mTOR controls kidney epithelia in health and disease

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ABSTRACT

Renal epithelial function is the cornerstone of key excretory processes performed by our kidneys. Most of these tasks need to be tightly controlled to keep our internal environment in balance. Recently, the mTOR signalling network emerged as a key pathway controlling renal epithelial cells from the glomerular tuft along the entire nephron. Both mTOR complexes, mTORC1 and mTORC2, regulate such diverse processes as glomerular filtration and the fine tuning of tubular electrolyte balance. Most importantly, dysregulation of mTOR signalling contributes to prevalent kidney diseases like diabetic nephropathy and cystic kidney disease. The following review shall summarize our current knowledge of the renal epithelial mTOR signalling system under physiological and pathophysiological conditions.

Keywords: ADPKD, diabetic nephropathy, mTOR, podocyte, tubular transport

INTRODUCTION

The mammalian kidney is one of the most complex excretory organs, allowing fine-tuned excretion of water, salt and waste products over a highly dynamic range of urinary volume thereby conserving our internal environment [1]. This amazing epithelial structure was a prerequisite for mammalian life to conquer virtually every natural habitat on our planet [2]. Although our kidneys usually perform a silent job, once failing we are strikingly reminded of the tremendous workload that is imposed on our excretory system. With an ageing population and stressors, i.e. hypertension, atherosclerosis and diabetes mellitus on the rise, kidney injury and failure are increasing worldwide and renal replacement therapy is imposing huge costs on our health care systems [3, 4]. Therefore, deciphering and understanding the pathways that govern renal epithelial function in health and disease is a prerequisite to develop new strategies to combat chronic kidney disease.

One of these signalling cascades is the mechanistic target of rapamycin (mTOR) pathway which has been initially described as a cell cycle inductor in yeast but is highly evolutionarily conserved [5–9] (Figure 1). Rapamycin, a known inhibitor of target of rapamycin (TOR), forms a complex with FK506 Binding Protein 12 (FKBP12) to abolish TOR activity. This notion is supported by mutations in yeast TOR1, TOR2 or FKBP 12 which lead to resistance to the growth-inhibitory properties of rapamycin [10–12]. The mammalian homologues of TOR1 and TOR2 are named mechanistic target of rapamycin complex 1 and 2 (mTORC1 & mTORC2), respectively, and are intracellular multiprotein complexes. Their common backbone consists of the mTOR kinase, DEP domain-containing mTOR-interacting protein (DEPTOR) and mammalian lethal with Sec13 protein 8 (mLST8). In the case of mTORC1, this backbone interacts with regulatory-associated protein of mTOR (RAPTOR) and is rapamycin sensitive. In contrast, mTORC2 interacts with mammalian stress-activated protein kinase-interacting protein 1 (mSIN1), rapamycin-insensitive companion of mTOR (RICTOR), proline rich protein 5 (PROTOR 1) and proline rich protein 5 like (PRR5L) and is rapamycin insensitive. mTORC1 integrates a wide variety of vital nutrient cues including growth factors, amino acids, cellular energy content and cellular stress. To regulate cellular growth, cell division, lipid and mitochondrial biogenesis, mTORC1 phosphorylates a set of substrates such as p70 S6Kinase (p70S6K), eukaryotic translation initiation factor 4E-binding protein 1 (EIF4EBP1), Lipin1 and peroxisome proliferator activated receptor gamma coactivator 1
FIGURE 1: Overview of mTOR pathway. mTOR forms two protein complexes with RAPTOR (mTORC1) or RICTOR (TORC2). Activation of the mTOR kinase via nutrients, growth factors or cell stress leads to phosphorylation of downstream targets involved in cell growth, proliferation, metabolism and aging. AKT, protein kinase B; AMPK, AMP-activated protein kinase; Deptor, DEP domain-containing mTOR-interacting protein; 4E-BP1, eukaryotic translation initiation factor 4E-binding protein; ERK1/2, extracellular signal-regulated kinase 1/2; FKBP12, FK506-binding protein of 12 kDa; FOXO, forkhead box O1; IGF, insulin growth factor; LKB1, serine threonine kinase 11; MEK1/2, mitogen-activated protein kinase kinase mLST8, mammalian lethal with Sec 13 protein 8; mSIN1, mammalian stress-activated protein kinase-interacting protein; mTOR, mammalian target of rapamycin complex; p70S6K, p70 ribosomal S6 kinase; PI3K, phosphoinositide-3 kinase; PGC1-α, PPARγ coactivator 1-α; PKCα, protein kinase Cα; PRR5L, proline rich protein 5 like; Rag, Ras-related GTP-binding protein; Raptor, regulatory-associated protein of TOR; Rheb, Ras homologue enriched in brain; Rictor, rapamycin-insensitive companion of mTOR; SGK-1, serum- and glucocorticoid-induced protein kinase 1; TK, tyrosine kinase; TSC, tuberous sclerosis complex; TSC1, hamartin, TSC2, tuberin; ULK1, unc-51-like kinase 1.
alpha (PGC1-α) [13, 14]. mTORC2 in contrast seems to be mainly activated by insulin and related pathways, and controls several downstream AGC kinases such as AKT, serum- and glucocorticoid stimulated kinase (SGK) and protein kinases C (PKCs) by phosphorylating their hydrophobic motif [15–18]. By initiating and enhancing their activation, mTORC2 plays an important role in cellular survival and organization of the cytoskeleton. For a comprehensive and detailed general overview of mTOR signalling cascades we refer to several recent reviews and review series [19–22].

The aim of this review was to specifically delineate the emerging physiological and pathophysiological function of mTOR in specialized kidney epithelial cells. Although the kidney has been a longstanding pharmaceutical target of mTOR inhibitors to either prevent renal allograft rejection or treat renal cell carcinoma, the diverse and specific roles of the two different mTOR complexes in renal epithelial cells are just about to be appreciated.

mTOR in Glomerular Health and Disease

Podocyte size control by mTORC1

Podocytes form the most vulnerable part of all functional kidney compartments. Genetic, toxic, immunological or metabolic insults to podocytes result in a stereotypic injury sequence of foot process effacement, podocyte loss and ultimately glomerulosclerosis [23]. Clinically, these events are in direct and causative relationship to the degree of proteinuria. Podocytes are postmitotic cells and although potential stem cell niches of podocytes have been identified, podocyte regeneration seems to be very limited and might only be confined to rare clinical conditions [24]. However, experimental podocyte depletion models did not only unravel that podocyte loss is coupled to the development of glomerulosclerosis, but also indicated that the threshold for podocyte loss resulting in glomerulosclerosis is surprisingly high with an ∼20% reduction of podocyte numbers required to cause glomerulosclerosis [25, 26]. While this ‘podocyte depletion hypothesis’ [27] is well correlated with clinical observational studies and has been experimentally proven by the use of elegant transgenic diphtheria toxin models [25, 26], the mechanisms of podocyte adaptation remained only poorly understood. Current knowledge suggests that differentiated podocytes do have at least some capacity to adjust to an altered glomerular architecture. With podocyte mitosis and regeneration being virtually absent in most pathological settings, podocyte hypertrophy has entered the focus of glomerular research. Recent findings now highlight mTOR as a key regulator of podocyte size control [28–30]. Podocyte-specific disruption of the mTORC1 complex resulted in a pathological picture, which clinically most closely resembles secondary focal and segmental glomerulosclerosis (FSGS) with quite stable proteinuria and slow progression [29]. Interestingly, this phenotype was strictly dependent on the timepoint of mTORC1 disruption with the FSGS phenotype only occurring when mTORC1 signalling was interrupted during glomerular development, but not at adult stages [29]. In fact, deficiency of mTORC1 signalling during glomerular development led to apparently smaller podocytes compared with wild-type littermates [29]. Mechanistically, this points to the importance of mTORC1 stimulated podocyte growth during development allowing podocytes to adjust to the expanding glomerular surface. Failure of podocytes to increase their cell size during this developmental period causes secondary focal segmental glomerulosclerosis. Limitations of podocyte growth adaptation also likely contribute to the increased prevalence of FSGS in growth-related processes such as obesity, glomerulomegaly, nephronopenia and large body size. Recently, this hypothesis was tested using a transgenic rat model expressing dominant negative EIF4EBP1, which acts downstream of mTORC1 to modulate cap-dependent translation and cell hypertrophy [30]. In fact, proteinuria and glomerulosclerosis were directly related to increasing body weight and levels of dominant negative EIF4EBP1 expression in different experimental models, highlighting that mTOR signalling sets the threshold for the adaptive growth capacity of podocytes [30]. However, glomerulosclerosis could be prevented if dietary calorie restriction limited weight gain and glomerular enlargement suggesting a role of mTOR signalling in obesity-associated FSGS [30].

Compensatory podocyte size increase: a hypothetical explanation for rapamycin-induced proteinuria

Although experimentally not proven yet, mTOR-dependent podocyte size control can likely compensate—at least within certain limits—for podocyte losses due to any kind of podocyte injury [31](Figure 2A). In this context, it is interesting that mTOR inhibition by rapamycin treatment often results in proteinuria in humans [32, 33]. Examples include patients with chronic allograft nephropathy receiving rapamycin [34] or patients receiving rapamycin de novo after renal transplantation [35]. Hereby, not only the time point but also the dose of mTOR inhibitors appears to directly influence podocyte function including the expression of podocyte slit diaphragm molecules and the development of proteinuria [36–38]. Furthermore, in some animal models of chronic hyperfiltration, rapamycin was shown to have deleterious effects [39] on renal function. Thus, in situations where mTORC1 sustains an adaptive compensatory action in response to any underlying glomerular stress/injury, rapamycin might present a ‘second hit’ leading to proteinuria (Figure 2B). Furthermore, it was demonstrated very recently that mTORC2 via Akt2 also plays a critical role for podocyte survival [40]. Strikingly, the mTORC2-Akt2 survival signal was abolished in patients receiving rapamycin thereby contributing to the deleterious effects of rapamycin in certain clinical settings [40]. A future question, however, will be how these underlying compensatory actions of mTORC1/2 can be predicted, if proteinuria is not yet present and histological biopsies are not available.
Role of mTORC1 in diabetic nephropathy

While the inhibition of mTORC1 activity is associated with an increased occurrence of proteinuria in some clinical settings, rapamycin treatment has also been demonstrated to ameliorate pathological phenotypes of many different renal diseases in rodent models (reviewed in [41, 42]) such as minimal change disease [43], FSGS [44], membranous nephropathy [45, 46], crescentic glomerulonephritis [47] and diabetic nephropathy (DN) [48]. Diabetes was shown to correlate with increased mTORC1 activity in both diabetic animals and humans [28, 29] and rapamycin prevented glomerular hypertrophy, mesangial expansion, glomerular basement membrane (GBM) thickening and renal macrophage recruitment in diabetic animal models [48]. As a general theme, podocyte injury appears to be reactivating developmental programmes such as Notch [49], Wnt [50–54] and mTOR pathways. However, while transient mTORC1 activation seems to compensate in certain settings for lost podocytes by increasing the size of remaining podocytes, persistently activated mTORC1 might cause deregulated cell hypertrophy ultimately leading to podocyte loss and glomerulosclerosis. This has now recently been proven for experimental diabetic models; genetic reduction of mTORC1 in podocytes prevented the development of DN in animals [28, 29], highlighting that mTORC1 hyperactivation is a key determinant for the development of DN (Figure 2C). In agreement with these findings, deletion of the tuberous sclerosis complex (TSC1) gene, a major negative regulator of mTORC1 resulting in constitutive mTORC1 hyperactivation, mirrored several features of DN such as thickening of the GBM, mesangial expansion, foot process effacement, podocyte de-differentiation, redistribution of slit diaphragm proteins, podocyte loss and proteinuria [28]. The precise downstream mechanisms of mTOR signalling in podocytes contributing to podocyte loss are still only very
incompletely understood. However, podocyte dysfunction was directly associated with mTORC1-induced endoplasmic reticulum (ER) stress and the reduction of ER stress by using the chemical chaperon (4-phenylbutyrate) abolished podocyte loss in podocyte-specific TSC1 knock-out (KO) mice [28]. Interestingly, mTORC1-induced ER stress has also been reported in rat models of minimal change disease [43, 55]. Furthermore, high glucose has recently been demonstrated to cause mTOR-dependent increased levels of NADPH oxidase type 1 and 4 activity in podocytes in vitro [56] underlining a role of mTOR-dependent cellular stress signalling pathways such as reactive oxygen species (ROS) production and ER stress. mTORC1 is also known for blocking the initial steps of autophagosome formation [57]. In fact, inhibition of autophagy itself causes a degenerative glomerulopathy [58]. However, although a podocyte-specific complete mTOR KO results in impaired autophagic flux [59], the phenotypes of constitutively mTOR hyper-activated podocytes and autophagy-depleted podocytes have only very little overlap suggesting that autophagy is not the major downstream pathway of mTOR in mediating podocyte injury and loss.

In summary, substantial mTORC1 activation can already be detected at early stages of diabetes leading to an unbalanced cell hypertrophy, de-differentiation, podocyte detachment and ultimately progressive glomerulosclerosis (Figure 2C). Future studies will have to delineate more precisely the podocyte-specific mTOR downstream targets involved in this process to identify novel and more specific drug targets, which might offer new avenues for the therapy of DN.

Role of mTORC2 for glomerular injury

In contrast to mTORC1, very little is known regarding the potential function of mTORC2 in glomerular maintenance and disease. This is mainly due to the fact that rapamycin has long been regarded as a highly specific mTORC1 inhibitor. However, recent work indicates that prolonged application of rapamycin can also inhibit mTORC2 and not only mTORC1 [60–62]. Knowing this, some of the functions that have been only attributed to mTORC1 might also be present, at least partially, been mediated by mTORC2. First insights in the function of mTORC2 on glomerular biology came from a genetic model demonstrating that mTORC2 is dispensable for normal podocyte development [29]. However, simultaneous podocyte-specific mTORC2 and mTORC1 KO significantly aggravated the mTORC1 KO phenotype [29]. In addition, the mTORC2 KO sensitized mice towards injury models such as bovine serum albumin overload suggesting that mTORC2 plays an important role in podocyte stress surveillance and survival [29]. Consistently, it could be demonstrated that mTORC2 and its downstream target Akt2 are essential for survival of remaining podocytes in response to nephron reduction [40]. Importantly, the mTORC2-Akt2 activation could also be observed in biopsy tissue from kidney transplant patients [40]. Rapamycin was shown to prevent the activation of mTORC2-Akt2 in such patients, which was associated with increased glomerular apoptosis [40]. Thus, unexpectedly, there seems to be a significant role of mTORC2 inhibition in the proteinuric effect of sirolimus. In summary, these results identify mTORC2-Akt2 as an important podocyte survival factor that likely contributes to rapamycin-induced proteinuria in concert with mTORC1. The future will show whether novel mTORC1-specific inhibitors will cause less glomerular side effects and proteinuria than currently used mTOR inhibitors.

Potential role of mTORC1 for parietal epithelial cells and crescent formation

Parietal epithelial cells entered centre stage of glomerular research when they were identified as potential progenitor cells of podocytes and as the most abundant cell-type within glomerular crescents [63–66]. So far, not much is known about the regulation of parietal epithelial cells with regard to their progenitor cell properties. Based on other epithelial tissues, it might be speculated that mTORC1 could give cues for cell growth followed by subsequent cell division [13]. Crescent formation occurs as a pathologic proliferative response in several forms of glomerulonephritis [64, 67]. The heparin-binding epidermal growth factor-like signalling pathway is important to induce both podocyte and parietal epithelial cell proliferation [66]. It is conceivable to speculate that mTORC1 could mediate effects downstream of the epidermal growth factor receptor especially regarding hypertrophy and proliferation [68]. These events could possibly present a potential pharmaceutical target to dampen and halt crescent formation, which is regarded as a key pathogenetic feature determining the decline of renal function.

mTOR-regulating tubular function and maintenance

Although mTORC1 is an established drug target for renal cell carcinoma and renal transplantation, information regarding the physiological function of mTORC1 and mTORC2 within the kidney’s tubular apparatus is scarce. Initially, several studies explored the acute and chronic effects of sirolimus on glomerular filtration rate (GFR), renal blood flow (RBF) and tubular function. In contrast to cyclosporine, there was no decrease in GFR as well as RBF, and renal function in pigs was not altered [69]. With regard to GFR, these findings could be shown in patients receiving kidney allografts as well [70]. In addition, it was noted that patients treated with sirolimus but not cyclosporine presented with hypophosphatemia and hypokalemia [70]. A subsequent manuscript demonstrated that the observed hypokalemia was due to renal potassium loss [71]. The also detectable hypophosphatemia seems to be more difficult to explain. Despite having been observed in humans, mice and rats, the signalling cascade leading to phosphaturia remains unclear [70, 72, 73]. In contrast to available in vitro data, at least for NaPi-IIa, mTORC1 inhibition in vivo does not seem to influence the apical phosphate reabsorption machinery consisting of NaPi-IIa, NaPi-IIc and Pit-2 in the proximal tubule [72, 73]. Due to systemic application of rapamycin, hormonal effects could potentially mediate the
observed phosphaturic effect but again no conclusive differences in major pathways regulating phosphate transport, i.e. 1,25(OH)2 D3, PTH or FGF23, could be detected between treated and untreated mice [72]. As phosphaturia is seemingly a consistent finding, one could speculate whether mTORC1 either decisively regulates the basolateral efflux pathways in proximal tubular cells or influences a hitherto not identified hormonal component of phosphate homeostasis. Conditional cell specific ablation of mTORC1 within the proximal tubule could possibly help to decipher which of the two possibilities will prove correct.

In addition to phosphaturia, mice treated with rapamycin for 3 days presented with a 40% increase in glucose excretion and a doubling of aminoaciduria giving the picture of a mild Fanconi-like phenotype [72]. Taken together, these pharmacologic findings could possibly be explained by inhibition of mTORC1s anabolic action, although definitive proof has yet to be accomplished.

Due to the lack of a specific mTORC2 inhibitor there are no mechanistic insights into its in vivo role in renal tubules. Indeed, it can only be speculated on its putative in vivo function based on in vitro experiments [74]. In these it could be demonstrated that mTORC2 is critical to phosphorylate the hydrophobic motif of SGK1 at Thr 422 which in turn is a prerequisite for PDK1 to phosphorylate and activate the kinase domain of SGK1 at Thr 256 [16, 74]. This mechanism is similar to the phosphorylation of AKT on Ser 473, which has become a signature phosphorylation for mTORC2. SGK1 is known to be intimately involved in the regulation of aldosterone-sensitive distal tubular sodium reabsorption [75]. When distal tubular cells are stimulated with aldosterone and insulin together, mTORC2 deficiency or inhibition (with a Pan-mTOR inhibitor) led to a significant reduction in SGK1 phosphorylation at Thr 422 and concomitant decrease of amiloride sensitive short-circuit current. In subsequent studies it could be shown that SIN1 mediates the association of mTORC2 and SGK1 and that PROTOR 1 seems to be critical in phosphorylating the HM of SGK1 [76, 77]. These findings indicate that mTORC2 might be involved in the regulation of Na+ balance which is highly pathophysiologically relevant for salt-sensitive hypertension or volume overload occurring with congestive heart failure (Figure 3A). Yet, although these in vitro data nicely fit together, they need to be genetically proven in conditionally targeted animals to appreciate their in vivo relevance.

Role of mTOR under ischaemic conditions

When rapamycin entered trials in renal transplantation it became rapidly apparent that administration too early after transplantation led to a significant increase in delayed graft function. Subsequent studies in humans and rodents showed that repair after ischaemia/reperfusion injury is delayed with rapamycin but that finally there is recovery of acute tubular necrosis [78]. Although several studies have addressed this clinically relevant finding, there are still uncertainties regarding the effect of mTOR inhibition on the different types of renal and immune cells involved during this process. Another area of dispute is whether mTOR inhibition does not only delay repair but also increases injury [79]. These questions are not only relevant to the field of transplantation but also to patients taking mTORC1 inhibitors on the basis of one of the extending oncologic indications who sustain an acute kidney injury.

mTOR in hereditary and cystic kidney diseases

Tuberous sclerosis is a relatively frequent monogenic disease with an incidence of 1:6000 which is caused by mutations in either TSC1 or TSC2 leading to hyperactivation of mTOR. Clinical manifestations classically include Vogt’s triad of mental retardation, facial angiofibromas and intractable epilepsy. Renal involvement is characterized by cystic lesions as well as angiomyolipomas. In addition to neuronal and renal sequelae, one in three patients suffers from lymphangioleiomyomatosis of the lung [80, 81]. Pathophysiologically, inhibition of the mTOR pathway seems a logical and causative treatment approach. In a collaborative European effort, the EXIST-1 and EXIST-2 Trials examined the efficacy and safety of the mTOR inhibitor everolimus in halting the progression of tuberous sclerosis [82, 83]. In a multicentred, randomized, double-blinded and placebo controlled trial approach EXIST-2 recruited 118 participants and subsequently divided them into two groups. In the treatment group the angiomyolipoma response rate was 42 versus 0% in the control group with an acceptable safety profile (mean drug trough levels near 8.5 ng/mL). Hence, mTOR inhibitors might prove to be the first effective and feasible approach to treat this rare and debilitating disease and to preserve renal function in these patients [84].

Autosomal dominant polycystic kidney disease (ADPKD) is the most common inherited monogenic disease and affects between 1:1000 and 1:4000 of the population [85]. Until the last decade, there was hardly anything known on the altered signalling pathways during cystogenesis and cyst progression. Today it is known that several well-known cell proliferation pathways are stimulated including amongst others the mTOR pathway (Figure 3B). In 2005, a seminal paper treating Han:SPRD rats with rapamycin for 5 weeks demonstrated that cyst increase was reduced by >40% while the number of proliferating cell nuclear antigen positive cells was reduced [86]. Soon afterwards these results were repeated with other mTOR inhibitors and in other rodent models of cystic kidney disease [87–91]. Although there are parallels to renal cell cancer, where mTOR inhibitors have gained importance as a targeted therapeutic approach, there seem to be obvious differences in intracellular signalling mechanisms which lead to mTOR activation [92, 93]. In ADPKD, it was initially shown that components of the mTOR pathway including mTOR kinase itself and the downstream target Phospho-S6 were activated only within the cyst lining epithelium but not in normal appearing renal epithelium of polycystic kidney disease (PKD) specimens from human and several mouse models. Later on, two concepts arose on how mTOR activation in ADPKD could be mediated. First, it was shown that native PKD1 inhibits MEK-dependent mTORC1 activation by dampening activation of the MEK/ERK pathway. Mutated PKD1, however, leads to activation of the MEK/ERK pathway which
in turn phosphorylates Tuberin on Serin 664 and finally leads to increased activation of mTORC1 [94]. Secondly, based on ciliary function as a flow sensor it was demonstrated that under normal ciliary function and flow-dependent ciliary bending LKB1 is activated, phosphorylates AMP Kinase (AMPK) which in turn inhibits Rheb and thereby decreases its stimulatory effect on mTORC1. When ciliary function is disrupted, e.g. through disruption of the kinesin subunit Kif3a, urinary flow does not lead to stimulation of the LKB1/AMPK axis which leads to disinhibition of Rheb and hence increased mTORC1 activity [95] (Figure 3B). In line with these experiments, stimulation of AMPK by the well-known anti-diabetic drug metformin led to reduced cyst growth in two mouse models of PKD [96].

Although preclinical evidence for a crucial involvement of the mTOR pathway in ADPKD is eye-catching, the results of three randomized clinical trials in ADPKD patients are more difficult to interpret [97–99]. In the SIRENA (The Sirolimus Treatment in Patients with Autosomal Dominant Polycystic Kidney Disease: Renal Efficacy and Safety) trial, 21 patients were randomized in a crossover trial comparing the effect of 6-month treatment with sirolimus to 6-month standard care [97]. In the 15 patients who completed the study, cyst volume and total kidney volume (TKV) increased less and renal parenchymal volume increased more in the sirolimus-treated group (average drug trough levels 7.7 ± 2.2 ng/mL). No effect of sirolimus could be detected on GFR decline, while albuminuria increased significantly. Ten out of fifteen patients suffered from aphthous stomatitis, a well-known side effect of sirolimus. In the SWISS ADPKD study, 100 patients were randomized to receive either sirolimus or conventional care [99]. In the 15 patients who completed the study, cyst volume and total kidney volume (TKV) increased less and renal parenchymal volume increased more in the sirolimus-treated group (average drug trough levels 7.7 ± 2.2 ng/mL). No effect of sirolimus could be detected on GFR decline, while albuminuria increased significantly. Ten out of fifteen patients suffered from aphthous stomatitis, a well-known side effect of sirolimus. In the SWISS ADPKD study, 100 patients were randomized to receive either sirolimus or conventional care [99]. After a treatment period of 18 months, there was no difference in TKV. While eGFR remained stable in sirolimus-treated patients, there was a trend (P = 0.07) to a decline in placebo treated patients. Adverse events were more common in sirolimus-treated patients but infection rates were similar in both groups.

In the third and largest trial (Everolimus in ADPKD) 433 patients were enrolled in a multicentre, double blind, placebo controlled trial and randomly assigned to receive either everolimus or placebo [98]. TKV was reduced in the everolimus group at 1 year and marginally failed significance at 2 years (P = 0.06) while parenchymal volume increased less in sirolimus-treated patients at 1 year but not at 2 years. Functional decline estimated with eGFR was not different between the two groups. As in the other two studies drug-specific adverse events were more common in everolimus-treated patients. While the SIRENA trial and the Everolimus in ADPKD trial report a reduction in TKV in treated patients, no such effect was seen in the SWISS ADPKD study. This difference might be explained by the TKV at the beginning of each study, which was around 2000 mL in the former two and ~1000 mL in the latter. Hence, advanced disease might be necessary to detect volumetric changes. In addition, as reported very recently, other hitherto unknown cystpromoting cues exist which are directly cilia-dependent and might drive cyst growth once mTOR is inhibited [100]. Furthermore, mTOR inhibitors do not only seem to affect renal cysts but also have profound effects on the remaining parenchyma. In fact, in the Everolimus in ADPKD trial, decline in renal function tended to be more pronounced in everolimus- versus placebo-treated patients at 1 year suggesting that mTOR activity supports a hypertrophy of the remaining functional nephrons to sustain GFR. This assumption is corroborated by the SWISS ADPKD trial where sirolimus-treated patients had a stable eGFR while placebo treated patients suffered from a steady functional decline almost reaching significance after 18 months (P = 0.07).

These trials underline common difficulties in translational research: (i) Preclinical models often only mirror human

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**FIGURE 3:** Role of mTOR for tubular epithelial function and cystic kidney disease. (A) Close up of a cell from the collecting duct: putative mTORC2-dependent regulation of sodium and potassium balance. (B) Possible mechanisms of activation of the mTORC1 pathway in cystic cells. mTORC1 is activated either due to loss of PC1 inhibition on MEK1/2 or due to loss of flow-dependent ciliary activation of LKB1.
disease to a certain degree and even robust positive treatment effects in rodent models have to be extrapolated to humans with caution. For example cyst growth occurs within weeks to months in rodents but needs decades in humans. Hence the proportion and sequence of proliferative versus secretory pathways will be dramatically shifted. In addition, all rodent modeling leading to PKD are caused by recessive mutations while ADPKD is an autosomal dominant disease. (ii) mTOR seems to act in a cell type-specific and context-dependent manner. This is exemplified with everolimus’ positive effects on delaying renal angiomyolipoma growth in TSC patients, while ADPKD patients so far did not show detectable functional benefits.

CONCLUSION

mTOR complexes have been recognized to regulate an increasing variety of renal epithelial processes ranging from podocyte size control within the glomerular tuft, over regenerative capacity of proximal tubular cells during acute kidney injury to the fine tuning of water and salt transport in the collecting duct. Strikingly, in many kidney diseases, mTOR activity is altered turning its physiological functions into disease-driving mechanisms. Therefore, further insights into the upstream regulation of mTOR as well as the identification of kidney-specific downstream targets of mTOR might allow the development of precise and targeted therapies to maintain and safeguard renal epithelial function for the most common acquired and hereditary kidney diseases.

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CONFLICT OF INTEREST STATEMENT

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REFERENCES

2. Smith HW. From Fish to Philosopher. Boston: Little, Brown, 1953
14. Peterson TR, Sengupta SS, Harris TE et al. mTOR complex 1 regulates lipin 1 localization to control the SREBP pathway. Cell 2011; 146: 408–420


88. Shillingford JM, Murcia NS, Larson CH et al. The mTOR pathway is regulated by polycystin-1, and its inhibition reverses renal cystogenesis in polycystic kidney disease. Proc Natl Acad Sci USA 2006; 103: 5466–5471
89. Wahl PR, Serra AL, Le Hir M et al. Inhibition of mTOR with sirolimus slows disease progression in Han:SPRD rats with autosomal dominant polycystic kidney disease (ADPKD). Nephrol Dial Transplant 2006; 21: 598–604

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