibitors have been shown to be effective in treating ADPKD in animal models, for patients with ADPKD, the optimal time points and the dosage for mTOR inhibitors still need to be determined.

### RCC

RCC, which originates in the renal cortex, accounts for 2%–3% of all adult malignancies. The mutations of the VHL gene lead to the upregulation of hypoxia-inducible factor, vascular endothelial growth factor, PDGFβ, and TGFβ to stimulate tumor angiogenesis and proliferation (86). The signaling of the vascular endothelial growth factor receptor
and PDGF receptor activate the PI3K-AKT-mTORC1 pathway. In turn, the mTOR pathway may activate hypoxia-inducible factor 1α to constitute a positive feedback loop between VHL and mTOR in RCC (13). Hence, mTOR signaling may be a potential therapeutic target in RCC (87).

Several mTOR signaling inhibitors, including mTOR kinase domain inhibitors (16), mTOR/PI3K dual inhibitors (88), PI3K-selective inhibitors, and a proapoptotic protein named programmed cell death 6 have been used in the treatment of RCC (Table 1) (89). A novel dual mTORC1/2 inhibitor AZD2014 disrupts mTORC1/2 assembly and activation to promote RCC cell death (90). Unfortunately, in a randomized phase 2 study, the progression-free survival and overall survival after treatment with AZD2014 are inferior to everolimus in refractory metastatic RCC (Table 1) (91).

**Conclusion**

Over the last few decades, numerous studies have demonstrated that mTOR signaling plays an important role in renal physiology and disease. Transient mTOR signaling activation in tubular cells or fibroblasts is beneficial for renal repair after AKI, but continuing activation of mTOR signaling stimulates extracellular matrix deposition and renal fibrosis in mice. Similarly, mTORCs are indispensable for maintaining glomerular homeostasis, and aberrant activation of mTORC1 leads to podocyte dysfunction and albuminuria. In ADPKD and RCC, mTOR signaling activation plays a pathogenic role through promoting renal cyst formation and RCC cell survival. Although the role of mTOR in renal physiology and disease has been considerably explored, much still remains to be uncovered. The usage of mTOR inhibitors in kidney transplantation, ADPKD, and RCC need more assessment. Further exploration is needed to define optimal treatment time points, dosage, combined therapies, and cell type-specific medications in treating patients with various type of kidney disease. Notably, the intertwined mechanisms between mTORC1 and mTORC2 signaling in kidneys need to be deciphered.

**Disclosures**

All authors have nothing to disclose.

**Funding**

This work was supported by National Science Foundation of China grants 81570611/H0503 and 81770675/H0503.

**Author Contributions**

C. Dai provided supervision and was responsible for investigation; C. Dai and Y. Gui reviewed and edited the manuscript; and Y. Gui wrote the original draft and was responsible for formal analysis.

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Received: June 15, 2020 Accepted: September 2, 2020