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The role and mechanism of TSC in kidney diseases: a literature review

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Abstract

Background Tuberous sclerosis complex (TSC) is an autosomal dominant genetic disorder characterized by multisystem involvement, primarily caused by loss-of-function mutations in the *TSC1* or *TSC2* genes. TSC is a key integrator of metabolic signaling and cellular stress and has become an important regulator in several kidney diseases.

Summary *TSC1* and *TSC2* can be used not only as genetic markers for disease diagnosis, but also as potential immunotherapeutic targets for kidney disease. Recent studies on the pathogenesis of TSC may provide guidance for developing new treatment strategies for kidney diseases.

Key messages Therefore, we systematically reviewed the molecular biology of TSC and their signaling pathway, regulation of cell metabolism, and immune response in acute renal injury, chronic kidney disease, diabetic kidney disease, renal cysts, benign and malignant intrarenal tumors, and renal angiomyolipomas. We also summarize the efficacy and adverse effects of mTOR inhibitors in the treatment of TSC-related kidney diseases.

Keywords Tuberous sclerosis complex, Kidney diseases, mTOR inhibitors, Immunotherapeutic

Introduction

The kidney plays a central role in metabolic, excretory, and endocrine functions to keep the fluid balance [1]. Chronic kidney disease (CKD) and kidney failure are substantial contributors to the increasing global health burden [2]. The progression of most kidney diseases are determined by immune mechanisms, one of the immediate causes is a persistent and excessive inflammatory response, which led to the tissue damage [3]. Inflammation caused by immune cells plays an important role in the occurrence and development of kidney diseases [4].

Tuberous sclerosis is an autosomal dominant disease caused by loss-of-function mutations in one of two genes, *TSC1* or *TSC2*, in which multiple organs are involved [5, 6]. Tuberous sclerosis complex (TSC) is a key integrator of metabolic signals and cellular stress. Diseases caused by TSC mutations included extensive tumors of the

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brain, heart, kidneys, lungs, skin, retina, and neurological manifestations [7, 8]. However, renal manifestations, which are among the most prevalent pathogenic factors, have previously been identified as a leading cause of mortality in adult patients with TSC [9, 10]. Therefore, we systematically describe the role and research progress of TSC genes and its pathway in acute and chronic kidney injury, kidney tumors, and other related kidney diseases in this review (Fig. 1).

Molecular biology of TSC

TSC1 and *TSC2* are pathogenic genes of TSC, while *TSC1* is located on subsegment 34 of the long arm of chromosome 9 and encodes 8.6 kb of RNA, which is 55 kb in DNA and contains 23 exons. *TSC2* is located at subposition 13.3 on the short arm of chromosome 16 and encodes 5.5 kb of RNA, which is a 40 kb DNA and contains 41 exons [11]. The *TSC1* gene encodes harmatin and *TSC2* encodes tuberin [6, 12].

The *TSC1* protein has a molecular weight of 130 KDa and is composed of 1164 amino acids, which exhibits a characteristic distribution in the cytoplasm and co-localizes with p75NTR-associated cell death executor (NADE) [13]. In addition, hamartin contains a putative transmembrane domain at amino acids 127–144 and a coiled helix domain spanning amino acids 719–998 [14], and the amino acids 145–510 residues is the key to activating Rho GTPase [15].

The *TSC2* protein has a molecular weight of 198 KDa and is composed of 1784 amino acids, with substantial

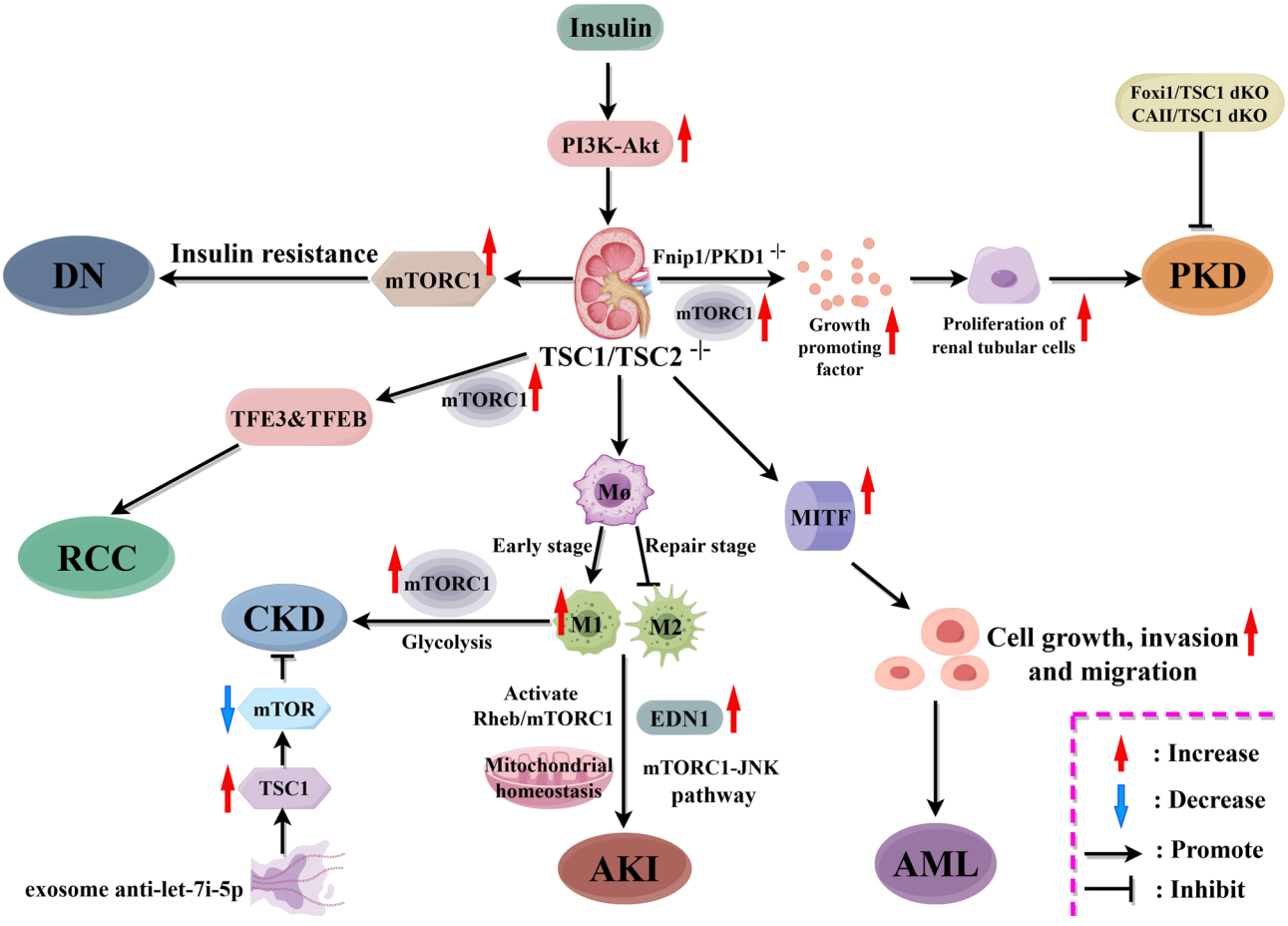

Fig. 1 Patterns of *TSC1/2* role in kidney disease (by Figdraw). The deletion of *TSC1/2* in the kidney results in a range of renal disease responses. *TSC1/2* deletion results in the activation of the mTORC1 signaling pathway, which subsequently induces insulin resistance, increases the secretion of growth-promoting factors, and enhances the activity of *TFE3* and *TFEB*. Concurrently, increased *MITF* expression promotes cell growth, invasion, and migration, consequently accelerating the progression of DKD, PKD, RCC, and RAML. In the context of AKI and CKD, the early stages of IRI are characterized by *TSC1* deletion-induced macrophage polarization toward the M1 phenotype, which in turn leads to increased renal dysfunction. Conversely, in the repair stage of IRI, *TSC1* deletion reduced macrophage polarization toward M2, thereby attenuating renal fibrosis. Additionally, the mTORC1-JNK signaling pathway, mitochondrial homeostasis, and glycolysis play a role in the occurrence and development of AKI and CKD. DKD: Diabetic kidney disease; PKD: Polycystic kidney disease; RCC: Renal cell carcinoma; RAML: Renal angiomyolipomas; AKI: Acute kidney injury; CKD: Chronic kidney disease; *MITF*: Microphthalmia transcription factor; *TSC1/TSC2*-|-: *TSC1/TSC2* deletion; *Fnip1/PKD1*-|-; *Fnip1/PKD1* deletion; *Foxi1/TSC1* dKO: *Foxi1/TSC1* double knockout; *CAII/TSC1* dKO: *CAII/TSC1* double knockout

homology to the Rap1-GAP protein. The small G protein family associated with Ras can be inhibited by tuberin [16]. In addition, a calmodulin binding site has been found at the carboxyl terminus of tuberin, which plays a key role in Ca^{2+}-dependent signal transduction pathways and participates in various intracellular signal transduction pathways, and regulates cell growth and metabolism by modulating the inhibitory effect of *TSC2* on mTORC1 [17, 18].

Both TSC genes are tumor suppressors, and the TSC complex plays an important role in negatively regulating mammalian target of rapamycin (mTOR)-dependent proteins, thereby inhibiting tumor cell growth by prolonging or stagnating the G1 phase [19].

TSC signaling pathways and their immune response

Upstream molecules that affect TSC

The TSC complex is located in the cytoplasm, and many signaling pathways affect the TSC complex, which in turn affect cell metabolism, growth, and death. The PI3K-Akt and mTOR pathways are important pathways that affect protein synthesis and cell growth, and the TSC complex is an important molecule that connects these two pathways. Cellular regulation involves the activation of PI3K by growth factors. This activation leads to the generation of phosphatidylinositol (3,4)-bisphosphate (PIP3), a molecule that engages Akt and *PDK1*, and facilitates the phosphorylation and subsequent activation of Akt by *PDK1*. Akt exerts its inhibitory effect on the TSC complex, achieved through the multi-faceted phosphorylation of the *TSC2* subunit, thereby modulating cellular pathways (Fig. 2) [20, 21]. Moreover, during autophagy, adenosine 5′-monophosphate-activated protein kinase (AMPK) and mTOR bind to unc–51-like kinase 1 (*ULK1*) and regulate the production of phosphatin inositol 3-phosphate (PI3P), which is mediated by WD repeat domain phosphoinositide-interacting protein (WIPI) protein to autophagosome formation (Fig. 2). In addition, WIPI3 binds to AMPK-activated TSC complex on lysosomes, regulating mTOR [22]. CXXC-type zinc finger protein 5 (CXXC5) protein is classified as part of the CXXC family, characterized by their zinc finger motifs, which are integral to their function in protein-DNA interactions. *TSC1* can be transcriptionally inhibited by the CXXC5-CRL4B-NURD complex, thus promoting tumor cells [23].

TSC1/mTOR signaling pathway

The TSC complex integrates signals from multiple pathways, such as the reception of insulin-Akt signaling that activates *TSC1* in TGFβ-Smad2/3 signaling [24]. The TSC complex is a recognized upstream inhibitor of mTOR, which is achieved by inhibiting ras homolog enriched in brain(*Rheb*) (Fig. 2) [25, 26]. *Rheb* is a critical component in the mTOR signaling pathway, serves as a downstream target for the GTPase activation domain of *TSC2*. Upon binding to GTP, *Rheb* triggers the activation of Mechanistic Target of Rapamycin Complex 1 (mTORC1), which subsequently initiates a series of phosphorylation reactions. This results in the activation of 70 kDa ribosomal protein S6 kinase (p70S6K) and the phosphorylation of both ribosomal protein S6 and eIF4E-binding protein 1 (4EBP1), which affects protein synthesis (Fig. 2) [5]. Subsequently, the mTOR pathway, which is responsive to amino acid levels, facilitates the activation of S6K, a pivotal event for initiating translation and propelling cellular proliferation (Fig. 2) [27].

Other *TSC1* signaling pathway

In addition, under adequate nutritional conditions, mTOR promotes autophagy inhibition via phosphorylation and inactivation of the autophagy initiator, ULK1 [28]. *TSC1* exerts an inhibitory effect on M1 polarization by impeding the Ras-Raf1-MEK-ERK signaling cascade. Conversely, *TSC1* fosters M2 characteristics via the mTOR-dependent activation of the CCAAT/enhancer binding protein-β (C/EBP-β) pathway [29]. In a murine model of hepatic ischemia/reperfusion injury, loss of myeloid-specific *TSC1* inhibits AKT and MST1 phosphorylation and reduces the accumulation of NRF2, while activating the TLR4/NF-κB pathway, leading to increased liver inflammation [30]. *TSC1*-deficient osteoclasts released high levels of interleukin–34 (IL–34), which could effectively induce acute myeloid leukemia cell differentiation and prevent its progression [31].

Role of TSC in kidney-related diseases

Acute kidney injury

Acute kidney injury (AKI) is frequently precipitated by a steep decline in renal function over a short period, which results in the accumulation of metabolic waste products. This phenomenon is associated with a markedly increased risk of morbidity and mortality [32].

Renal fibroblasts have been demonstrated to exert a substantial influence on the survival of tubular cells and prognosis of AKI. However, the precise mechanism by which this occurs remains unclear. Gui et al. demonstrated that fibroblasts in a mouse renal ischemia-reperfusion injury (IRI) model exhibited a substantial increase in the abundance of p-Akt and p-S6 6 and 24 h after IRI, as determined by western blotting. This suggests that after IRI in mouse kidneys, the activation of mTORC1 and mTORC2 signaling occurs (Fig. 3) [33]. Loss of *TSC1* signaling or activation of mTORC1 signaling may help protect renal tubular cells from acute injury, and the mTORC1 pathway is expected to be a potential target for the treatment of acute kidney injury (AKI).

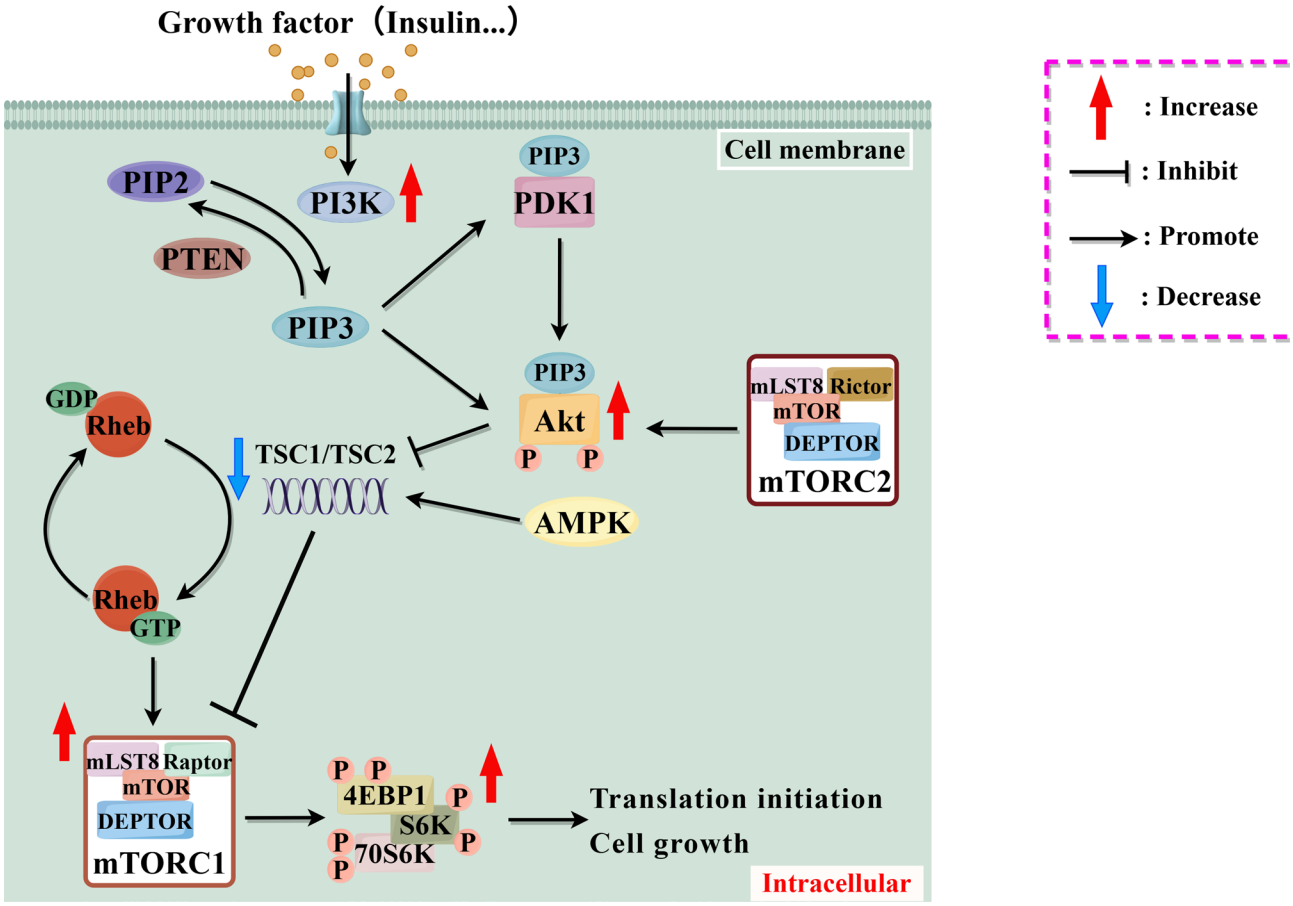

Fig. 2 Schematic diagram of the *TSC1/2* signaling pathway (by Figdraw). TSC genes is localized in the cytoplasm and serves as a critical molecular hub that integrates the PI3K-Akt and mTOR signaling pathways. During cellular regulation, growth factors, such as insulin, activate PI3K, PI3K then phosphorylates PIP2 to PIP3. PIP3 phosphorylates and activates Akt through the intermediary action of the kinase *PDK1*. PI3K activity is negatively modulated by *PTEN*, a phosphatase that dephosphorylates PIP3 to PIP2. Akt regulates the function of TSC by phosphorylating multiple sites on the *TSC2* subunit, thereby inhibiting its suppressive effect on mTORC1. Additionally, TSC negatively regulates mTORC1 by inhibiting *Rheb*, an activator of mTORC1. AMPK activates the TSC, thereby enhancing its inhibitory effect on mTOR. Upon binding to GTP, *Rheb* triggers mTORC1 activation, which initiates a series of phosphorylation reactions. This leads to p70S6K activation and 4EBP1 phosphorylation, promoting protein synthesis and cell proliferation. PI3K: Phosphoinositide 3-kinase; PIP2: Phosphatidylinositol (4,5)-bisphosphate; PIP3: Phosphatidylinositol (3,4)-bisphosphate; *PTEN*: Phosphatase and tensin homolog; *PDK1*: Pyruvate dehydrogenase kinase 1; Akt: Protein kinase B; *Rheb*: Ras homolog enriched in brain; GDP: Guanosine diphosphate; GTP: Guanosine triphosphate; AMPK: AMP-activated Protein Kinase; *TSC1/TSC2*: Tuberous sclerosis complex 1/2; mTORC1/2: Mechanistic target of rapamycin complex 1/2; 4EBP1: Eukaryotic initiation factor 4E-binding protein 1; S6K: Ribosomal S6 kinase; 70S6K: 70 kDa ribosomal S6 kinase; P: Phosphorylation

TSC1 is a GTPase-activating protein of *Rheb*, that inhibits the activation of *Rheb* and mTORC1 signaling (Fig. 2) [34]. This was corroborated by Gui et al., who discovered that ablation of *TSC1* resulted in the activation of *Rheb*/mTORC1 signaling in fibroblasts. This activation exerted a protective effect against AKI and tubular epithelial cell death in mouse kidneys subjected to IRI (Fig. 3) [33]. As a target in the rapamycin signaling pathway, hamartin, the gene product of *TSC1*, has been demonstrated to be involved in hypoxia and hypoxic neuroprotection. The mTOR signaling pathway is activated in fibroblasts from the kidneys of mice with IRI, and ablation of fibroblast *TSC1* prevents renal tubule cell death and IRI in mice [33]. Subsequently, Lu et al. further investigated the protective mechanism of *Rheb* against AKI. In a mouse model of cisplatin-induced AKI, the deletion of *Rheb1* signaling in renal tubular cells resulted in tubular cell death, mitochondrial defects, and worsening of AKI. This suggests that *Rheb1* in renal tubules exerts its protective effects against AKI and renal tubular cells by maintaining mitochondrial homeostasis (Fig. 3) [35]. The activation of the mTORC1 in fibroblasts following the fibroblast-specific knockdown of the *TSC1* has been observed to enhance renal function in an LPS-induced mouse model of AKI. These findings suggest that mTORC1 signaling in renal fibroblasts plays a role in infectious AKI. Shen et al. demonstrated that mTORC1-JNK-dependent ECE1 upregulation improved renal function in LPS-induced fibroblast *TSC1*/AKI mice. This mechanism involves the upregulation of *EDN1* in renal

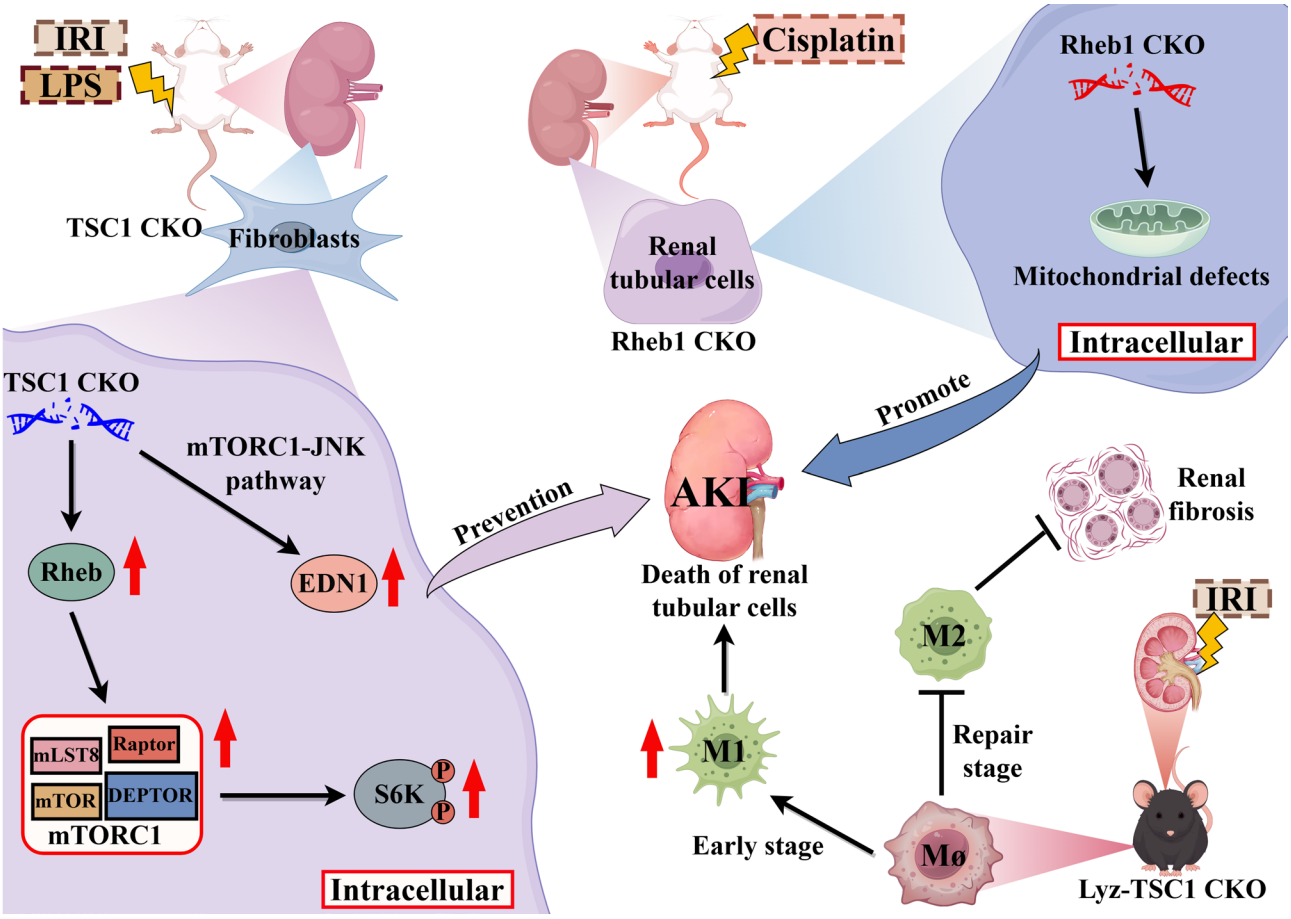

Fig. 3 Schematic illustration of the molecular interactions and signaling pathways involved in the association between TSC and acute kidney injury development (by Figdraw). In mouse kidneys undergoing IRI or LPS-induced injury, deletion of *TSC1* in fibroblasts caused activation of *Rheb*, which in turn activated the mTORC1 signaling pathway, causing a significant increase in the downstream abundance of p-S6, and consequently protecting renal tubular cells from acute injury. Meanwhile, *EDN1* was upregulated in renal fibroblasts after *TSC1* knockdown and was dependent on the mTORC1-JNK pathway, which improved renal function in LPS-induced AKI mice. In a cisplatin-induced mouse model of AKI, deletion of *Rheb1* signaling in renal tubular cells resulted in tubular cell death, mitochondrial defects, and worsening of AKI. In the Lyz-*TSC1* CKO mouse model, deletion of *TSC1* promoted macrophage polarization toward M1 early in the disease, leading to increased renal impairment after IRI. In contrast, during the repair phase of IRI, *TSC1* deficiency led to a reduction in macrophage polarization toward the M2 type, which attenuated renal fibrosis. IRI: Ischemia-reperfusion injury; LPS: Lipopolysaccharides; *TSC1*: Tuberous sclerosis complex 1; *Rheb*: Ras homolog enriched in brain; p-S6: Phospho-S6; mTORC1: Mechanistic target of rapamycin complex 1; *EDN1*: Endothelin−1; mTORC1-JNK: Mechanistic Target of Rapamycin Complex 1-c-Jun N-terminal kinase; AKI: Acute kidney injury; Lyz-*TSC1* CKO: Myeloid cell-specific knockout of *TSC1*; *Rheb1*: Ras homolog enriched in brain 1

fibroblasts after *TSC1* knockdown and is dependent on the mTORC1-JNK pathway (Fig. 3) [36]. Overall, *TSC1* deletion led to the activation of *Rheb*/mTORC1 signaling in fibroblasts, which had a protective effect against AKI and tubular epithelial cell death.

Renal IRI is usually accompanied by tubular cell death due to necrosis and apoptosis, and the immune system plays a crucial role in promoting the process of renal IRI [37]. Following renal IRI, there is an increase in the recruitment of monocytes and neutrophils, which results in the development of AKI [38]. The level of *TSC1* expression in macrophages has been demonstrated to exert a substantial influence on the development of IRI. In the Lyz-*TSC1* CKO (myeloid cell-specific knockout of *TSC1*) mouse model, deletion of *TSC1* has been observed to promote macrophage polarization toward M1. In the early stages of the disease, *TSC1* deletion results in increased renal dysfunction after IRI. Conversely, in the reparative stage of IRI, *TSC1* deficiency leads to a decrease in macrophage polarization toward M2, thereby attenuating renal fibrosis. Thus, *TSC1* influences renal IRI processes by regulating macrophage polarization (Fig. 3) [39]. By regulating the polarization of macrophages, especially the action of *TSC1*, the immune response and repair process of the kidney can be improved. Future therapeutic strategies may improve the immune environment of renal IRI, reduce renal fibrosis and promote renal function recovery by regulating *TSC1*.

Rapamycin has a good preventive effect on cisplatin-induced AKI. In a study where

rapamycin perfluorocarbon nanoparticles (PFC NP) were pre-injected into mice to prevent cisplatin-induced AKI, the renal structure and function of mice were retained 48 h after cisplatin-induced injury. Similarly, the weight loss of mice also slowed down. Among them, rapamycin plays a preventive role in AKI by up-regulating autophagy and reducing the expression of apoptotic protein Bax to inhibit apoptosis [40]. During the treatment of AKI, rapamycin can also play a role in alleviating the symptoms of AKI. It has been confirmed that rapamycin can improve renal injury induced by ioxanol in diabetic rats, up-regulate autophagy and down-regulate the apoptotic protein Bax through the mTOR/p70S6K signaling pathway. Meanwhile, it upregulates the anti-apoptotic protein BCl-2 and inhibits apoptosis [41]. Rapamycin not only alleviates the severity of AKI by regulating autophagy and apoptosis, but also inhibits the inflammatory response during AKI by reducing the proportion of immune cells. When rapamycin is used to treat AKI caused by ischemia-reperfusion in Balb/c mice, the proportion of NKT cells in the spleen is significantly decreased, and the proportion of NKT cells in the peripheral blood and kidneys is significantly increased. In addition, the CXCR3+ NKT cells in the kidneys of the rapamycin treatment group increased significantly. Chemokines CXCL9 and CXCL10, as ligands of CXCR3, also increased in the kidneys treated with rapamycin [42]. Coincidentally, the mitochondrial metabolism in dendritic cells treated with rapamycin was reprogrammed, and a tolerance phenotype that inhibits inflammation was produced, thereby alleviating AKI [43]. Overall, mTOR inhibitor achieves therapeutic effects on AKI by regulating autophagy and apoptosis of renal cells and altering the function of lymphocytes.

Renal fibrosis and CKD

Approximately 700 million people worldwide are affected by CKD, a health issue that has garnered global attention. CKD often leads to a series of severe complications, including cardiovascular disease, end-stage renal failure, metabolic disorders, anemia, and premature mortality [44–46]. Renal fibrosis, defined as the accumulation of scarring within the renal parenchyma and represents the common final pathway of most chronic and progressive kidney diseases, ultimately leading to end-stage renal disease [47, 48].

mTOR pathway is one of the most important regulatory pathways involved in renal fibrosis. In a study, overexpression of *TRIM6* leads to higher ubiquitination levels of *TSC1* and *TSC2*, which promotes proteasome-dependent degradation of both proteins and leads to activation of the mTORC1 pathway, thereby exacerbating renal fibrosis (Fig. 4) [49]. In addition, other study reports that *TSC1*-associated mTORC1 signaling mediates the progression of renal interstitial fibrosis by regulating the glycolysis of proximal renal tubular epithelial (RTE) cells (Fig. 4) [50]. Notably, phosphorylated ribosomal protein S6 (rpS6) inhibits fibrosis and cystogenesis in *TSC1*-deleted kidneys [51]. The activation of the *TSC1*/mTOR signaling pathway by mesenchymal stem cell (MSC)-derived exosomal anti-let-7i-5p was demonstrated through an in-vitro TGF-β-induced renal fibrosis model, which resulted in the attenuation of renal fibrosis. Furthermore, *TSC1* was identified as a binding target for anti-let-7i-5p exosomes in unilateral ureteral obstruction (UUO) -induced renal fibrosis in vivo (Fig. 4) [52]. *TSC1* significantly affects the progression of renal fibrosis by regulating the mTORC1 pathway, and the intervention of this pathway may become a new strategy for the treatment of renal fibrosis. Macrophage infiltration is a common occurrence in cases of renal fibrosis. In a previous study, Hu et al. demonstrated that Lyz-*TSC1* CKO mice exhibited reduced levels of renal fibrosis and lower levels of M2 markers during the repair phase of IRI (Fig. 4) [39]. This implies that *TSC1* may be involved in the immune cell-mediated fibrosis process. By regulating the function of immune cells, especially the role of *TSC1*, it may help to slow or reverse renal fibrosis.

Patients with TSC develop CKD earlier and more frequently than the general population [53]. In addition, studies have reported a strong correlation between angiomyolipomas (AML) size and CKD [53, 54]. When a large lesion involves the renal parenchyma it often leads to a decline in renal function or the patient develops a renal AML bleeding and subsequent interventions (embolization, nephrectomy, etc.) [53, 55]. Everolimus is an mTOR-I inhibitor that has been developed into a commercially available immunosuppressant. Extensive clinical and experimental evidence demonstrates that mTOR-I exhibits beneficial therapeutic effects on a wide range of diseases [56, 57]. Kronick et al. reported on 2 patients with concomitant glomerular filtration rate (GFR) decline in the absence of bulky AML and the presence of a TSC nephrotic variant phenotype observed under microscopy. After treatment with everolimus, the 1st patient achieved an improvement in renal function, from stage G4 to stage G3b, which remained stable for 5 years after initiation of everolimus therapy [58]. This suggests that future studies should focus on the pathophysiologic features of CKD in patients with TSC, the prevalence of micronephropathy in this patient population, and the therapeutic effects of mTOR inhibitors beyond the current indications in an expanded patient population.

Diabetic kidney disease

Diabetic kidney disease (DKD), a substantial contributor to end-stage renal disease, is a prevalent concern in both developing and developed countries [59]. The incidence

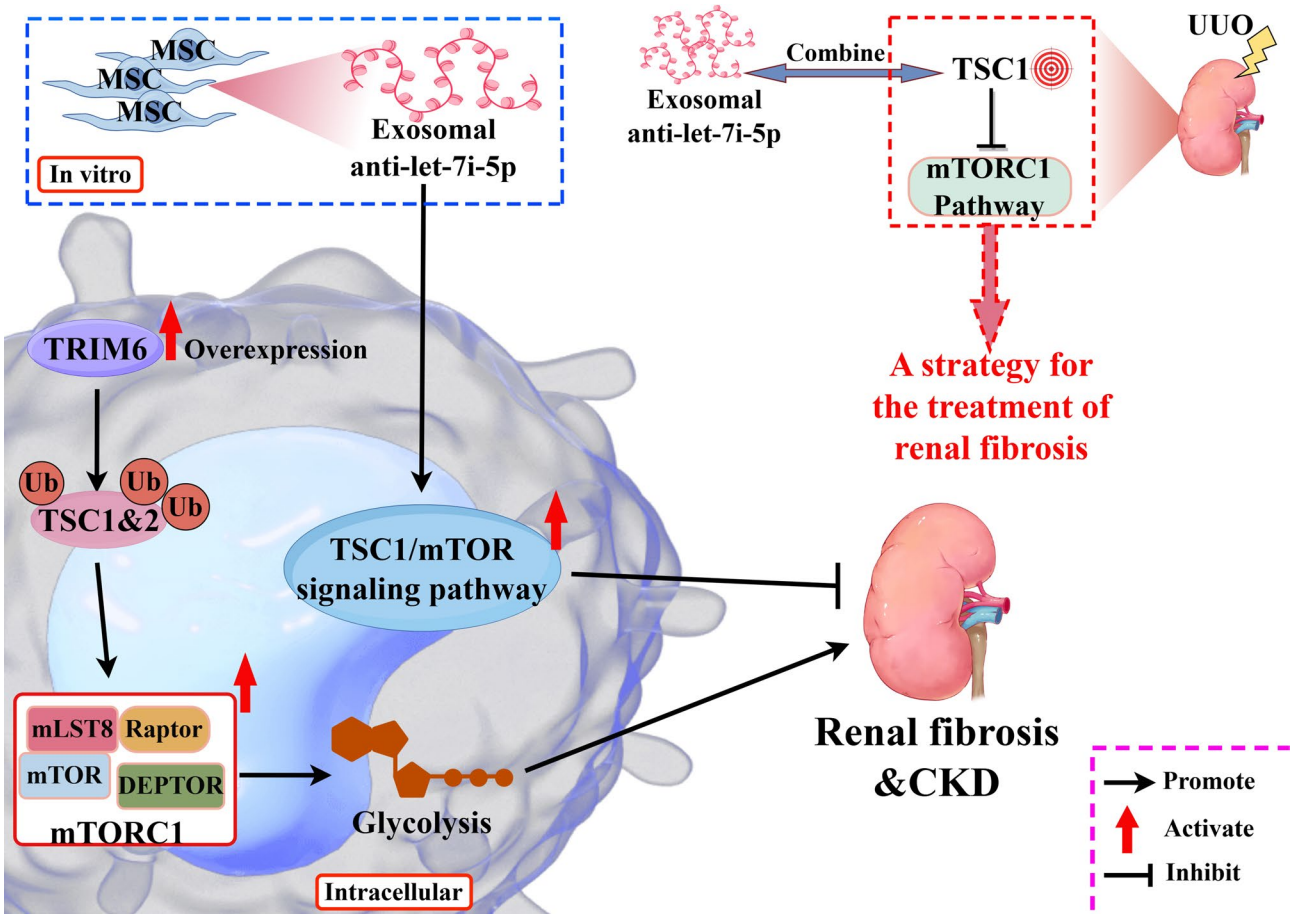

Fig. 4 Schematic illustration of the molecular interactions and signaling pathways involved in the association between TSC and renal fibrosis and CKD development (by Figdraw). Overexpression of *TRIM6* leads to increased ubiquitination levels of *TSC1* and *TSC2*, thereby promoting activation of the mTORC1 pathway and exacerbating renal fibrosis. Additionally, *TSC1*-related mTORC1 signaling mediates the progression of renal interstitial fibrosis by regulating glycolysis in RTE cells. Notably, in an in vitro model of TGF-β-induced renal fibrosis, exosomes derived from mesenchymal stem cells (MSC) carrying anti-let−7i−5p activate the *TSC1*/mTOR signaling pathway, thereby alleviating renal fibrosis. Furthermore, in a UUO-induced renal fibrosis model, *TSC1* serves as the binding target for the anti-let−7i−5p exosomes. *TSC1* significantly impacts the progression of renal fibrosis by regulating the mTORC1 pathway, and targeting this pathway may offer a novel therapeutic strategy for renal fibrosis. *TRIM6*: Tripartite Motif Containing 6; *TSC1/TSC2*: Tuberous sclerosis complex 1/2; mTORC1: Mechanistic target of rapamycin complex 1; RET: Proximal tubular epithelial; TGF-β: Transforming Growth Factor β; UUO: unilateral ureteral obstruction; CKD: Chronic kidney disease; *TSC1&2* Ub: *TSC1&2* Ubiquitination

of microvascular complications caused by DKD is high, which substantially increases the mortality of patients [60]. DKD is the leading cause of kidney disease in patients starting kidney replacement therapy, affecting approximately 40% of people with type 1 and 2 diabetes, and it increases the risk of death [61].

TSC is a key suppressor of mTORC1, and abnormal activation of mTORC1 in glomerular podocytes leads to glomerular dysfunction and may contribute to the development of glomerular diseases, including DKD (Fig. 5) [62]. Constitutive activation of the mTORC1 pathway has been identified as one of the key mechanisms of insulin resistance. *TSC1–2* is essential for maintaining insulin-mediated PI3K signaling. In cell culture systems, when *TSC1–2* function is impaired, insulin-induced PI3K-Akt signaling is attenuated in tumor cells, leading to a tendency toward low malignancy potential. This may be due to the inability of TSC-mutant cells to activate PI3K and its downstream effector proteins. This suppression can be reversed by treatment with rapamycin (Fig. 5) [63, 64].

Mutations in TSC and the activation of mTOR have been implicated in the pathogenesis of multiple kidney diseases, such as DKD (Fig. 5) [65–67]. Diabetes may activate the TSC-mTOR signaling by inactivating AMPK, thereby inhibiting the ULK kinase complex and preventing autophagy initiation. Autophagy is induced by reducing the expression of mTOR and stimulating the expression of *TSC1* and LC3 (Fig. 5) [68]. Nevertheless, the effects of glucose transporter 1 (GLUT1) on mTOR activity persist in cells lacking functional *TSC2*, as demonstrated by Buller et al., indicating that the effects of

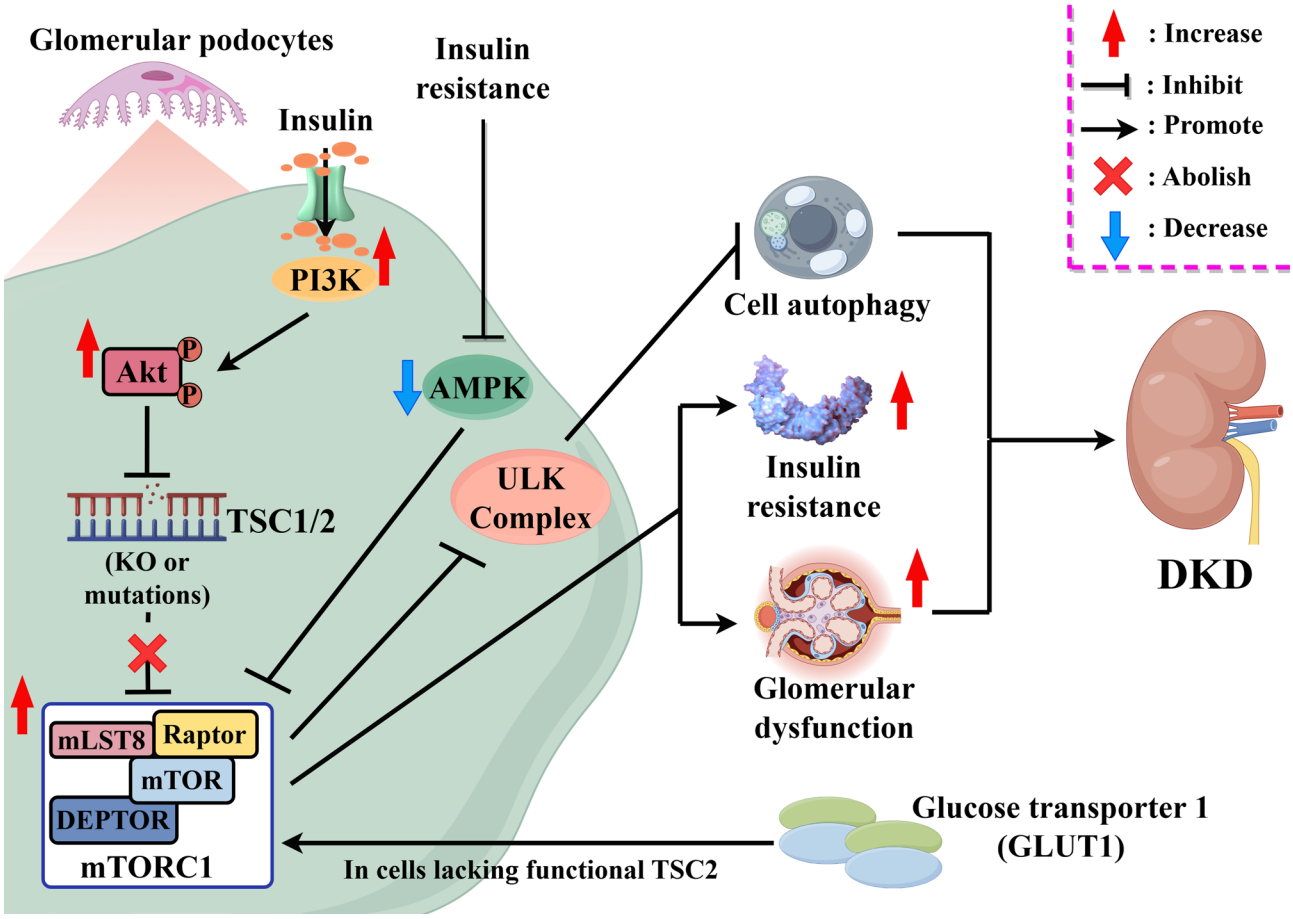

Fig. 5 Schematic illustration of the molecular interactions and signaling pathways involved in the association between TSC and diabetic kidney disease development (by Figdraw). TSC is a key inhibitor of mTORC1. Abnormal activation of mTORC1 in glomerular podocytes leads to glomerular dysfunction, as well as induces insulin resistance and may promote the progression of diabetic kidney disease. In vitro, insulin-induced activation of PI3K-Akt signaling inhibits *TSC1* or *TSC2* signaling, which promotes activation of the mTORC1 pathway. Furthermore, in cells with knocked-out *TSC1* or *TSC2*, the mTORC1 pathway was significantly activated, leading to the progression of diabetic kidney disease. When the AMPK pathway is inactivated, it activates the TSC-mTOR signaling pathway, which inhibits the ULK kinase complex and prevents autophagy initiation, thereby exacerbating diabetic kidney disease. TSC: Tuberous sclerosis complex; mTORC1: Mechanistic target of rapamycin complex 1; PI3K-Akt: Phosphoinositide 3-kinase-Protein kinase B; *TSC1/TSC2*: Tuberous sclerosis complex 1/2; AMPK: AMP-activated Protein Kinase; ULK: Unc–51 Like Autophagy Activating Kinase; DKD: Diabetic kidney disease

GLUT1 are not mediated by TSC [69]. TSC serves as a crucial inhibitor of mTORC1, and the aberrant activation of mTORC1 in glomerular podocytes may result in glomerular dysfunction. Simultaneously, the upregulation of mTORC1 inhibits ULK kinase complexes and obstructs the initiation of autophagy, thereby exacerbating diabetic kidney disease.

Diabetes can induce dysregulation of renal autophagy, thereby leading to renal damage. Rapamycin treatment can prevent the early renal structural changes in diabetic experimental rats, thereby preventing the early steps in the development of diabetic kidney disease. mTOR blockade may be beneficial for the treatment of diabetic kidney disease. Rapamycin treatment reduced albuminuria, glomerular enlargement, glomerular basement membrane thickening, renal macrophage recruitment and renal mRNA expression levels of proliferating cell nuclear antigen, transforming growth factor -β1, vascular endothelial growth factor and monocyte chemoattractant protein-1 in experimental diabetic rats [70]. The excessive activation of the mTOR pathway and oxidative stress are considered to play a role in the imbalance of autophagy induced by diabetes [71]. Therefore, as an mTOR inhibitor, rapamycin is a therapeutic drug for diabetic kidney disease with potential clinical value. In diabetic kidney disease, the impairment of podocyte function is an important pathogenesis. A study on the phenotypic transformation and injury of podocytes in diabetes indicated that the activation of mTORC1 in podocytes is a key event inducing DKD, and the reduction of mTORC1 activity in podocytes is a potential therapeutic strategy for preventing DKD [72]. Overall, mTOR inhibitors enhance autophagy by inhibiting the mTOR pathway in

renal cells, especially podocytes, thereby reducing the severity of diabetic kidney disease.

Renal cysts and polycystic kidney disease

Renal cysts and masses are prevalent complications in patients with TSC, and their prevalence substantially increases with age [73]. Notably, renal cysts are more common than angiomyolipomass in children [55, 74]. In patients with tuberous sclerosis, benign tumors may manifest not only in the brain and skin but also in the kidneys, where they can lead to the formation of cysts [75].

The deletion of *TSC1* tends to exert its effect on the kidney through the activation of mTOR signaling and can be partially reversed by rapamycin. However, *TSC1* haploinsufficiency, without the activation of mTOR signaling, also successfully induces cyst formation in the mouse kidney [76]. Therefore, the specific mechanism of *TSC1* gene deletion in the development of renal cysts requires further investigation.

In a study by Fang et al., renal proximal tubule *TSC1*-specific knockout ($TSC1^{pt\ KO}$) mice demonstrated proliferation and hypertrophy of renal proximal tubule cells, accompanied by cystic degeneration. In vivo, treatment with metformin resulted in increased AMPK phosphorylation; activation of the pro-apoptotic factors, BAD and cleaved caspase–3; and blockade of the pro-survival factor Akt. This inhibited *TSC1* deletion-induced aberrant renal proliferation. However, the effect of metformin was reversed by the AMPK inhibitor dosomorphine (Fig. 6) [77]. The role of AMPK signaling in metformin treatment provides new ideas for the treatment of renal cysts induced by *TSC1* deletion. Activation of AMPK inhibits abnormal proliferation and slows renal cyst formation.

Interestingly, a localized cystic environment can also be observed in *Fnip1*-deficient mice, where increased infiltration of immune cells and cell adhesion molecules enhances the tendency of cells to form spherical structures during division. This cellular behavior promotes the development of polycystic kidney disease (PKD). In mouse RTE cells, deletion of *Fnip1* resulted in decreased AMPK activity and increased mTORC1 activity. Notably, the mTORC1 signaling pathway was substantially activated when *Fnip1* and *TSC1* were co-deleted, leading to a substantial acceleration of PKD development compared with deletion of *Fnip1* or *TSC1* alone (Fig. 6) [78]. This study suggests that mTORC1 plays a critical role in the pathogenesis of PKD, and that mitigating its hyperactivation may help slow the progression of the disease. Further investigations are needed to elucidate the interplay between the AMPK pathway and mTOR signaling, as well as to identify other mechanisms through which *TSC1* deletion promotes cyst formation.

In addition to mTORC1's role, *PKD1*, which encodes polycystin 1, also plays a crucial role in renal cystic degeneration. When *PKD1* is ablated in renal interstitial cells, the kidney progresses toward cystic degeneration (Fig. 6) [79]. Furthermore, mutations in *TSC1* and *PKD1* in renal mesangial cells may induce the secretion of growth-promoting factors, thereby promoting tubular cell proliferation and accelerating the development of renal cystic degeneration (Fig. 6). Wu et al. produced a cyst from the loop of Henle after labeling kidney fibroblasts/stromal cells with *Prx1*, a marker of MSC, and ablated the *TSC1* gene in these cells. This suggests that stromal cells play a role in the development of renal cysts in patients with TSC [80]. The role of AMPK signaling in metformin treatment provides new ideas for the treatment of renal cysts caused by *TSC1* loss. By activating AMPK, abnormal cell proliferation can be inhibited, thereby slowing the formation of renal cysts.

Additionally, cortical cysts consisting of hyperproliferating A-intercalated cells were observed in *TSC1*-specific principal cell-deficient mice. In contrast to *PKD1* mutant mice, *TSC1*-knockout (*TSC1*-KO) mice exhibit elevated levels of forkhead transcription factor 1 (*Foxi1*) in the cystic epithelium. Histologic and MRI analyses of kidneys from *TSC1/Foxi1*-double knockout (dKO) mice demonstrated that *Foxi1* deletion resulted in the complete elimination of the cystic load induced by *TSC1*-KO mice [8]. The *Foxi1* gene plays an important role in H^+-ATPase expression and A-intercalated cells activity, regulation, and expression of acid-base transport, including several vacuolar H^+-ATPase (V-H^+-ATPase) subunits (e.g., d2 and b1), carbonic anhydrase 2 (*CAII*), and AE–1 (Slc4a1) [81–83]. Specifically, *Foxi1* and its downstream targets, including apical H^+-ATPase and *CAII*, were strongly expressed in cystic epithelial cells of *TSC1*- (or *TSC2*-) KO mice, but not in *PKD1* mutant mice. The deletion of *CAII* plays a critical role in H^+-ATPase activation, and *CAII*/*TSC1*-dKO mice have a substantially lower cystic load and longer lifespan than *TSC1*-KO mice. This demonstrated the critical role of H^+-ATPase activation in TSC-associated renal cystic changes (Fig. 6) [8, 84]. These findings provide a new perspective to further understand the role of *TSC1* loss, mTORC1 signaling, AMPK pathway, and *PKD1* mutations in renal cysts and polycystic kidney disease, and provide a rationale for future clinical treatment strategies.

The efficacy of mTOR inhibitors in the treatment of TSC-related complications, including seizures, subependymal giant cell astrocytoma (SEGA), renal AML, and facial angiofibroma, has been demonstrated in previous cohort studies [85–88]. Everolimus has been shown to be effective not only in the treatment of renal AML but also in the treatment of renal cystic disease, especially in pediatric patients. In a case report, three pediatric patients

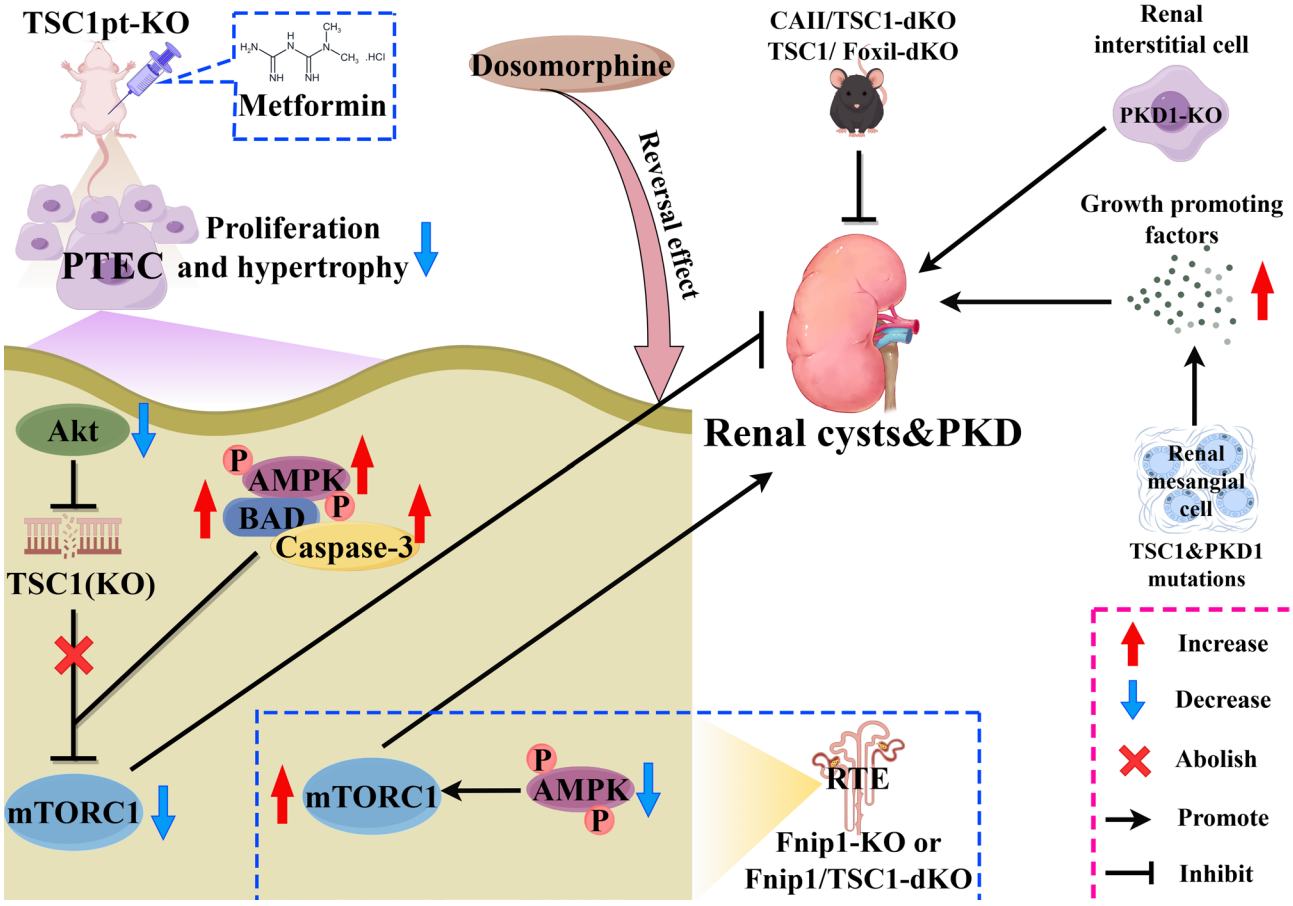

Fig. 6 Schematic illustration of the molecular interactions and signaling pathways involved in the association between TSC and renal cysts and polycystic kidney disease development (by Figdraw). *TSC1*pt-KO mice exhibited proliferation and hypertrophy of renal proximal tubule cells with cystic degeneration. In vivo, metformin treatment resulted in increased AMPK phosphorylation; activation of pro-apoptotic factors BAD and cleaved caspase−3; and concomitant blockade of the pro-survival factor Akt. This inhibited the aberrant renal proliferation induced by *TSC1* deletion, which was reversed by the AMPK inhibitor dosomorphine. In mouse RTE cells, knockdown of *Fnip1* resulted in decreased AMPK activity and increased mTORC1 pathway activity. Notably, when *Fnip1* and *TSC1* were co-deleted, the mTORC1 signaling pathway was massively activated, leading to a significant acceleration in the development of PKD compared to deletion of *Fnip1* or *TSC1* alone. When *PKD1* was knocked down in renal mesangial cells, the kidney was more likely to progress toward cystic degeneration.In addition, mutations in *TSC1* and *PKD1* in glomerular mesangial cells may exacerbate the development of renal cystic degeneration by inducing the secretion of growth-promoting factors, which promotes the proliferation of renal tubular cells. It is noteworthy that *TSC1/Foxil*-dKO and *CAII/TSC1*-dKO mice have a lower cystic load than *TSC1*-KO mice. *TSC1*pt-KO: Renal proximal tubule *TSC1*-specific knockout; AMPK: AMP-activated Protein Kinase; BAD: Bcl−2 Associated Agonist of Cell Death; Akt: Protein kinase B; *TSC1*: Tuberous sclerosis complex 1; RTE: Proximal renal tubular epithelial; *Fnip1*: Folliculin Interacting Protein 1; mTORC1: Mechanistic target of rapamycin complex 1; PKD: polycystic kidney disease; *PKD1*: Polycystic Kidney Disease 1; *Foxil*: Forkhead transcription factor 1; *TSC1/Foxil*-dKO: *TSC1/Foxil* double knockout; *CAII*: Carbonic anhydrase 2; *CAII/TSC1*-dKO: *CAII/TSC1* double knockout; *TSC1*-KO: *TSC1*-knockout

with SEGA control and seizures were included. After treatment with everolimus, renal cysts completely disappeared in patients 1 and 3, while reducing the number of renal cysts in patient 2. Notably, renal function improved in all 3 patients, and eGFR improved while creatinine levels returned to normal levels [89]. This demonstrates the clinical value and application prospect of everolimus in the treatment of patients with TSC combined with renal cystic disease and the maintenance of patients' basic renal function. In addition, in an animal study, rats with polycystic kidney disease treated with everolimus significantly reduced the size of renal cysts compared with the pre-treatment period, while alleviating their declining renal function within 5 weeks [90].

Renal cell carcinoma

The incidence of renal cell carcinoma (RCC) is increasing worldwide, with more than 430,000 new cases and 150,000 deaths by 2022 [91]. Kidney cancer has many different molecular subtypes that are driven by different molecular factors [92].

Recently, low-grade oncocytic tumors (LOTs) of the kidney have been considered potential novel tumors. To determine the molecular signature of LOT, Williamson et al. analyzed 17 tumors that matched the IHC

characteristics and morphology of renal oncocytoma using next-generation sequencing of 324 of the associated cancer genes and found that all tumors had at least one mutation in *TSC1* ($n=7$, 41%), *TSC2* ($n=2$, 12%), *PIK3CA* ($n=4$, 24%), or mTOR ($n=5$, 29%) [93]. An analysis by Ricci et al. based on the eight reviewed studies and an aggregate cohort of 79 LOT samples similarly found that most (57/79 [72.1%]) had at least one of the mutated genes, *TSC1*, *TSC2*, and mTOR,. The persistence of mutations in genes associated with the *TSC1*/*TSC2*/mTOR signaling pathway in LOT of the kidney indicates the potential for considering it a specific genetic tumor. Furthermore, *GATA3* develops a localized or differential expression pattern in chromophobe RCC (chRCC) [94]. Moreover, in a study conducted by Ricci et al., it was demonstrated that *GATA3* maintains consistent reactivity in all LOT samples. These findings support the reliability and validity of *GATA3* as a molecular marker for LOT [93, 95]. Overall, these studies suggest that LOTs may represent a tumor type driven by mutations in the *TSC1*/*TSC2*/mTOR signaling pathway, with *GATA3* potentially serving as a reliable molecular marker for the diagnosis and differentiation of LOTs.

Renal cystadenoma formation has been observed in a mouse model with a heterozygous deletion of *TSC2* and spontaneous inactivation of the second allele. Some of cystadenomas develop into oncocytic high-grade RCCs as the mice age [96]. Therefore, *TSC2* gene deletion is a potential risk factor for renal cystic lesions and RCCs development, especially in older patients, and the development of renal cystadenoma to more aggressive RCCs needs to be vigilant. Asrani et al. demonstrated that the activation of mTORC1, which is triggered by *TSC2* deletion, increases the activity of *TFE3* and *TFEB* (Fig. 7)

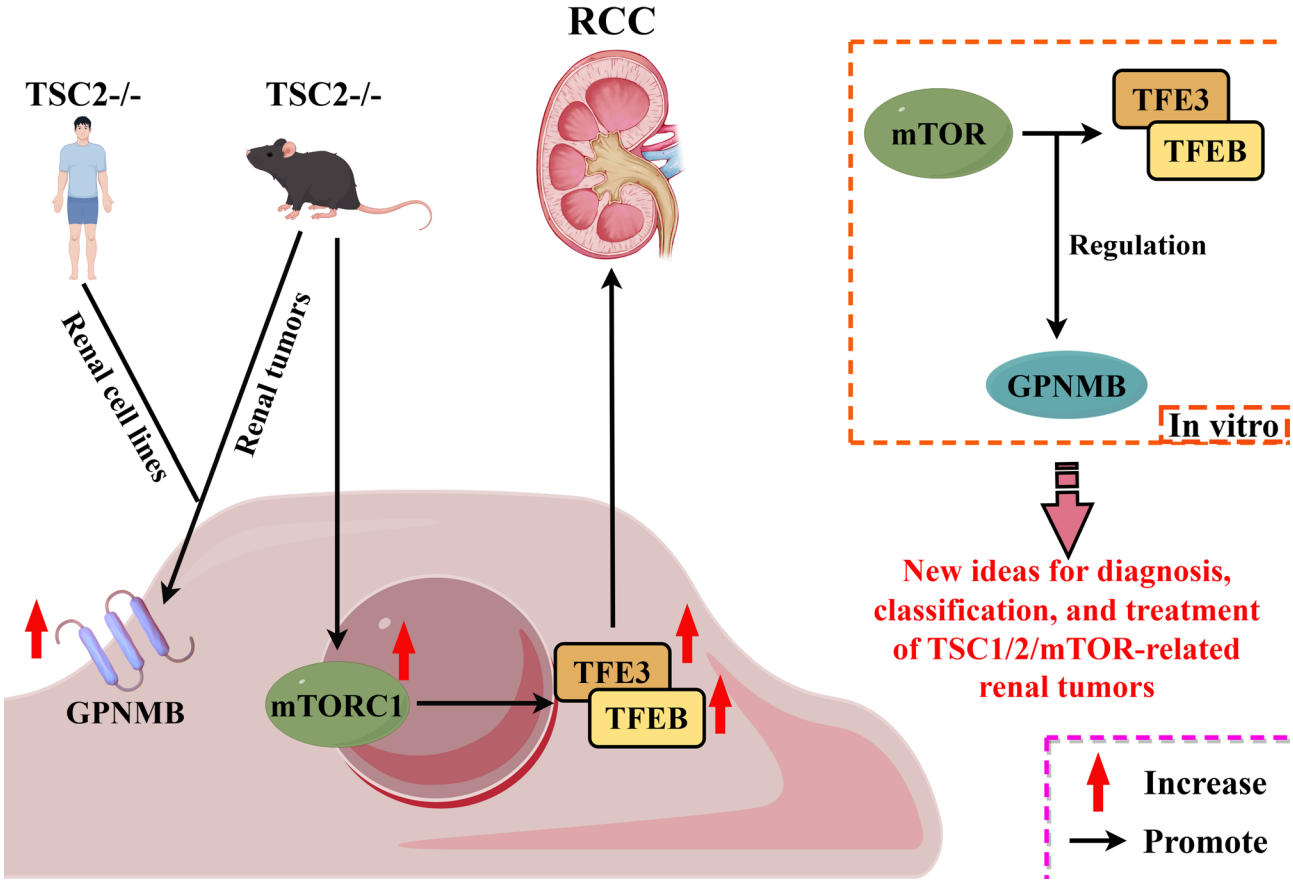

Fig. 7 Schematic illustration of the molecular interactions and signaling pathways involved in the association between TSC and renal cell carcinoma development (by Figdraw). In a mouse model with heterozygous deletion of *TSC2* and spontaneous inactivation of the second allele, some cystadenomas developed into eosinophilic high-grade RCC as the mice aged. *TSC2* gene deletion is a potential risk factor for the development of renal cystic lesions and renal cell carcinomas, especially in elderly patients. Activation of mTORC1 triggered by *TSC2* deletion increases the activity of *TFE3* and *TFEB*. At the molecular level, mTOR and *TFE3/TFEB* may be located in a mutually driven feed-forward signaling loop. In addition, *GPNMB* was highly expressed in *TSC2*-deficient mouse renal tumors and human renal cell lines, and *GPNMB* expression was regulated by mTOR kinase and *TFEB/TFE3* in vitro. These findings provide new ideas for the diagnosis, typing and treatment of *TSC1/2*/mTOR-associated renal tumors. *TSC1/2*: Tuberous sclerosis complex 1/2; RCC: renal cell carcinoma; mTORC1: Mechanistic target of rapamycin complex 1; *TFE3*: Transcription Factor E3; *TFEB*: Transcription Factor EB; *GPNMB*: Glycoprotein nonmetastatic B

[97]. At the molecular level, it is possible that mTOR and *TFE3*/*TFEB* may be located in a mutually driven feed-forward signaling loop. Translocation RCCs (tRCC) have been observed to exhibit elevated levels of mTOR activity compared to clear cell RCC (Fig. 7) [98]. In a study conducted by Salles et al., it was discovered that glycoprotein nonmetastatic B (*GPNMB*) is highly expressed in *TSC2*-deficient mouse renal tumors and human renal cell lines. Furthermore, *GPNMB* expression is regulated in vitro by mTOR kinase and *TFEB*/*TFE3* (Fig. 7). It is evident that the use of *TFE3*/*TFEB* as secondary diagnostic markers for typing these tumors may help avoid phenotypic overlap. Concurrently, *GPNMB* can be employed as a clinical marker to differentiate *TSC1/2*/mTOR alteration-associated renal tumors from tRCC, which can assist in distinguishing these renal tumors from other prevalent conventional subtypes [99]. These findings provide new ideas for the diagnosis, classification and treatment of *TSC1/2*/mTOR related renal tumors. By monitoring mTORC1 signaling activation, *TFE3/TFEB* activity and *GPNMB* expression, different types of renal tumors can be effectively distinguished, especially between TSC-associated renal tumors and tRCC, thus providing powerful support for the individualized treatment.

Patients with TSC tend to produce many malignant and benign tumors and can occur in different tissues and organs. In addition, studies have shown an increased risk of RCC in patients with TSC syndrome [100, 101]. mTOR inhibitors have been approved by the U.S. FDA as first- and second-line agents for the treatment of advanced kidney cancer [102, 103]. However, the efficacy and response time of mTOR inhibitors in patients with TSC-associated renal cancer have not been clearly elucidated. Alsidawi et al. described a case of a patient with *TSC2*-associated metastatic renal cancer harboring the H1620R and Y1650C mutations, who received everolimus which produced an aberrant response to first-line therapy and continued to benefit from an mTOR inhibitor 2 years after receiving treatment [104]. Similarly, in two clinical cases, a female patient with both tuberous sclerosis and recurrent RCC and a male patient with TSC that progressed to chest wall fibromatosis and bilateral multifocal RCC were reported to be treated with everolimus and sirolimus, respectively. It is noteworthy that the female patient's symptoms related to recurrent RCC and tuberous sclerosis improved simultaneously after treatment with everolimus. Meanwhile, the male patient's tumor condition of fibromatosis and RCC also significantly resolved within 6 months of treatment with mild adverse effects [105, 106]. This provides a clinical case and rationale for the use of mammalian target of rapamycin (mTOR) inhibitors, such as everolimus, as a first-line treatment option for TSC-associated RCC.

Although several targeted agents, including inhibitors of the vascular endothelial growth factor pathway and mammalian target of rapamycin protein (mTOR) inhibitors, have been clinically approved for the treatment of metastatic or advanced RCC [107, 108]. However, the use of anti-PD−1 immunotherapies and anti-angiogenic agents for the treatment of patients with TSC-associated renal cancers has been found to produce greater therapeutic efficacy and clinical benefit compared to mammalian target of rapamycin (mTOR) inhibitors in recent clinical case studies. For example, among treated patients with advanced RCC, nivolumab had longer overall survival and fewer grade 3 or 4 adverse events compared to everolimus [109]. This result was confirmed in a randomized, open-label, phase 3 trial in which 658 patients with renal cell carcinoma were randomized into two groups to receive everolimus and cabozantinib. The results showed that cabozantinib better improved progression-free survival in kidney cancer patients (7.4 months vs. 3.8 months). Notably, the objective tumor remission rate was higher in the cabozantinib treatment group than in the everolimus group (21% vs. 5%) [110]. It can be seen from this that anti-PD−1 immunotherapy and anti-angiogenic drugs have demonstrated more powerful therapeutic effects in patients with renal cancer. They are expected to become a more effective alternative therapy, playing an important clinical role in the treatment of patients with renal cancer and improving their prognosis.

Renal angiomyolipomas

Renal angiomyolipomas (RAML) is a benign, but potentially life-threatening tumor. It consists of a mixture of different tumor cells that resemble smooth muscle (myo-), fat (lipo-), and blood vessels (angio-) at the molecular and tissue level [111]. In a single RAML, these different classes of tumor cells are clonal and able to differentiate into different lineages, suggesting a high probability of tumor stem cell origin [112, 113].

RAML is a major risk factor in patients with TSC. In patients with *TSC1* mutations, RAML generally develops at a later age with a relatively small lesion size and growth potential, whereas *TSC2* mutations predispose patients to RAML at an early age and have a higher probability of bleeding and hematuria complications (Fig. 8). The peak incidence of new-onset RAML is between the ages of 18 and 40 years, but lifelong surveillance is necessary because RAML can occur later in life [74]. In TSC, up to 80% of patients develop secondary bilateral kidney and multiple angiomyolipomas, which seriously jeopardizes their physical and mental health [114].

Gonçalves et al. used a reverse tumor engineering approach to study the origin of human and mouse RAML cells. It was found that some cells and cell lines in human RAML have molecular characteristics of proximal tubule

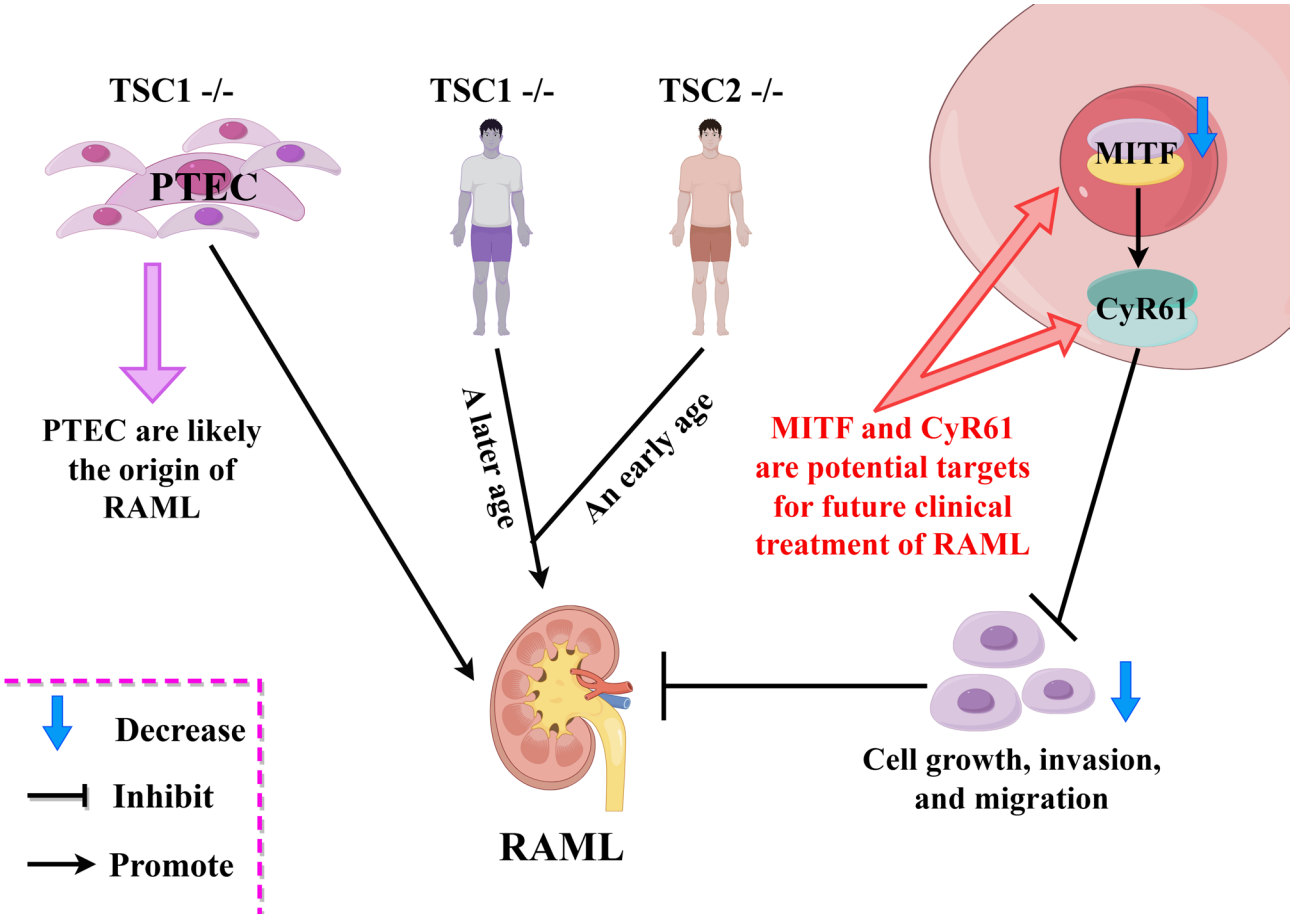

Fig. 8 Schematic illustration of the molecular interactions and signaling pathways involved in the association between TSC and renal angiomyolipomas development (by Figdraw). In patients with *TSC1* mutations, RAML usually occurs at a later age with relatively small lesion size and growth potential, whereas patients with *TSC2* mutations are predisposed to develop RAML at an earlier age and have a higher probability of bleeding and hematuria complications. Studies suggest that human proximal renal tubular epithelial cells may be the origin of RAML. Interestingly, reduced expression of *MITF* negatively regulates RAML development by inhibiting cell growth, invasion, and migration. It can act by regulating *CyR61*, which suggests that *CyR61* is a novel target for *MITF* to regulate RAML progression, and both *MITF* and *CyR61* are expected to be potential targets for future clinical treatment of RAML. *TSC1/2*: Tuberous sclerosis complex 1/2; RAML: Renal angiomyolipomas; *MITF*: Microphthalmia transcription factors; *CyR61*: Cysteine-Rich Angiogenic Protein 61

epithelial cells. This suggests that proximal tubule epithelial cells are likely the origin of RAML (Fig. 8). Furthermore, after renal epithelial *TSC1*-specific deletion (either pure or heterozygous deletion), some of the cells exhibited lesions with detectable RAML markers. This supports the ability of renal epithelial cells to potentially transform into RAML, providing a tumor model of RAML [115].

RAML and lymphangiomas (LAM) are common and predominant clinical manifestations in patients with tuberous sclerosis, and may also occur in patients with non-tuberous sclerosis. Genetic variants of *TSC1* or *TSC2* can be detected in RAML, but the probability of having a *TSC1* mutation is much lower than that of having a *TSC2* mutation [116, 117]. LAM is very rare, and its etiology is currently unknown. LAM can occur independently or in association with TSC. RAML occurs in 70% of patients with tuberous sclerosis and 50% of patients with LAM. Smolarek et al. tested the *TSC1* and *TSC2* genes for RAML in 13 women with LAM and showed that heterozygous deletions of *TSC2* were present in seven (54%) patients with RAML and in eight retroperitoneal lymph nodes, which supports the possibility of a common molecular basis for the pathogenesis of TSC, LAM, and angiomyolipomas [118]. In summary, these findings emphasize the critical role of *TSC2* mutations in the development of RAML and LAM, and they suggest a common molecular mechanism underlying these disorders, particularly in the context of tuberous sclerosis.

Interestingly, Zarei et al. found that the reduced expression of microphthalmia transcription factors (*MITF*) negatively regulates the development of renal angiomyolipomas by inhibiting cell growth, invasion, and migration. It was able to act by regulating *CyR61* (Fig. 8) [119]. This suggests that *CyR*61 is a novel target for *MITF* in regulating the progression of RAML and, both *MITF* and

CyR61 are expected to be potential targets for future clinical treatment of RAML. These studies have revealed the molecular mechanism of RAML in patients with non-tuberous sclerosis, highlighted the critical role of *TSC2* gene mutations in the development of these lesions, and also provided new therapeutic targets (such as *MITF* and *CyR61*) for the clinic. Understanding the clinical characteristics of different gene mutations and the molecular mechanism of TSC can provide an important reference for early diagnosis, personalized treatment and disease monitoring of patients with non-tuberous sclerosis.

The preservation of renal function and the prevention of other dangerous complications, such as bleeding, represent the primary clinical objectives in the management of patients with RAML. In cases of large, symptomatic, and/or hemorrhagic AML, embolization or surgical intervention have traditionally been considered standard treatment options. Nevertheless, mTOR inhibitors have recently emerged as a noninvasive alternative for the treatment of such conditions [120]. In 2018, the U.S. Food and Drug Administration (FDA) approved everolimus for the treatment of TSC-related indications, including SEGA, partial seizures, and renal angiomyolipoma in adults and children aged over 2 years [121].

A Phase III, double-blind, placebo-controlled trial involving 107 patients with TSC and renal angiomyolipoma demonstrated a response rate of 54% following treatment with everolimus. The proportion of patients achieving a reduction in renal angiomyolipoma volume of ≥ 30% and ≥ 50% increased over time, reaching 81.6% (62/76) and 64.5% (49/76), respectively, by week 96. Notably, no cases of renal hemorrhage were reported during the treatment period. Most adverse events (AEs) were graded as mild to moderate (Grade 1/2), and the incidence of new or severe AEs decreased over time [122]. These findings indicate that everolimus effectively reduces the volume of renal angiomyolipoma in patients with TSC and angiomyolipoma, thereby improving patient outcomes. This conclusion has been corroborated by numerous single-center and multicenter clinical trials conducted in various countries [123–128].

Furthermore, a clinical case series involving 5 years of continuous everolimus treatment in patients with TSC-associated angiomyolipoma (TSC-AML) demonstrated that among 28 treated patients, everolimus facilitated AML regression in all patients who were able to tolerate the treatment for more than 6 months. Additionally, baseline renal function remained stable over a period of 3 years [129]. This study corroborated the efficacy of everolimus in treating TSC-AML and provided valuable "real-world" data regarding patient tolerance, which holds significant clinical relevance. Notably, in both the EXIST–1 and EXIST–2 studies, patients with TSC and RAML exhibited stable mean estimated glomerular filtration rate (eGFR) over time following everolimus treatment [130]. Consequently, everolimus does not appear to exhibit nephrotoxicity and can effectively control or even reverse renal growth while preserving or enhancing renal function in patients with TSC and RAML [130, 131].

Other studies have demonstrated that mTOR inhibitors serve as an effective therapeutic option for patients with aggressive malignant renal epithelioid angiomyolipoma (EAML). A retrospective analysis was conducted on the clinical data of seven EAML patients treated with everolimus. Following three months of oral administration of everolimus (10 mg, once daily), five patients exhibited partial remission, while two patients showed stable disease. The mean maximum tumor diameter decreased from 9.6 cm to 5.2 cm [132]. Additionally, the response to everolimus in TSC patients was found to be superior compared to non-TSC patients [132, 133]. Both sirolimus and everolimus are clinically recognized as mTOR inhibitors; however, it has been observed that in the treatment of TSC-AML patients, the everolimus group demonstrated a more significant reduction in TSC-AML volume at both six and twelve months compared to the sirolimus group [134]. These findings align with the results of a double-blind, placebo-controlled, phase 3 trial of everolimus, which revealed that 55% of everolimus-treated patients achieved a ≥ 50% reduction in target angiomyolipoma lesion volume from baseline, while 80% of patients achieved a ≥ 30% reduction [86].

Limitations of mTOR inhibitor therapy

During the treatment of diseases, it is necessary to consider the adverse reactions of drugs, as some drugs need to be taken for a long time to achieve therapeutic effects. The treatment of tuberous sclerosis syndrome is no exception, as drug withdrawal will lead to the rebound growth of tumors (SEGA, RAML, cardiac rhabdomyolipoma and skin lesions) in most patients [125, 135]. mTOR inhibitors such as rapamycin and its derivatives are key drugs for the treatment of tuberous sclerosis syndrome. However, oral mTOR inhibitor treatment may be associated with an increased risk of infection. The most common adverse reactions are oral inflammation. Acne, amenorrhea and laboratory abnormalities have also been reported [136, 137]. Oral mucositis is the most common dose-limiting toxicity observed in mammalitic rapamycin target (mTOR) inhibitors (everolimus, tisirolimus and sirolimus). Its characteristic is avatar-like lesion, which is very different from the lesions caused by chemotherapy and radiotherapy. These single or multiple well-defined circular superficial painful ulcers are confined only to the non-keratinized mucosa and are occasionally surrounded by erythema halos [138]. It is noteworthy that the mechanism by which rapamycin aggravates pneumonia may

be that it aggravates Staphylococcus aureus pneumonia by increasing the inflammatory level of macrophages. Inhibition of the mTOR-RPS6 pathway upregulates the expression of cytokines and chemokines in macrophages, thereby increasing inflammatory cell infiltration and exacerbating tissue damage [139]. When rapamycin is used to treat polycystic kidney disease, it can cause dose-dependent hyperlipidemia (with a 30–72% increase in triglycerides) and thrombocytopenia (with an incidence of 10–20%). Blood drug concentration needs to be monitored to reduce the risk [140]. mTOR inhibitors sirolimus and everolimus, when used as immunosuppressants in solid organ transplantation, can cause serious adverse reactions. However, in interstitial pneumonia, the tolerance of everolimus seems to be superior to that of sirolimus [141]. Serious adverse events associated with sirolimus, as reported by Bissler [142], were attributed to pre-existing poor physical conditions in affected patients. This highlights the necessity for cautious clinical application of sirolimus in chronic disease management, ensuring thorough evaluation of drug safety and appropriateness based on comprehensive patient health assessments. Finally, mTOR inhibitors may cause systemic non-nephropathic side effects, including oral ulcers, blood disorders, bone marrow suppression, metabolic abnormalities, adverse skin reactions, lymphedema, and fertility/gonadal toxicity [143, 144]. It is evident that mTOR inhibitors may still induce mild to severe adverse drug reactions during the treatment of TSC associated with various renal diseases. This highlights the necessity for appropriate monitoring and management of patients in clinical practice. Simultaneously, emphasis should be placed on the development of individualized targeted therapies for patients to optimize efficacy while minimizing side effects.

Conclusions and clinical perspectives

TSC is a crucial regulatory molecule involved in the complex network that controls cell growth, proliferation, and survival. Numerous studies have confirmed that TSC plays a significant role in various renal diseases. Loss-of-function mutations in the *TSC1* or *TSC2* genes lead to abnormal activation of the mTOR pathway, which, while essential for cell metabolism and stress responses, is also closely linked to the pathogenesis of renal dysfunction. Notably, in TSC-associated renal diseases such as DKD, RAML, RCC, renal cysts, and CKD, excessive activation of the mTOR pathway is a hallmark feature. Interestingly, while mTOR promotes cell growth and survival in TSC-related renal lesions, dysregulated activation of the pathway results in the progression of renal damage, thereby exacerbating renal failure.

Extensive animal model studies have demonstrated activation of the mTOR pathway in various TSC-related renal diseases. mTOR inhibitors, such as rapamycin and its derivatives, have shown potential to halt disease progression and prevent further renal damage in these models. In patients with TSC, mTOR inhibitors have proven to be highly effective in controlling the growth of renal cysts, RAML, and RCC. Numerous clinical trials have evaluated the therapeutic potential of mTOR inhibitors in TSC-associated renal diseases, including RAML, RCC, and renal cysts. These studies highlight the clinical significance of mTOR inhibitors, such as everolimus, in the treatment of TSC, demonstrating their ability to significantly slow the progression of renal failure in patients with TSC.

In conclusion, our understanding of the mechanisms by which TSC contributes to renal disease has advanced considerably. Targeting the mTOR pathway provides a new therapeutic approach for TSC-related renal diseases. As research progresses, it is essential to further explore the long-term effects and safety of mTOR inhibitors, particularly in managing TSC-related chronic kidney disease and improving patient prognosis.

Author contributions

Q.L.L. and X.G.Z. designed and wrote the manuscript. Y.X., Y.X.F., H.D.Z., T.F.L., X.Q.Z., L.K.P., and X.J. revised the manuscript. H.L.D. contributed to the concept and outline of manuscript, the revision of this manuscript, and the final approval of the version to be published. All authors have read and approved the final manuscript.

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Data availability

No datasets were generated or analysed during the current study.

Declarations

Ethical approval

None required for reviews.

Informed consent to participate

It does not apply to reviews.

Competing interests

The authors declare no competing interests.

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