Rapamycin and dexamethasone during pregnancy prevent tuberous sclerosis complex–associated cystic kidney disease

Morris Nechama,1 Yaniv Makayes,1 Elad Resnick,1 Karen Meir,2 and Oded Volovelsky1

1Pediatric Nephrology Unit and 2Department of Pathology, Hadassah-Hebrew University Medical Center, Jerusalem, Israel.

Chronic kidney disease is the main cause of mortality in patients with tuberous sclerosis complex (TSC) disease. The mechanisms underlying TSC cystic kidney disease remain unclear, with no available interventions to prevent cyst formation. Using targeted deletion of TSC1 in nephron progenitor cells, we showed that cysts in TSC1-null embryonic kidneys originate from injured proximal tubular cells with high mTOR complex 1 activity. Injection of rapamycin to pregnant mice inhibited the mTOR pathway and tubular cell proliferation in kidneys of TSC1-null offspring. Rapamycin also prevented renal cystogenesis and prolonged the life span of TSC newborns. Gene expression analysis of proximal tubule cells identified sets of genes and pathways that were modified secondary to TSC1 deletion and rescued by rapamycin administration during nephrogenesis. Inflammation with mononuclear infiltration was observed in the cystic areas of TSC1-null kidneys. Dexamethasone administration during pregnancy decreased cyst formation by not only inhibiting the inflammatory response, but also interfering with the mTORC1 pathway. These results reveal mechanisms of cystogenesis in TSC disease and suggest interventions before birth to ameliorate cystic disease in offspring.

Introduction

Tuberous sclerosis complex disease (TSC) is a genetic disorder affecting various organs, including the brain, kidney, skin, and heart, with an estimated prevalence of approximately 1:6,000 in all populations studied (1–4). De novo or inherited autosomal dominant mutations in TSC1 or TSC2 result in inactivation of TSC composed of hamartin and tuberin proteins, respectively. The complex acts as a tumor suppressor and inhibits the activity of mTOR complex 1 (mTORC1), a central regulator of various cellular functions such as protein translation, proliferation, metabolism, and autophagy (5–7). However, the hamartin–tuberin complex also has mTORC1-independent cellular effects through distinct pathways. For instance, we have previously shown that the effect of improved nephron endowment by TSC1 hemizygous deletion in nephron progenitor cells (NPCs) is independent of mTORC1 activity (8, 9). Interruption of the hamartin–tuberin complex by TSC2 deletion also has mTORC1-independent effects on prostaglandin production and NOTCH activity (10–12). PAK2 and TGF-β–Smad2/3 signaling pathways were suggested to mediate this independent effect as well (13, 14). Moreover, in cancer cells, TSC2 was shown to regulate VEGF gene expression in a mTOR-independent pathway (15). Indeed, some of the clinical manifestations of TSC disease respond only partially to mTOR inhibitors (2, 13, 14, 16, 17).

TSC has debilitating neurological effects in childhood, including convulsions and autism spectrum disorder, which usually stabilize in late adolescence (18, 19). Chronic kidney disease (CKD) is the main cause of morbidity in adult patients with TSC (20, 21). The renal manifestations of TSC include renal angiomylipoma (AML), benign vascular lesions, and cystic disease of the kidneys, which appear in the majority of patients (21, 22). AML of large dimensions poses an increased risk of bleeding if left untreated. Cystic kidney disease leads to a gradual loss of renal parenchyma, aggravated by the decline in nephron number consequent to multiple surgical procedures for AML resections and ablations. As a result, patients with TSC are exposed to complications of CKD at an earlier age than the general population. About 40% of patients with TSC eventually develop advanced CKD (stage III–V) (23). The burden of CKD in patients with TSC ranges in severity from a single renal cyst to a severe polycystic phenotype, especially in deletion disrupting both TSC2 and PKD1 genes and PKD1 (24, 25). The precise pathomechanism by which this autosomal...

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dominant disorder causes cystic renal disease remains poorly understood. Furthermore, although mTOR inhibitors reduce the size and bleeding risk of large AML (16, 26), their therapeutic effects and, even more critical, their preventive effects on TSC cystic kidney disease are still controversial (21, 27, 28).

We have previously generated a mouse model of TSC by specific deletion of TSC1 in NPCs using Cre-Lox recombination in mice (8). NPCs differentiate into the various segments of the nephron, forming the basic filtering unit. They originate from the embryonic metanephric mesenchyme and disappear in the third postpartum day in mice, with no subsequent regeneration. Although TSC1 hemizygous deletion induces nephrogenesis, complete deletion of TSC1 leads to a lethal cystic phenotype characterized by severe injury to the renal proximal tubular cells (PTCs) (8).

We now show that mononuclear inflammation, especially macrophage infiltration, plays a central role in cystogenesis in CKD of TSC. The cyst formation is paralleled by increased mTOR activity and cystogenic processes, such as increased c-Myc expression and cell proliferation. Furthermore, the cystic disease in offspring can be prevented using mTOR inhibitors and corticosteroids during pregnancy. Our findings identify mechanisms and therapeutic targets to overcome TSC cystic kidney disease.

**Results**

We have previously shown that complete deletion of TSC1 in NPCs induces tubular damage as early as E15.5 with swollen cellular appearance and occluded lumen. Cyst formation was evident at E17.5 (Figure 1A and B) (8). Dissection of kidneys from TSC1-null embryos at various time points during embryogenesis herein identified increased phosphorylation of ribosomal protein S6 (pS6), the main downstream target of mTORC1 at E15.5 and E17.5 (Figure 1C). The increased levels of pS6 were evident in the damaged PTCs, as was determined by lotus tetragonolobus lectin–positive (LTL-positive) cyst-lining epithelial cells (Figure 1D and Supplemental Figure 1; supplemental material available online with this article; https://doi.org/10.1172/jci.insight.136857DS1). The cysts appeared in the late stages of pregnancy, beginning at E17.5. Therefore, the increased pS6 staining initially appeared in the injured tubular cells and then in the cyst-lining epithelial cells, which originate from the PTCs. Therefore, TSC1 deletion in NPCs leads to tubular injury and cyst formation, associated with mTORC1 activation.

Next, we examined whether mTORC1 inhibition alleviates TSC tubular damage and cystogenesis. TSC1fl/fl female mice were mated with Six2 Cre+/+ TSC1fl/+ males to generate Six2 Cre+/+ TSC1fl/fl pups with NPC-specific TSC deletion (25% of offspring). We injected pregnant TSC1fl/fl females with rapamycin, a potent and specific inhibitor of mTORC1 or vehicle, on embryonic days E12.5, E14.5, and E16.5 (Figure 2A). We measured tubular damage and cyst formation in kidneys of the TSC1–/– offspring. Rapamycin prevented cellular injury and cystogenesis, as demonstrated by a decline in cyst area and number (Figure 2, B and C). While mTOR activity dramatically increased in TSC1–/– animals compared with control littersmates, rapamycin decreased mTORC1 activity, as shown by reduced pS6 levels in both null and hemizygous TSC1 kidneys compared with controls. pS6 levels decreased by both immunofluorescent (IF) staining and Western blot analysis (Figure 2, B, D, and E, and Supplemental Figure 3).

C-Myc is a potent transcription factor involved in diverse cellular activities, previously demonstrated to be involved in cystogenesis in autosomal dominant polycystic kidney disease (ADPKD) (29, 30). In addition to the increase in mTORC1 activity, c-Myc levels increased in both hemizygous and TSC1-null kidneys. Furthermore, c-Myc predominantly expressed in cyst-lining epithelial cells (Figure 2B). Rapamycin decreased c-Myc levels, as observed by both IF staining and Western blot analysis (Figure 2, B, D, and E). Cell proliferation was also inhibited by rapamycin, as measured by decreased expression of the proliferation marker Ki67. Therefore, rapamycin ameliorates cystic kidney disease by reducing mTOR activity, c-Myc expression, and cell proliferation, all of which have been previously shown to be involved in cyst formation in other renal cystic diseases (29, 30).

NPC-specific TSC1-null mice die 2 days after birth (8). We therefore investigated whether rapamycin improves the survival of TSC mice. To that end, we monitored the effect of rapamycin administered during pregnancy on offspring survival. Rapamycin prolonged the survival of pups up to the age of P14, demonstrating a significant effect of rapamycin after birth (Figure 2F). At P14, the effect of rapamycin was no longer evident, and the mice had a high cystic burden and increased pS6 expression in the proximal tubules due to TSC1 ablation (Supplemental Figure 2). Thus, rapamycin administration during pregnancy prolonged the life span of newborn TSC mice by transient inhibition of renal tubular damage and cystogenesis.
We then examined the effect of TSC1 deletion on global gene expression and intracellular pathways in PTCs and the effect of rapamycin on both. For this purpose, we sort Prominin1-positive PTCs from control and Six2 Cre tg/+ TSC1fl/fl mice (31), with and without rapamycin as above (Figure 2A and Figure 3A). Gene expression was analyzed by RNA sequencing to identify genes and pathways that are affected by TSC1 deletion and rescued by rapamycin treatment during pregnancy (Figure 3B). Among these pathways, we found an increase in the expression of genes related to inflammatory response and complement pathway activation in TSC1-null mouse PTCs compared with controls. The expression of these genes was downregulated in PTCs from pups of mothers injected with rapamycin during pregnancy (Figure 3, C–E). Many of these genes are associated with macrophage polarization and chemotaxis to the inflammation site (32–34).

Inflammation with high mononuclear cell infiltration was evident in cystic kidneys of TSC1-null P14 mice (Figure 2G). Cystic kidneys of TSC1-null P0 mice demonstrated increased levels of the NF-κB P65 subunit protein (Figure 4, A–C). Increased macrophage infiltration in P0 mice was also demonstrated by high levels of the F4/80 macrophage marker expression by immunostaining as well as high levels of F4/80+ cells in dissociated TSC1–/– kidneys, as reflected by FACS analysis (Figure 4, A and D). Therefore, cystic kidneys in TSC show an inflammatory response and macrophage infiltration.

We next examined whether antiinflammatory treatment during pregnancy prevents the TSC cystic disease in a similar manner as mTOR inhibition. Corticosteroids are potent antiinflammatory medications that are frequently used during high-risk preterm pregnancies for various indications, including acceleration of lung maturation (35). Dexamethasone was injected to pregnant TSC mice carrying TSC1-null embryos, at the same time course as rapamycin (E12.5, E14.5, and E16.5) (Figure 2A). Dexamethasone administration during gestation improved renal cystic burden (Figure 4, E and F) and significantly reduced macrophage infiltration, as indicated by F4/80 immunostaining and FACS analysis (Figure 4, E, G, and H). However, dexamethasone did not rescue the injured proximal tubular morphology, as these still demonstrated swollen epithelial cells and occluded lumens. Dexamethasone decreased mTORC1 activity but not P65 in TSC cystic kidneys (Figure 4, I–K). Therefore, inflammation plays a central role in the pathogenesis of TSC1 deletion in NPCs is associated with mTOR pathway activation in PTCs. Kidney sections at different embryonic ages as indicated were stained with (A) H&E. (B) Enlarged kidney sections as in A. (C) Phosphorylated S6 analyzed by IF. (D) LTL antibody by IHC. Scale bar: 50 μm. n = 3. Black arrows indicate the injured PTCs with swollen appearance and occluded lumen. TSC, tuberous sclerosis complex; NPCs, nephron progenitor cells; PTCs, proximal tubular cells; IF, immunofluorescent; LTL lotus tetragonolobus lectin.
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of cystic TSC kidney disease and corticosteroid administration during gestation prevents cystogenesis in TSC by inhibiting both mTOR activation and inflammation.

Discussion

In our TSC mouse model, TSC1 deletion in NPCs induced proximal tubule cell damage and cyst formation, starting as early as E15.5. Based on our findings, mTORC1 hyperactivation in TSC-null proximal renal tubules leads to tubular cell damage and proliferation, also through c-Myc activation. Administration of steroids during pregnancy prevented cyst formation in TSC offspring by not only hindering the inflammatory process, as the high macrophage infiltration, but also downregulating mTORC1 activity (Figure 5).

Various murine models were based on TSC1 or TSC2 deletions in distinct portions of the nephron. In the current model, elimination of both TSC1 alleles was introduced specifically in the progenitor cells, and therefore it was present in most segments of the nephron in the offspring. Using this strategy we also showed that the resulting cysts originate from the proximal tubules. Moreover, the severe cystic disease appeared only after homozygous deletion of TSC1 in our model. It has been previously suggested that a second hit, which leads to loss of heterozygosity, takes place in the cystic cells as reported in TSC-AML and pulmonary lymph-angiomatosis. However, the reports on loss of heterozygosity in TSC renal cysts are not consistent (36, 37).

Rapamycin has been successfully used during pregnancy in small clinical trials to prevent TSC manifestations, such as cardiac tumors (38, 39). Benefits from the early use of rapamycin were also demonstrated in neurological manifestations of TS, such as developmental delay (40). However, the safety of rapamycin during pregnancy has not been studied thoroughly in human studies and teratogenic effects have been

Figure 2. mTOR inhibition by rapamycin prevents proximal tubular damage and cyst formation and prolongs survival of Six2 CreERT/ TSC1fl/fl mice. (A) Experimental time course. TSC1fl/fl females were mated with Six2 CreERT/ TSC1fl/+ males. Rapamycin or vehicle (DMSO) was injected at the indicated gestational ages. Kidneys of Six2 CreERT/ TSC1fl/+ P0 pups were removed. (B) Renal sections from Six2 CreERT/ TSC1fl/fl mice were stained with H&E, IHC for LTL and Ki-67, and IF for phospho-S6 (pS6) and c-Myc. Scale bar: 50 μm. n = 3 in each group. (C) Quantitative analysis of cyst area and number of cyst per section as in B. *P < 0.05, compared with vehicle, DMSO (n = 5); rapamycin (n = 6). (D) Western blots of kidney extracts from control, Six2 Cre TSC1fl/+, Six2 CreERT/ TSC1fl/fl mice treated with rapamycin or vehicle as in A, analyzed for pS6, GAPDH, and c-Myc, showing elevated pS6 and c-Myc expression in heterozygous and homozygous renal extracts compared with control and reduction in the expression of these proteins upon rapamycin treatment. (E) Quantification of the Western blot as in D. *P < 0.05, compared with control (WT). n = 3 in each group. (F) Kaplan-Meier curve showing improved survival rate of rapamycin-treated Six2 CreERT/ TSC1fl/fl mice (n = 6), compared with DMSO (n = 6). (G) H&E staining of P14 offspring of rapamycin-treated Six2 CreERT/ TSC1fl/fl mice as in A, demonstrating mononuclear infiltrate. The dashed circle indicates mononuclear inflammation site. Scale bar: 500 μm. n = 3. Unpaired t test was used for C and 1-way ANOVA statistical analysis was used followed by Duncan’s post hoc test for E. TSC, tuberous sclerosis complex; IF, immunofluorescent; LTL lotus tetragonolobus lectin.
reported in animal models (39, 41). Our findings suggest that the use of antiinflammatory agents such as corticosteroids may reduce kidney damage induced by cystogenesis. Indeed, corticosteroids such as dexamethasone and betamethasone are commonly used during pregnancy and may therefore serve as an alternative approach for the treatment of TSC.

Previous studies demonstrated that the catabolic effect of glucocorticoids on skeletal muscle is also mediated by inhibition of the mTOR pathway. Steroid administration led to decreased levels of phosphorylated 4EBP1 and S6 kinase 1, the main downstream targets of mTORC1. It has been suggested that glucocorticoids enhance the expression of REDD1, a mTORC1 suppressor (42–44). Our study is a report of the effect of glucocorticoids on TSC disease, such as cystic kidney disease. We showed that the effect of glucocorticoids is direct through modulation of mTORC1 activity as well as by the inhibition of the inflammatory response, which is essential for TSC cystogenesis (42–44).

Our results indicated increased c-Myc expression in TSC cystic epithelial cells that is mTOR dependent. A relationship between the hamartin–tuberin complex, acting as a tumor suppressor gene, and c-Myc has been studied in tumors (45–47). c-Myc, as a potent oncogene, induces expression of genes involved in cell proliferation in various tumors. On the one hand, c-Myc is a direct repressor of TSC2 expression—encoding tuberin, the coprotein of hamartin. On the other hand, the loss of TSC, by loss of tuberin, enhances the expression of c-Myc, thereby causing an oncogenic loop. The effect of loss of TSC is mediated by hyperactivation of mTORC1. mTORC1 is required for proper translation of c-Myc.
as well as its posttranscriptional modification to enhance c-Myc stability (48). Lately, a similar relationship of c-Myc with hamartin encoded by \textit{TSC1} was revealed in c-Myc–driven tumors such as Burkitt’s lymphoma, also in a mTORC1 dependent manner (46). Indeed, renal cystogenesis is characterized by an increased proliferation rate of tubular cells. Analysis of ADPKD kidney biopsies also confirmed increased c-Myc expression specifically in the cystic epithelial cells concurrent with an increased proliferation rate (29, 30, 49, 50). Inhibition of c-Myc significantly reduced cystogenesis in vivo (51). Here, we report that interruption of the hamartin–tuberin complex in NPCs by \textit{TSC1} deletion during pregnancy is associated with high c-Myc activity and proliferation rate in TSC cyst-lining cells.

The role of inflammation in TSC manifestation was also demonstrated in TSC-related tumors. Rapamycin leads to only partial regression of TSC tumors, including AML, with a high risk of relapse of the tumor after cessation of rapamycin therapy. The adequate response of the immune system to the tumors may also be impaired by the effect of rapamycin on the immune system. Immunotherapy by PD-1 and CTLA-4 blockade leads to substantial regression of TSC-related tumors with long-term remission, which may be further improved by combination therapy with mTORC1 inhibitors (52–57).

Our findings on the role of inflammation in TSC cystic disease are compatible with new evidence demonstrating that macrophages play a central role in cyst formation and progression in ADPKD (58, 59).
Previous studies have shown that infiltrating macrophages are initially of the proinflammatory classically activated M1-like type, which exacerbate tubular injury due to their release of reactive oxygen and nitrogen intermediates (60). Later, the M1-like macrophages are converted to alternatively activated M2-like macrophages, which then stimulate the proliferation of tubular epithelial cells for the apparent purpose of facilitating the repair of the sustained injury. Whether the same molecular mechanisms by which macrophages induce cytogenesis in ADPKD are activated in TSC awaits further investigation.

We demonstrated that hyperactivation of mTOR plays a central role in TSC cystic kidney disease by increasing PTC proliferation rate, which is also mediated by enhancing c-Myc activity. We also showed that increased inflammation, mainly macrophagic infiltration, contributes to cyst formation in TSC, as demonstrated in other cystic kidney disease. Steroid administration diminishes cystogenesis by not only modulating the inflammatory response but also interfering with mTORC1 activity, as has been demonstrated in other organs. Our findings raise an alternative strategy for early intervention during pregnancy to prevent long-term kidney disease as a result of cyst formation in TSC. Our data may also contribute to the understanding of other aspects of TSC disease, such as AML, in the kidney as well as in other organs.

**Methods**

**Animals.** All mice were maintained in the Hebrew University Specific-Pathogen-Free (SPF) Animal Facility Unit. The transgenic mice were a gift from Raphael Kopan’s lab from the division of developmental biology from Cincinnati Children’s Hospital Medical Center. The following CD1 transgenic mice lines were used: Tg(Six2-EGFP/cre)1Amc (herein Six2 CreE/+ ) and TSC1fl/fl (8). For heterozygous deletion of TSC1 in NPCs, 6- to 8-week-old Six2 CreE/+ male mice were mated with 6- to 8-week-old TSC1fl/fl females. To generate homozygous TSC1 deletion (Six2 CreE/+ TSC1fl/fl ) mice, 6- to 8-week-old heterozygous Six2 CreE/+ TSC1fl/+ males were backcrossed with 6- to 8-week-old TSC1fl/+ females. The pregnancy date was determined by vaginal plug expulsion. The morning of plug detection was designated as day 0.5 of pregnancy. At different embryonic dates, pregnant females were sacrificed using CO2 and cervical dislocation. The embryos and newborn pups were dissected and kidneys were excised for histopathology evaluation. In some experiments, rapamycin (0.2 mg/kg), dexamethasone (0.1 mg/kg), or vehicle (DMSO or PBS, respectively) were i.p. injected.

**Histology.** Embryonic or newborn mouse kidneys were dissected on ice-cold PBS and fixed overnight in fresh 4% formaldehyde in PBS. Kidneys were embedded in paraffin for sectioning. For overall histology, tissue sections were stained with hematoxylin/eosin and PAS staining. IHC and IF staining were performed as previously described (8, 61). Briefly, paraffin-embedded tissue sections (4–6 μm) were deparaffinized, hydrated, and incubated overnight at 4°C with the following antibodies according to the manufacturer’s instructions: biotin anti-LTL (B-1325, Vector); rabbit anti–phospho-S6 ribosomal protein (2211, Cell Signaling Technology); mouse anti-c-Myc (Sc-40, Santa Cruz Biotechnology);
Biotechnologies). For FACS analysis of F4/80 + cells, kidneys were chopped as described above and washed twice with cold HBSS. For PTC isolation, the cells were stained with PE-conjugated anti-CD133/prominin-1 antibody (12-1331-82, Invitrogen) according to manufacturer’s instructions. PE + and PE − cells were isolated by cell flow cytometry–based cell sorting (Hebrew University Faculty of Medicine Core Facility). The cells were washed and total RNA was extracted using peqGOLD TriFast (PeqLab, Santa Cruz Biotechnology); rabbit anti-P65 (8242, Cell Signaling Technology); and mouse anti-GAPDH (MAB374, MilliporeSigma). See complete unedited blots in the supplemental material.

FACS of PTCs. Kidneys were excised in ice-cold HBSS buffer. The kidneys were sliced and chopped into pieces (~0.5–1 mm) on ice using a surgical scalpel. The chopped kidneys were transferred into HBSS solution containing 1 μg/μL collagenase/dispace (10269638001, MilliporeSigma) and incubated for 25 minutes at 37°C. The cells were filtered through a 40-μm nylon cell strainer (Corning) and washed twice with cold HBSS. For PTC isolation, the cells were stained with PE-conjugated anti-CD133/prominin-1 antibody (12-1331-82, Invitrogen) according to manufacturer’s instructions. PE + cells were isolated by cell flow cytometry–based cell sorting (Hebrew University Faculty of Medicine Core Facility). The cells were washed and total RNA was extracted using peqGOLD TriFast (PeqLab Biotechnologies). For FACS analysis of F4/80 + cells, kidneys were chopped as described above and stained with APC-conjugated anti-F4/80 antibody (130-117-509, Macs Miltenyi Biotec). The cells were washed twice with HBSS before analysis by LSRII flow cytometry.

RNA sequencing and pathway analysis. The RNA quality was evaluated in TapeStation using the RNA ScreenTape kit (Agilent Technologies) and quantified using a Qubit apparatus (Qubit DNA HS Assay kit, Invitrogen). Libraries were prepared from RNA samples using a KAPA Stranded mRNA-Seq Kit (KK8401, Roche). The libraries were barcoded and pooled for multiplex sequencing (1.5 pM total, including 1.5% PhiX control library). The pooled cDNA was loaded on a NextSeq 500 High Output v2 kit (75 cycles) cartridge (Illumina) and sequenced on Illumina NextSeq 500 System, using sequencing conditions of 75 cycles, single-read. Library preparation and sequencing were performed at the Core Facility of the Hebrew University Faculty of Medicine. For further validation, RNA was extracted and used for quantitative PCR (qPCR) with the following primers: IL-6 forward, 5′-ATGACGGGCCAGTGAGAATG-3′; CXCL10 forward, 5′-ATGACGGGCCAGTGAGAATG-3′; TNFA forward, 5′-TAGCCCAAGTCGTAGCAGAAC-3′; TNFA reverse, 5′-ACAAGGTCAACCCACCACTG-3′; CXCL10 forward, 5′-ATGACGGGCCAGTGAGAATG-3′; CXCL10 reverse, 5′-TCTGTG-3′; and CCL4 forward, 5′-CTGTGCAACCTACCATCCGA-3′; CCL4 reverse, 5′-AGGTCAGAGCCCATTGGT-3′.

Gene set enrichment analysis. Differential expression data from TSC1-KO versus TSC1-KO-Rapamycin were subjected to gene set enrichment analysis (GSEA, Broad Institute). GSEA uses ranked differential expression data (cutoff independent) to determine whether a priori–defined sets of genes show statistically significant and concordant differences between 2 biological states. GSEA was run against the hallmark gene sets collection from the molecular signatures database.

Statistics. The numbers of biological samples were determined based on effect size or sample variation. No statistical method was used topredetermine the sample size. No animals or samples were excluded from any analysis. Animals were randomly assigned to groups for in vivo studies; no formal randomization method was applied when assigning animals for treatment. Values are reported as means± SEM unless otherwise stated. The data were analyzed by a Student’s 2-tailed t test. In experiments as indicated, 1-way ANOVA analysis was performed followed by Duncan’s post hoc test. The significance was set at a P value of less than 0.05. The data are presented using the GraphPad Prism version 7.

Study approval. All animal studies were carried out in compliance with the ethical regulations approved by the Animal Care Committee of the Hebrew University Medical School (ethical approval no. MD-17-15368-4).
Author contributions
MN and OV conceived the study, designed the experiment, and wrote the manuscript. MN conducted most of the experiments and supervised the rest of the experiments. YM and ER conducted some of the experiments. KM helped to conceive the study and critically reviewed the manuscript.

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Address correspondence to: Oded Volovelsky, Pediatric Nephrology Unit, Hadassah-Hebrew University Medical Center, Ein-Kerem Campus, Jerusalem, 91120, POB 12000, Israel. Phone: 972.2.6777023; Email: odedvo@hadassah.org.il.


56. Chun HS, et al. A Novel mTORC1-Dependent Mechanism Controls Diabetes Induced by Leptin.

55. Li Q, et al. Regulating mammalian target of rapamycin to tune vaccination-induced CD8(+) T cell responses for tumor immunotherapy.


53. Babcock JT, Nguyen HB, He Y, Hendricks JW, Wek RC, Quilliam LA. Mammalian target of rapamycin complex 1 (mTORC1) and the unfolded protein response.


48. Petrovic-Djergovic D, Popovic M, Chittiprol S, Cortado H, Ransom RF, Partida-Sánchez S. CXCL10 induces the recruitment of macrophages into kidney, which aggravate purumycin aminonucleoside nephrosis.

47. Schmidt EV, Ravitz MJ, Chen L, Lynch M. Growth controls connect: interactions between c-myc and the tuberous sclerosis complex.


45. Lam HC, Nijmeh J, Henske EP. New developments in the genetics and pathogenesis of tumours in tuberous sclerosis complex.

44. Babcock JT, Nguyen HB, He Y, Hendricks JW, Wek RC, Quilliam LA. Mammalian target of rapamycin complex 1 (mTORC1) and the unfolded protein response.

43. Lam HC, Nijmeh J, Henske EP. New developments in the genetics and pathogenesis of tumours in tuberous sclerosis complex.

42. Shah OJ, Anthony JC, Kimball SR. Glucocorticoids oppose translational control by leucine in skeletal muscle.


37. Onda H, Lueck A, Marks PW, Warren HB, Kwiatkowski DJ. Tsc2(−/−) mice develop tumors in multiple sites that express gelatinase B.

36. Petrovic-Djergovic D, Popovic M, Chittiprol S, Cortado H, Ransom RF, Partida-Sánchez S. CXCL10 induces the recruitment of macrophages into kidney, which aggravate purumycin aminonucleoside nephrosis.


34. Lam HC, Nijmeh J, Henske EP. New developments in the genetics and pathogenesis of tumours in tuberous sclerosis complex.

33. Lam HC, Nijmeh J, Henske EP. New developments in the genetics and pathogenesis of tumours in tuberous sclerosis complex.

32. Lam HC, Nijmeh J, Henske EP. New developments in the genetics and pathogenesis of tumours in tuberous sclerosis complex.


27. Lam HC, Nijmeh J, Henske EP. New developments in the genetics and pathogenesis of tumours in tuberous sclerosis complex.


25. Lam HC, Nijmeh J, Henske EP. New developments in the genetics and pathogenesis of tumours in tuberous sclerosis complex.


23. Lam HC, Nijmeh J, Henske EP. New developments in the genetics and pathogenesis of tumours in tuberous sclerosis complex.

22. Lam HC, Nijmeh J, Henske EP. New developments in the genetics and pathogenesis of tumours in tuberous sclerosis complex.


20. Lam HC, Nijmeh J, Henske EP. New developments in the genetics and pathogenesis of tumours in tuberous sclerosis complex.


18. Lam HC, Nijmeh J, Henske EP. New developments in the genetics and pathogenesis of tumours in tuberous sclerosis complex.

17. Lam HC, Nijmeh J, Henske EP. New developments in the genetics and pathogenesis of tumours in tuberous sclerosis complex.

16. Lam HC, Nijmeh J, Henske EP. New developments in the genetics and pathogenesis of tumours in tuberous sclerosis complex.

15. Lam HC, Nijmeh J, Henske EP. New developments in the genetics and pathogenesis of tumours in tuberous sclerosis complex.


12. Lam HC, Nijmeh J, Henske EP. New developments in the genetics and pathogenesis of tumours in tuberous sclerosis complex.

11. Lam HC, Nijmeh J, Henske EP. New developments in the genetics and pathogenesis of tumours in tuberous sclerosis complex.

10. Lam HC, Nijmeh J, Henske EP. New developments in the genetics and pathogenesis of tumours in tuberous sclerosis complex.